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Program and Abstracts

The Eighteenth International Conference on Antiviral Research

Sponsored by:

The International Society For Antiviral Research

Princesa Sofia Hotel

Barcelona, Spain

April 11–14, 2005

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and
Eighteenth International Conference on Antiviral Research

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Local Host

José A. Esté

Retrovirology Laboratory IrsiCaixa
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Badalona, Barcelona, Spain

Introduction to Sponsor

The International Society for Antiviral Research (ISAR)

The Society was organized in 1987 as a non-profit scientific organization for the purpose of advancing and disseminating knowledge in all areas of antiviral research. To achieve this objective, the Society organizes an annual meeting. The Society is now in its 18th year of existence, and has about 600 members representing 30 countries. For membership application forms or further information, please contact Dr. Amy Patick, Secretary, ISAR; Pfizer Global R&D, Department of Virology, 10777 Science Center Drive, San Diego, CA 92121, USA; Phone: +1 858 622 3117; fax: +1 858 678 8182; E-mail: amy.patick@pfizer.com. Membership application forms will also be available at the Conference Registration desk, or from our website www.isar-icar.com.

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Office of AIDS Research, National Institutes of Health, Bethesda, MD, USA

SATELLITE SYMPOSIUM

Clinical Update on Antiviral Drugs

Sunday, April 10, 2005
14:00–17:00
Catalunya Room
Princesa Sofia Hotel

SOCIAL EVENTS

Opening and Clinical Symposium Reception

Sunday, April 10, 2005
18:00–20:00.
Top City Room
Floor 19
Princesa Sofia Hotel

Conference Banquet

Wednesday, April 13, 2005

Reception

19:30
Mezzanine Level

Dinner and Program

20:00–22:00
Catalunya Room
Princesa Sofia Hotel

Final Program

Eighteenth International Conference on Antiviral Research

Sponsored by:

International Society for Antiviral Research

Princesa Sofia Hotel

Barcelona, Spain

April 11–14, 2005

2005 International Conference on Antiviral Research

Monday, April 11, 2005

Opening Greetings

Catalunya Room

09:00 Welcome to the 18th I.C.A.R., John A. Secrist, III, President I.S.A.R.
Welcome to Barcelona, José A. Esté, Local Host

Oral Session I: Retroviruses
Chairs: Amy K. Patick and María-José Camarasa

09:15 Plenary Speaker
Manos Perros, Anti-infectives Discovery, Pfizer Global R&D, Sandwich Laboratories, UK
"Inhibitors of HIV Entry targetting CCR5"

09:45 1. Novel "lock-in" modified *cyclo*Sal nucleotides (II): Application of the AM- and the POM-group
Chris Meier, Christian Ducho, Henning J. Jessen, Jan Balzarini
University of Hamburg, Institute of Organic Chemistry, Hamburg, Germany; Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium

10:00 2. Deoxythreosyl Phosphonate Nucleosides as Selective Anti-HIV Agents
Tongfei Wu, Matheus Froeyen, Veerle Kempeneers, Christophe Pannecouque, Roger Busson, Erik De Clercq, Piet Herdewijn
Laboratory of Medicinal Chemistry, Rega Institute for Medical Research, Minderbroedersstraat 10, B-3000 Leuven, Belgium; Laboratory of Virology, Rega Institute for Medical Research, Minderbroedersstraat 10, B-3000 Leuven, Belgium

10:15 3. Comparative Evaluation of Twelve Pyrimidinedione Inhibitors of HIV-1 For Further Preclinical and Clinical Development
Robert W. Buckheit Jr., Tracy L. Hartman, Karen M. Watson
ImQuest BioSciences, Inc., Frederick, MD, USA

10:30 *Break*

11:00 4. Selective Removal of Superoxide Anions is Crucial for HIV Replication in Human Primary Macrophages and Prevents Peroxynitrite Mediated Apoptosis in Neurons
S. Aquaro, C. Muscoli, M. Pollicita, A. Ranazzi, T. Granato, MC. Bellocchi, A. Modesti, D. Salvemini, V. Mollace, C.F. Perno
Department of Experimental Medicine University of Tor Vergata Rome, Italy; Faculty of Pharmacy University of Catanzaro Magna Grecia, Roccelletta di Borgia Catanzaro, Italy; MetaPhore Pharmaceuticals, Inc., St. Louis, MO, USA

11:15 5. Substrate Dependence of HIV RNase H Activity and Inhibition by Active Site and Allosteric Site Binding Compounds
Julie Qi Hang, Yu Li, Yanli Yang, Stan Tsing, Jim Barnett, Nick Cammack, Joseph A. Martin, Klaus Klumpp
Roche Palo Alto LLC, Palo Alto, CA, USA

11:30 6. Homology Modeling of HIV-1 gp120 and Docking of Molecules on its Surface Agree with Experimental Data
Mercedes Armand-Ugón, Imma Clotet, Cristina Tintori, Fabrizio Manetti, Bonaventura Clotet, Maurizio Botta, José A. Esté
Retrovirology Laboratory irsiCaixa, Hospital Universitari Germans Trias i Pujol, 08916 Badalona, Spain; Dep. Farmaco Chimico Tecnologico, Università degli Studi di Siena, Siena, Italy

11:45 7. Dioxolane–Thymine Nucleoside is Active Against a Variety of Clinically Relevant NRTI Drug-resistant HIV-1 Strains

Chung K. Chu, Vikas Yadav, K.L. Rapp, Mervi Detorio, Raymond F. Schinazi
The University of Georgia College of Pharmacy, Athens, GA 30602, USA; Emory University/VA Medical Center, Decatur, GA 30033, USA

12:00 8. Reduced Susceptibility to Lopinavir due to V32I/I47A Mutations in HIV-1 Protease
Kirsten Stray, Andrew Mulato, Holly MacArthur, Stephanie Leavitt, Christopher Baer, Xiaohong Liu, Christian Callebaut, Gong-Xin He, Martin McDermott, Tomas Cihlar
Gilead Sciences, Foster City, CA, USA

12:30 *Lunch*
Mitre Room, Lobby Level
Princesa Sofia Hotel

Monday, April 11, 2005

Elion Award Lecture
Catalunya Room

14:00 Presentation of Award: John A. Secrist, III, President I.S.A.R.
Awardee Lecture: Erik De Clercq, Rega Institute, Katholieke Universiteit Leuven, Leuven Belgium
"Antiviral Drug Development: Where Chemistry meets Biology and Medicine"

Oral Session II: Hepadnaviruses
Chairs: Fabien Zoulim and John D. Morrey

15:00 9. Analysis of the Evolution of the HBV Quasi-Species During Sequential Therapy Shows the Emergence of Multiple Drug Resistant Virus
S. Villet, C. Pichoud, M.N. Brunelle, J.P. Villeneuve, C. Trépo, F. Zoulim
INSERM U271, Lyon, France; Hôpital St. Luc, Montréal, Canada

15:15 10. Synthesis and Properties of Novel Types of Chiral Open-Ring Acyclic Nucleoside Phosphonates
Antonin Hol'y, Petra Dolkov
Institute of Organic Chemistry and Biochemistry, Academy of Science, Praha, Czech Republic

15:30 11. Anti-HBV Activity and Intracellular Metabolism of Tenofovir In Vitro
William E. Delaney, Xiaoping Qi, Adrian S. Ray, Huiling Yang, Michael D. Miller, Shelly Xiong
Gilead Sciences, Foster City, CA, USA

15:45 12. Characterisation of Hepatitis B Virus Adefovir Resistance Mutations Outside the Polymerase Active Site Background/Aims: Resistance to ADV was Originally Found in the D Domain of the HBV Polymerase at rtN236T
Angeline Bartholomeusz, Stephen Locarnini, Anna Ayres, Geoff Thompson, David Chalmers, Michael Kuiper
Victorian Infectious Diseases Reference Laboratory, North Melbourne, Vic., Australia; Victorian College of Pharmacy, Parkville, Vic., Australia; Victorian Partnership for Advanced Computing, Carlton, Vic., Australia

Monday, April 11, 2005

Poster Session I: Retroviruses, Hepatitis Viruses, Respiratory Viruses, West Nile Virus, Virological Methods
Baleares Room

16:00–18:00

39. Multi-Targeting the Entrance Door to Block HIV-1 by Aminoglycoside–Arginine Conjugates (AACs)
Aviva Lapidot, Gadi Borkow
The Weizmann Institute of Science, Organic Chemistry, Rehovot, Israel

41. Alkoxyalkyl Esters of (*S*)-HPMPA are Potent Inhibitors of HIV-1 In Vitro
Kathy A. Aldern, James R. Beadle, William B. Wan, Stephanie L. Ciesla, Karl Y. Hostetler
Department of Medicine, San Diego VA Healthcare System and the University of California, San Diego, La Jolla, CA 92093-0676, USA

43. External Qi and Qi Water of Yan Xin Life Science Technology (YXLST) Potently Inhibit HIV-1 Replication
Xin Yan, Hua Shen, Liping Wang, Hongjian Jiang, Xinqi Wu, Jun Wang, Dan Hu, Delia Wolf, Zhaoxiong Yang, Ming Dao, Peihua Ni, Chengsheng Zhang
New Medical Science Research Institute, New York, NY, USA; Dana-Farber Cancer Institute, Boston, MA, USA; Harvard Medical School, Boston, MA, USA; Brigham and Women's Hospital, Boston, MA, USA; Children's Hospital, Boston, MA, USA; Mass General Hospital, Boston, MA, USA; Massachusetts Institute of Technology, Boston, MA, USA; University of Connecticut, Storrs, CT, USA; McMaster University, Hamilton, Ont., Canada

45. Potent and Selective Inhibition of HIV-1 Transcription by a Novel Naphthalene Derivative
Xin Wang, Kazunobu Yamataka, Mika Okamoto, Satoru Ikeda, Masanori Baba
Division of Antiviral Chemotherapy, Center for Chronic Viral Diseases, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima 890-8544, Japan; Japan Tobacco Inc., Osaka 569-1125, Japan

47. Exploring a New Approach in AIDS Therapy. Design, Synthesis and Biological Evaluation of Potential Dimerization Inhibitors of HIV-1 Reverse Transcriptase
Carlos García-Aparicio, Fatima Rodriguez-Barrios, Federico Gago, Erik De Clercq, Jan Balzarini, María-José Camarasa, Sonsoles Velazquez
Instituto de Química Médica, Juan de la Cierva 3, Madrid, Spain; Departamento de farmacología, Universidad de Alcal, Madrid, Spain; Rega Institute for Medical Research, K.U. Leuven, Leuven, Belgium

49. "Borano-Nucleotides" as Molecular Tools to Circumvent Nucleosidic Drugs Resistance of HIV-1 RT
Karine Alvarez, Jèrùme Deval, Karine Barral, Cèline De Michelis, Bruno Canard
AFMB, UMR 6098, Marseille, France

51. Dimerization Inhibitors of Wild-Type and Mutated HIV-1 Proteases: A Pathway to Circumvent Resistances to Classical Antiproteases
Ludovic Bannwarth, Sandrine Ongeri, Nicole Boggetto, Naôma Merabet, Bruno Collinet, Sames Sicsic, Michèle Reboud-Ravaux
Institut Jacques Monod, UMR 7592, CNRS-Univ. Paris 6 et 7, 2 place Jussieu, 75251, Paris Cedex 05, France; Biocis, UMR-CNRS C8076, Facultè de pharmacie, Univ. Paris 11, 5 rue JB Clèment, 92296, Ch, tenay-Malabry Cedex, France

53. Inhibitors of HIV Integrase: New Diketo Structures with Heterocyclic Scaffolds
Vasu Nair, Guochen Chi, Vinod Uchil
University of Georgia, Department of Pharmaceutical and Biomedical Sciences, Athens, GA 30602, USA

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Alessandra Cordeiro, Maria Cruz Bonache, Erik De Clercq, Jan Balzarini, María-José Camarasa, Ana San-Félix
Instituto de Quimica Médica (CSIC), Madrid (Spain); Rega Institute for Medical Research, K.U. Leuven, Leuven (Belgium)

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Noah H. Duffy, Thomas W. Bell, Sreenivasa Anugu, Kaka Dey, Qi Jin, Meinrado F. Samala, Andrej Sodoma, Kurt Vermeire, Erik De Clercq, Dominique Schols
Department of Chemistry, University of Nevada, Reno, NV 89557, USA; Rega Institute for Medical Research, K.U. Leuven, B-3000 Leuven, Belgium

59. Synthesis and Study of 1-(2′-Deoxy-b-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide as an Anti-HIV-1 Mutagenic Agent
Valérie Vivet-Boudou, Jean-Christophe Paillart, Alain Burger, Roland Marquet

Laboratoire Structure des Macromolécules Biologiques et Mécanismes de Reconnaissance, UPR 9002 CNRS, IBMC, 15 rue René Descartes, 67084 Strasbourg, France; Laboratoire de Chimie Bioorganique, UMR 6001 CNRS, Université de Nice Sophia Antipolis, Parc Valrose, 06108 Nice Cedex 2, France

61. Effect of Non-Nucleoside Reverse Transcriptase Inhibitors on the HIV-1 Reverse Transcriptase Associated Ribonuclease H Activity
Enzo Tramontano, Francesca Esposito, Antonio Piras, Paolo La Colla
University of Cagliari, Department of Sciences and Biomedical Technologies, Cagliari, Italy

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Clara E. Cases-Gonzalez, Luis Menéndez-Arias
Centro de Biología Molecular Severo Ochoa (CSIC-UAM), Madrid, Spain

65. Molecular Mechanism for Suppression of Drug-resistant MMLV Reverse Transcriptase by (a-*P*-Borano)-2′,3′-dideoxycytidine-5′-triphosphate
Mikhail I. Dobrikov, Barbara Ramsay Shaw
Department of Chemistry, P.M. Gross Chemical Laboratory, Duke University, Durham, NC 27708-0346, USA

67. A Multiparametric Assay to Screen and Dissect the Mode of Action of Anti-HIV Envelope Drugs
Julia Blanco, Imma Clotet, Bonaventura Clotet, Jose A. Esté
Fundación irsiCaixa, Hosp Germans Trias i Pujol, Badalona, Spain

69. RNA Interference of p53 Blocks HIV Replication
Eduardo Pauls, Jordi Senserrich, Bonaventura Clotet, José A. Esté
Retrovirology Laboratory IrsiCaixa, Hospital Germans Trias i Pujol, Badalona, Spain

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V.S. Gallicchio, C.N. Mayhew, R. Sumpter, M.S. Inayat, M. Cibull, H.L. Elford
University of Kentucky, Department of Clinical Sciences, Lexington, KY, USA; University of Kentucky, Department of Pathology and Laboratory Medicine, Lexington, KY, USA; Molecules of Health Inc. Richmond, VA, USA

73. Second Generation Anti-HIV Short Hairpin RNA (Vif shRNAs and Decoy TAR RNAs) to Avoid RNAi-Mediated Escape Mutant Phenomenon
Hiroshi Takaku, Jacob S. Barnor, Kazuya Yamaguchi, Naoko Miyano-Kurosaki
Department of Life and Environmental Science, Chiba Institute of Technology, Chiba, Japan; High Technology Research Center, Chiba Institute of Technology, Chiba, Japan; Noguchi Memorial Institute for Medical Research, Department of Virology, Accra, Ghana

75. Comparison of Methodology to Assess Fitness and Replication Capacity of Reverse Transcriptase Inhibitor and Protease Inhibitor Resistant Viruses
Tracy L. Hartman, Robert W. Buckheit Jr.
ImQuest BioSciences, Inc., Frederick, MD, USA

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ImQuest BioSciences, Inc., Frederick, MD, USA; BioQual, Inc., Rockville, MD, USA; BioSyn, Inc., Huntingdon Valley, PA, USA

79. Cell-dependent Interference with Viral Transactivation by 6-Aminoquinolone Derivatives
Miguel Stevens, Oriana Tabarrini, Violetta Cecchetti, Erik De Clercq, Arnaldo Fravolini, Christophe Pannecouque
Rega Institute for Medical Research, K.U. Leuven, Leuven, Belgium; Dipartimento di Chimica e Tecnologia del Farmaco, Universita di Perugia, Perugia, Italy

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Kam Tong Leung, Lawrence Chi Ming Chiu, Samuel Sai Ming Sun, Vincent Eng Choon Ooi
The Chinese University of Hong Kong, Department of Biology, Hong Kong, China

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Infectious Disease Research Department, Southern Research Institute, Frederick, MD, USA

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Andrea Cuconati, Gael Westby, Anand Mehta, Timothy Block
Institute for Hepatitis and Virus Research of the Hepatitis B Foundation, Doylestown, 18901 PA, USA; Drexel University College of Medicine, Doylestown, 18901 PA, USA

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Thomas Jefferson University, Department of Biochemistry, Doylestown, PA 18901, USA; Drexel University College of Medicine, Department of Microbiology and Immunology, Doylestown, PA 18901, USA

89. Synthesis and In Vitro Anti-HBV and Anti-HCV Activities of Ring-Expanded ("Fat") Nucleobases and Nucleosides Containing the Imidazo[4,5-*e*][1,3]diazepine-4,8-dione Ring System
Peng Zhang, Brent E. Korba, Ramachandra S. Hosmane
Department of Chemistry and Biochemistry, University of Maryland (UMBC), Baltimore, MD 21250, USA; Division of Molecular Virology and Immunology, Georgetown University Medical Center, Rockville, MD 20850, USA

91. Determination of the Precise Mode of Action of Nucleotide Analog Inhibitors of the HCV-NS5B Polymerase
Hélène Dutartre, Joílle Boretto, Jean-Claude Guillemot, Bruno Canard
AFMB-CNRS Marseille France

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The University of Texas Medical Branch, Galveston, TX, USA; Pharmasset Inc., Tucker, GA, USA

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Hui-Ling Chiou, Chia-Jun Wu
School of Medical Technology, Chung Shan Medical University; Institute of Biochemistry, Chung Shan Medical University

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XTL Biopharmaceuticals Ltd., Rehovot, Israel; Department of Pathology, Stanford University School of Medicine, Stanford, CA, USA

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Infectious Disease Research Department, Southern Research Institute, Frederick, MD, USA; Intarcia Therapeutics, Inc., Emeryville, CA, USA

101. Synergistic Inhibition of Flaviridae Virus by Celgosivir in Combination with Ribavirin or Interferon-a
Dominique Dugourd, Raymond Siu, Jeremy Fenn, Jacob J. Clement, Richard Coulson
MIGENIX Inc., Vancouver, BC, Canada

103. Pharmacokinetics of Celgosivir (MX-3253), a Novel a-Glucosidase-1 Inhibitor, in Loperamide-Treated and Diarrhoea-Induced Rats
Doug Erfle, Evelina Rubinchik, Chris Pasetka, H.D. Friedland, Jacob J. Clement
MIGENIX Inc., Vancouver, BC, Canada

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Dominique Dugourd, Jeremy Fenn, Raymond Siu, Jacob J. Clement, Richard Coulson
MIGENIX Inc., Vancouver, BC, Canada

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Dimitrios P. Vlachakis, Colin Berry, Gareth Jones, Andrea Brancale
Medicinal Chemistry, Welsh School of Pharmacy, Cardiff University, Wales, UK; Cardiff School of Biosciences, Cardiff University, Wales, UK

109. Protective Action of Biologically Active Food Supplement Biotrit C during Experimental Influenza Infection
V. Lozitsky, A. Levitsky, O. Makarenko, A. Fedchuk, T. Gridina
Ukrainian Mechnikov Research Anti-Plague Institute, Odessa, Ukraine; Institute of Stomatology, Odessa, Ukraine

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Donald F. Smee, Kevin W. Bailey, Min-Hui Wong, Robert W. Sidwell
Institute for Antiviral Research, Utah State University, Logan, UT, USA

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Milka Mileva, Angel S. Galabov
Department of Biophysics, Medical University, 2 Zdrave Street, Sofia 1431, Bulgaria; Institute of Microbiology, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria

115. Investigation of Anti-influenza Activity Using Hierarchic QSAR Technology on the Base of Simplex Representation of Molecular Structure
Evgene N. Muratov, Anatoly G. Artemenko, Victor E. Kuz'min, Victor P. Lozitsky, Alla S. Fedchuk, Regina N. Lozytska, Yuri A. Boschenko, Tatiyana L. Gridina
A.V. Bogatsky Physico-Chemical Institute of the National Academy of Sciences of Ukraine, 86 Lustdorfskaya doroga, Odessa 65080, Ukraine, victor@farlep.net; Ukrainian Mechnikov Research Anti-Plague Institute, Odessa, Ukraine

117. A Novel Proteinaceous Protease Inhibitor from *Streptomyces chromophuscus* with Antiviral Activity
Julia Serkedjieva, Lidija Angelova, Michelle Dalgalarrondo, Jean Marc Chobert, Thomas Haertle, Iskra Ivanova
Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria; Department of Microbiology, Sofia University, Sofia, Bulgaria; INRA-LEIMA, BP71627, 44316 Nantes Cedex 3, France

119. Effect of a Plant Polyphenol Extract on Protease and Protease-Inhibitory Activities in Mice Lungs during Experimental Influenza Virus Infection
Julia Serkedjieva, Iskra Ivanova
Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria; Department of Microbiology, Sofia University, Sofia, Bulgaria

121. The Features of Antiviral Action of Arbidol—Selection and Characterization of Arbidol-Resistant Mutants
Irina A. Leneva, Alexander M. Shuster, Alan J. Hay, Robert G. Glushkov
Department of Chemotherapy of Infectious Diseases, Center of Chemistry of Drugs, Russian Chemical and Pharmaceutical Institute, Moscow, Russia; 'Masterlek', Moscow, Russia; National Institute for Medical Research, London, UK

123. Inhibition of Experimentally Induced Influenza Virus Infections by Barrogen, a Potent New Immunostimulant
Robert W. Sidwell, Donald F. Smee, Kevin W. Bailey, Min-Hui Wong, John W. Judge, Barnard Rosenberg
Institute for Antiviral Research, Utah State University, Logan, UT, USA; Barros Research Institute, Holt, MI, USA

125. Inhibition of Influenza A Virus in Cell Culture with Morpholino Oligomers
Qing Ge, David Stein, Andrew Kroeker, Herman Eisen, Patrick Iversen, Jianzhu Chen
Center of Cancer Research, MIT, Cambridge, MA, USA; AVI BioPharma Inc., Corvallis, OR, USA

127. Antiviral action of the bis-quarternary ammonium bases
V. Lozitsky, T. Gridina, Yu. Boschenko, A. Fedchuk, M. Lebeduk, G. Khorokhorina, V. Fedchuk, V. Paliy
Ukrainian Mechnikov Research Anti-Plague Institute, Odessa, Ukraine; State Medical University, Odessa, Ukraine; National Pirogov Medical University, Vinnitsa, Ukraine

129. Natural Inhibitor of Influenza A-Pr8 Extracted from Cinnamon
Irene Barak, Michael Ovadia
Department of Zoology, Tel Aviv University, Tel Aviv, Israel

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Odessa National University of I.I. Mechnikov, Odessa, Ukraine; Ukrainian I.I. Mechnikov Research Anti-Plague Institute, Odessa, Ukraine; A.V. Bogatsky Physics-Chemical Institute of NAS of Ukraine, Odessa, Ukraine

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Institute of Virology, School of Medicine, Wuhan University, Wuhan, Hubei, China; Centre of Experimental Animal, Wuhan University, Wuhan, Hubei, China

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Institute for Antiviral Research, Utah State University, Logan, UT, USA

137. Anti-Coronavirus Activities of Polyoxometalates
Shiro Shigeta, Shuichi Mori, Tatsuo Suzutani, Norio Yamamoto, Naoki Yamamoto, Toshihiro Yamase
Fukushima Medical University, Fukushima, Japan; Tokyo Medical and Dental University, Tokyo, Japan; Tokyo Institute of Technology

139. Structure Activity Relationship Studies on Biaryl Derivatives with Anti-Picornavirus Activity
Michaela Schmidtke, Vadim A. Makarov, Olga B. Riabova, Peter Wutzler
Institute of Virology and Antiviral Therapy, Friedrich Schiller University, D-07745 Jena, Germany; Department of Medicinal Chemistry, Research Center for Antibiotics, Moscow 117105, Russia

141. Discovery of Antiviral Agents against RNA Viruses: Correlation with Inhibition of IMPDH
Vasu Nair, Eric Bonsu, Mukta Gupta, Sherry Story
University of Georgia, Department of Pharmaceutical and Biomedical Sciences, Athens, GA 30602, USA

143. Antiadenoviral Activity of Plants Compounds
Lidiya N. Nosach, Nataliya S. Dyachenko, Valentina L. Zhavnovataya, Olga Yu Povnitsa, Ludmila D. Shipulina
Institute of Microbiology and Virology National Academy Science of Ukraine, Kyiv, Ukraine; NPO ‘VILAR’, Moskow, Russia

145. Discovery of West Nile Virus Inhibitors

Baohua Gu, Peter Mason, Lijuan Wang, Nigel Bourne, Shannon Rossi, Serguey Ouzounov, Andy Cuconati, Anand Mehta, Tim Block
Drexel Institute for Biotechnology and Virology Research, Drexel University College of Medicine, Doylestown, PA, USA; Department of Pathology, University of Texas Medical Branch, Galveston, TX, USA; Institute for Hepatitis and Virus Research, Doylestown, PA, USA; Department of Pediatrics and Sealy Center for Vaccine Development, University of Texas Medical Branch, Galveston, TX, USA

147. Reactions of Guanidine with Vinylogous Ester-Aldehydes: Synthesis and Anti-West Nile Virus Activity of a Novel Imidazole Nucleoside Containing a Diaminodihydro-*s*-triazine as a Substituent
Ravi K. Ujjinamatada, Yankanagouda S. Agasimundin, Peter Borowski, Ramachandra S. Hosmane
Department of Chemistry and Biochemistry, University of Maryland (UMBC), Baltimore, MD, USA; Abteilung fur Virologie, Bernhard-Nocht-Institut fur Tropenmedizin, Hamburg, Germany

149. Selective Functional Group Transformation: The Conversion of an Ester Group into an Amide or Acid in Vinylogous Ester–Aldehydes Attached to Aromatic or Heterocyclic Rings
Ravi K. Ujjinamatada, Ramachandra S. Hosmane
Department of Chemistry and Biochemistry, University of Maryland (UMBC), Baltimore, MD 21250, USA

151. The Three-Dimensional Structures of the Dengue Virus, West Nile Virus, Japanese Encephalitis and Yellow Fever Polymerase Proteins Predicted by Homology-Based Molecular Modeling
Dimitrios P. Vlachakis, Steven P. Oldfield, Andrea Brancale
Medicinal Chemistry, Welsh School of Pharmacy, Cardiff University, Wales, UK

153. The Inadequate Knowledge about Sexually Transmitted Diseases [STDs] and Risky Sexual Behaviour: The Risk Factors for Wild Spread of STDs Among Youth in Developing Countries
Oluwafemi I. Olawuyi, Adeyemi I. Falegan
University College Hospital, Medical Lab Science, Ibadan, Oyo, Nigeria; University College Hospital, Dentistry, Ibadan, Oyo, Nigeria

155. Genetic Screen for Monitoring Viral Proteases
Mariona Parera, Bonaventura Clotet, Miguel Angel Martinez
Fundacio irsiCaixa, Hospital Universitari Germans Trias i Pujol, 08916 Badalona, Spain

157. The Hierarchical QSAR Technology for Effective Virtual Screening and Molecular Design of the Promising Antiviral Compounds
Victor E. Kuz'min, Anatoly G. Artemenko, Evgene N. Muratov, Victor P. Lozitsky, Alla S. Fedchuk, Regina N. Lozytska, Yuri A. Boschenko, Tatiyana L. Gridina
A.V. Bogatsky Physico-Chemical Institute of the National Academy of Sciences of Ukraine, 86 Lustdorf-skaya doroga, Odessa 65080, Ukraine, victor@farlep.net; Ukrainian Mechnikov Research Anti-Plague Institute, Odessa, Ukraine

159. Screening Program Targeting Viral Enzymes: An Alternate Method To Discover Antiviral Drugs
Frédèric Peyrane, Claire Debarnot, Karine Barral, Barbara Selisko, Karine Alvarez, Jean-Claude Guillemot, Bruno Canard
Laboratory of Architecture and Function of Biological Macromolecules, CNRS-UMR 6098, Marseille, France

161. Luminescent Microscopy and Fractal Microscopy in Virus-Cell Imaging: A Comparative Study
Oleksandr P. Fedchuk, Andriy O. Fedchuk, Alla S. Fedchuk, Pavlo O. Fedchuk
I.I. Mechnikov Odesa National University, Odesa, Ukraine; I.I. Mechnikov Ukrainian Research Anti-Plague Institute, Odesa, Ukraine

163. Mathematical Analysis of Fractal Approach to General Cell Stability and Model Virus–Cell Interaction
Andriy O. Fedchuk, Oleksandr P. Fedchuk, Alla S. Fedchuk, Pavlo O. Fedchuk
I.I. Mechnikov Odesa National University, Odesa, Ukraine; I.I. Mechnikov Ukrainian Research Anti-Plague Institute, Odesa, Ukraine

165. Structural Genomics on Viral Replicative Proteins: A Tool for Antiviral Drug Discovery
Marie-Pierre Egloff, Bruno Coutard, Valérie Campanacci, Barbara Sélisko, Philippe Lieutaud, Sacha Grisel, Karen Dalle, Fabienne Tocque, Nicolas Brémond, Julie Lichière, Violaine Lantez, Christian Cambillau, Bruno Canard
Architecture et Fonction des Macromolécules Biologiques, UMR 6098 CNRS and Universités Aix-Marseille I and II, 31 Ch. Joseph Aiguier, 13402 Marseille Cedex 20, France

167. Simple and Rapid Method for the Simultaneous Quantification of Zidovudine and its Monophosphate in Cell Extract by High-Performance Liquid Chromatography
Isabelle Lefebvre, Jean Yves Puy, Catherine Perrin, Gilles Gosselin, Christian Périgaud
UMR 5625 CNRS-UM II, Université Montpellier II, Case Courrier 008, Place E. Bataillon, 34095 Montpellier Cedex 05, France; UMR 5625 CNRS-UM II, Laboratoire de chimie analytique, Faculté de pharmacie, Université Montpellier I, 15 Avenue Charles Flahault, Montpellier, France

Tuesday, April 12, 2005

Mini Symposium: Biodefense and Emerging Infections
Catalunya Room
Chairs: Earl R. Kern and Richard J. Whitley

09:00 George R. Painter, Chimerix, Inc., Durham, NC, USA
"Problems and Pitfalls in Smallpox Drug Development"

09:30 Mathias Heikenwälder, University Hospital of Zurich Institute of Neuropathology, Zurich, Switzerland
"Chronic Inflammation and Organ Tropism of Prions"

10:00 Albert Osterhaus, Department of Virology, Erasmus Medical Center, Rotterdam, Netherlands
"Influenza: Are we on the brink of the next pandemic?"

10:30 *Break*

11:00 Thibout Mukaba, University of North Carolina – DRC Program and Kinshasa School of Public Health Monkeypox Project, Kinshasa, Democratic Republic of the Congo
"Monkeypox in Africa: An Emerging Disease? Current Status and Research in Democratic Republic of the Congo."

11:30 Richard J. Whitley, University of Alabama at Birmingham, Department of Pediatrics, Division of Infectious Diseases, Birmingham, AL, USA
"West Nile Infection: Role of Genetic Susceptibility"

12:00 General Panel Discussion

12:30 *Adjourn*
Free Afternoon and Barcelona Tours

Wednesday, April 13, 2005

Prusoff Young Investigator Award Lecture
Catalunya Room

09:00 Presentation of Award: John A. Secrist, III, President I.S.A.R.
Awardee Lecture: Arianna Loregian, University of Padova, Padova, Italy
"Disruption of the Interactions Between the Subunits of Herpesvirus DNA Polymerases: Towards Novel Antiviral Agents"

Oral Session III: Herpesviruses and Poxviruses
Chairs: Johan Neyts and Mark N. Prichard

09:45 13. Maribavir Induces the Formation of Tegument Aggregates in Cells Infected with Human Cytomegalovirus
Mark N. Prichard, Caroll B. Hartline, William J. Britt, Earl R. Kern
University of Alabama School of Medicine, Department of Pediatrics, Birmingham, AL, USA

10:00 14. ST-246: A Potent and Specific Inhibitor of Orthopoxvirus Replication
Robert Jordan, Guang Yang, Sylvie Laquerre, Linda Barone, Daniel C. Pevear, Thomas R. Bailey, Susan Rippin, Marc S. Collett, Erik De Clercq, Johan Neyts, Kevin F. Jones, Tove Bolken, R.M. Buller, Erin Touchette, Kem Waller, Dennis E. Hruby
SIGA Technologies, Corvallis, OR; Rega Institute, Leuven, Belgium; Saint Louis University, St. Louis, MO; ViroPharma, Inc., Exton, PA, USA

10:15 15. Identification and Proposed Mechanism of Antiviral Nucleoside Metabolism by DNA Repair Enzymes
Philip L. Lorenzi, Christopher P. Landowski, Xueqin Song, Leroy B. Townsend, John C. Drach, Gordon L. Amidon
Departments of Pharmaceutical Sciences and Medicinal Chemistry, College of Pharmacy; Department of Biologic and Materials Sciences, School of Dentistry, University of Michigan, Ann Arbor, MI, USA

10:30 *Break*

10:50 Invitation to 19th ICAR, Earl R. Kern

11:00 ISAR Business Meeting

11:15 16. Cyclic HPMPC is a Highly Effective Therapy for CMV-Induced Deafness in a Guinea Pig Model
David R. White, Daniel I. Choo, Greg Stroup, Mark R. Schleiss
Cincinnati Children's Hospital, Departments of Otolaryngology and Pediatrics Cincinnati, OH; University of Minnesota School of Medicine, Department of Pediatrics, Division of Infectious Diseases, Minneapolis, MN, USA

11:30 17. Cyclic HPMPC Therapy Improves the Outcome of Guinea Pig Cytomegalovirus Congenital Infection and Decreases the Viral Load in the Placenta and Fetus
David I. Bernstein, Fernando J. Bravo, Rhonda D. Cardin
Cincinnati Children's Hospital Medical Center, Division of Infectious Diseases, Cincinnati, OH, USA

11:45 18. Development of an Aerosol Model of Rabbitpox: Experimental Infection and Comparative Pathogenesis
Chad J. Roy, Jason Paragas, Eric Mucker, Josh Shamblin, John Huggins, Don Nichols
Center for Aerobiological Sciences, Division of Virology, Division of Pathology, U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), Fort Detrick, MD, USA

12:00 19. Progressive Outer Retinal Necrosis in an AIDS Patient During the Era of Highly Active Anti-Retroviral Therapy (HAART): Successful outcome with Intravitreal Drugs and Monitoring with Quantitative PCR
S.K. Kurup, P.D. Yin, M. Wright, L. Kump, K. Moeller, G.L. Clarke, H.R. Coleman, J.A. Smith, S.H. Fischer, R.B. Nussenblatt
National Eye Institute, NIH; NIAID, NIH, Bethesda, MD, USA

12:30 *Lunch*
Mitre Room, Lobby Level
Princesa Sofia Hotel

Wednesday, April 13, 2005

Oral Session IV: Respiratory and West Nile Viruses
Catalunya Room
Chairs: David Kimberlin and Larisa V. Gubareva

14:00 Plenary Speaker
David W. Kimberlin, Division of Pediatric Infectious Diseases, University of Alabama at Birmingham, Birmingham, AL, USA
"Intrauterine – Acquired Viral Infections: The Hopes and Possibilities For Antiviral Interventions"

14:30 20. A Novel Broad-Spectrum Inhibitor of Influenza Virus Infections
Michael P. Malakhov, Laura M. Aschenbrenner, Larisa V. Gubareva, Vasiliy P. Mishin, Frederick G. Hayden, Donald F. Smee, Miles K. Wandersee, Robert W. Sidwell, Do H. Kim, Mang Yu, Fang Fang

NexBio Inc., 6330 Nancy Ridge Dr., Suite 105, San Diego, CA 92121, USA; Division of Infectious Diseases and International Health, Department of Internal Medicine, University of Virginia, Charlottesville, VA, USA; Institute for Antiviral Research, Utah State University, Logan, UT, USA

14:45 21. Effect of Hemagglutinin Glycosylation on Influenza A Virus Susceptibility to Neuraminidase Inhibitors: a Reverse Genetics Study
Vasiliy P. Mishin, Frederick G. Hayden, Larisa V. Gubareva
Division of Infectious Diseases & International Health, Department of Internal Medicine, University of Virginia, Charlottesville, VA, USA

15:00 22. Development, Validation and Optimization of a Luminescence-Based High Throughput Screen for Inhibitors of Severe Acute Respiratory Syndrome-associated Coronavirus
Colleen B. Jonsson, Nice Shindo, Thomas Fletcher, Mindy Sosa, Thomas Rowe, Jeffrey Hogan, Michael McDowell, Barbara Taggert, Nicole Kushner, Sara Cooley
Emerging Pathogens Department and High Throughput Screening Center, Southern Research Institute, Birmingham, AL, USA

15:15 23. Inhibition, Escape and Attenuation of SARS Coronavirus Treated with Antisense Morpholino Oligomers
Benjamin W. Neuman, David A. Stein, Andrew D. Kroeker, Michael J. Churchill, Alice M. Kim, Philip Dawson, Hong M. Moulton, Richard K. Bestwick, Patrick L. Iversen, Michael J. Buchmeier
The Scripps Research Institute, Neuropharmacology, La Jolla, CA, USA; AVI Biopharma Inc., Corvallis, OR, USA; The Scripps Research Institute, Cell Biology, La Jolla, CA, USA

15:30 24. Terminal Differentiation of Trophoblast Cells Serves as a Barrier to West Nile Virus Infection of the Fetus in Mice
Justin G. Julander, Pei-Yong Shi, Quinton A. Winger, Craig W. Day, Aaron L. Olsen, Robert W. Sidwell, John D. Morrey
Institute for Antiviral Research and Animal, Dairy, and Veterinary Sciences Department, Utah State University, Logan, UT, USA; and State University of New York, Wadsworth Center, Albany, NY, USA

15:45 25. Presumptive Identification of a Protein Associated with West Nile Virus Encephalitis in CSF of Hamsters
Aaron L. Olsen, Dong Chen, John D. Morrey
Institute for Antiviral Research, Animal, Dairy, and Veterinary Sciences Department; and Center for Integrated Biosystems, Utah State University, Logan, UT, USA

Wednesday, April 13, 2005

Poster Session II: Herpesviruses, Poxviruses, **Other Viruses**, **Prodrugs and New Antivirals**
Baleares Room

16:00–18:00

40. A Recombinant Guinea Pig Cytomegalovirus (GPCMV) Expressing the Human Cytomegalovirus (HCMV) *UL97* Gene Demonstrates Significantly Improved Susceptibility to the Antiviral Agent, Ganciclovir
Alistair McGregor, Nanette Huey, Greg Stroup, Mark R. Schleiss
Division of Infectious Diseases, Department of Pediatrics, University of Minnesota School of Medicine, Minneapolis, MN, USA; Departments of Molecular Genetics and Pediatrics, University of Cincinnati and CCHMC, Cincinnati, OH, USA

42. A New Family of Non-Nucleoside Inhibitors of Human Cytomegalovirus (HCMV) and Varicella-Zoster Virus (VZV) Based on the β-Keto-γ-Sultone Template
Sonia De Castro, Carlos García-Aparicio, Graziela Andrei, Robert Snoeck, Erik De Clercq, Jan Balzarini, María-José Camarasa, Sonsoles Velazquez
Instituto de Química Médica, Juan de la Cierva 3, 28006 Madrid, Spain; Rega Institute for Medical Research, K.U. Leuven, B-3000 Leuven, Belgium

44. The In Vitro Inhibition of Cytomegalovirus by Novel Ribonucleotide Reductase Inhibitors Didox and Trimidox

M.S. Inayat, V.S. Gallicchio, B.A. Garvy, H.L. Elford, O.R. Oakley
University of Kentucky, Department of Clinical Sciences, Lexington, KY, USA; University of Kentucky, Department of Infectious Disease, Lexington, KY, USA; Molecules for Health Inc., Richmond, VA, USA

46. Mechanism of Action against Human Cytomegalovirus of First and Second Generation Methylenecyclopropane Purines
John C. Drach, Julie M. Breitenbach, Katherine Z. Borysko, Gloria Komazin, Zhaohua Yan, Jiri Zemlicka
Department of Biologic & Materials Sciences, School of Dentistry and Department of Medicinal Chemistry, College of Pharmacy, University of Michigan, Ann Arbor, MI 48109, USA; Karmanos Cancer Institute, Wayne State University, School of Medicine, Detroit, MI 48201, USA

48. Inhibition of Drug-Resistant Human Cytomegalovirus Replication by Kampo (Japanese Herbal) Medicine
Tsugiya Murayama, Nobuo Yamaguchi, Yoshito Eizuru
Division of Persistent and Oncogenic Viruses, CCVD, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima 890-8520, Japan; Department of Serology, Kanazawa Medical University, Uchinada, Ishikawa 920-0293, Japan

50. Intracellular Localization of Herpes Simplex Virus Type 1 Thymidine Kinase in Virus-Infected Cells
Pan Kee Bae, Ju Ryung Nam, Hae Soo Kim, Myung-Jin Lee, In Kwon Chung, Chong-Kyo Lee
Korea Research Institute of Chemical Technology, Pharmaceutical Research Center, Taejon, South Korea; Yonsei University, Department of Biology, Seoul, South Korea

52. Intracellular Localization of Herpes Simplex Virus Type 1 Thymidine Kinase in Cells Infected with Virus and Treated with Various Antiviral Agents
Ju Ryung Nam, Pan Kee Bae, Hae Soo Kim, Myung-Jin Lee, Chong-Kyo Lee
Korea Research Institute of Chemical Technology, Pharmacology Research Center, Taejon, South Korea

54. Human UMP-CMP Kinase Specificity for Natural and Antiviral Analogs Using Competition Fluorescence Experiments
Dominique Deville-Bonne, Laurence Dugué, Sarah Gallois-Montbrun, Michel Veron, Sylvie Pochet
Institut Jacques Monod, UMR 7592, CNRS-Univ. Paris 6 & 7, 2, place Jussieu, 75251 Paris cedex 05, France; Unité de Chimie organique, Institut Pasteur, 25, rue de Dr. Roux, 75015 Paris, France; Unité de Régulation enzymatique des Activités cellulaires; Institut Pasteur, 25, rue du Dr Roux, 75015 Paris, France

56. Anti-Herpesvirus Activity of an Extract of Emodin
Hou Wei, Yang Z. Qiu, Li J. Jing, Cheng Li, Xiao Hong, Yang J. Jiang
Institute of Virology, School of Medicine, Wuhan University, Wuhan, Hubei Province, China; Virus Research Center, Chung Shan Medical University, Taiwan, China

58. Investigation of Antiherpetic Activity Using Hierarchic QSAR Technology on the Base of Simplex Representation of Molecular Structure
Anatoly G. Artemenko, Victor E. Kuz'min, Evgene N. Muratov, Victor P. Lozitsky, Alla S. Fedchuk, Regina N. Lozytska, Yuri A. Boschenko, Tatiyana L. Gridina
A.V. Bogatsky Physico-Chemical Institute of the National Academy of Sciences of Ukraine, 86 Lustdorfskaya doroga, Odessa 65080, Ukraine; E-mail: victor@farlep.net; Ukrainian Mechnikov Research Anti-Plague Institute, Odessa, Ukraine

60. Anti-Herpetic Activity of Synthetic Proteolysis Inhibitors and Their Analogues
Alla S. Fedchuk, Victor P. Lozitsky, Tatyana L. Gridina, Larysa I. Shitikova, Lyubov M. Mudryk, Victor E. Kuzmin, Regina M. Lozytska, John C. Drach
I.I. Mechnikov Ukrainian Research Anti-Plague Institute, Odesa, Ukraine; O.V. Bogatsky Physico-Chemical Institute, Odesa, Ukraine; School of Dentistry, University of Michigan, Ann Arbor, MI, USA

62. Complex Use of Myramistin and Interferon in Herpetic Stomatitis Treatment
Iryna G. Bartsykovska, Alla S. Fedchuk
Cosmetic Dental Clinic and Laboratory, Odessa, Ukraine; I.I. Mechnikov Ukrainian Research Anti-Plague Institute, Odessa, Ukraine

64. Influence of Doxorubicin and Etoposide on the Process of Cd95-Mediated Apoptosis in EBV-Infected Lymphoma Bl-41 and Dg-75 Cells
Svetlana D. Zagorodnya, Nadezhda V. Nesterova, Nataliya S. Dyachenko, Galina V. Baranova
Inst. of Microbiology & Virology Nat. Acad. Sci. of Ukraine, Kyiv, Ukraine

66. Studying of Anti Epstein-Barr Virus Activity of New Nitrogen-Containing Heterocyclic Compounds
Nadezhda V. Nesterova, Nataliya S. Dyachenko, Svetlana D. Zagorodnya, Galina V. Baranova, Inna V. Alexeeva, Larisa I. Palchikovskaya
Zabolotny Institute of Microbiology and Virology of NAS of Ukraine, Kyiv, Ukraine; Institute of Molecular Biology and Genetics of NAS of Ukraine, Kyiv, Ukraine

68. The EBV Transcription Profile Upon the Treatment with Acyclovir and Maribavir
Edward Gershburg, Dirk P. Dittmer, Joseph S. Pagano
Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7295, USA; Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA; Department of Medicine, University of North Carolina at Chapel Hill, NC 27599, USA

70. Smallpox Antivirals: In vitro Assay for Vaccinia Virus I7L Enzymatic Cleavage of Core protein Precursors
Chelsea M. Byrd, Dennis E. Hruby
Oregon State University, Molecular and Cellular Biology Program, Corvallis, OR, USA; SIGA Technologies, Corvallis, OR, USA

72. Efficacy of Smallpox Vaccination in the Presence of Antiviral Drugs, Cidofovir and Hexadecyoxypropyl-cidofovir
Robert M. Buller, Gelita Owens, Karl Y. Hostetler, Jill Schriewer
Saint Louis University, Department of Molecular Microbiology and Immunology, St. Louis, MO, USA; San Diego VAMC and the University of California, San Diego, La Jolla, CA, USA

74. Synergistic Combination Effect of Cidofovir and Idoxuridine on Vaccinia Virus Replication
Mimi Remichkova, Nikolaj Petrov, Angel S. Galabov
The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

76. Effect of Oral Treatment with HDP-(S)-HPMPA or ODE-(S)-HPMPA on Cowpox or Vaccinia Virus Infections in Mice
D.C. Quenelle, D.J. Collins, K.A. Keith, J. Trahan, J.R. Beadle, K.Y. Hostetler, E.A. Kern
University of Alabama, School of Medicine, Birmingham, AL, USA; San Diego VA Healthcare System and University of California, San Diego, La Jolla, CA, USA

78. Pharmacodynamics of Cidofovir, an Inhibitor of Poxvirus Replication, in an In vitro Hollow Fiber Model System
James J. McSharry, Kris M. Zager, Qingmei Weng, Mark R. Deziel, Arnold Louie, George L. Drusano
Ordway Research Institute, Emerging Infections and Host Defense, Albany, NY, USA

80. Antiviral Activity of Nucleoside Analogs Against Orthopoxvirus Replication is Limited Predominantly by Their Phosphorylation
Mark N. Prichard, Angela D. Williams, Emma A. Harden, Kathy A. Keith, Earl R. Kern
University of Alabama School of Medicine, Department of Pediatrics, Birmingham, AL, USA

82. Reduced Pathogenicity of Phenotypically and Genotypically Characterized Cidofovir (CDV)-Resistant Vaccinia Virus (VV)
G. Andrei, P. Fiten, E. De Clercq, G. Opdenakker, R. Snoeck
Rega Institute for Medical Research, K.U. Leuven, Leuven, Belgium

84. Group-Specific and Neutralizing Human SCFV to Orthopoxviruses from a Combinatorial Phage Library

Vera V. Morozova, Viktoria V. Voronina, Maia V. Shveigert, Eugeni F. Belanov, Alexander A. Ilyichev, Nina V. Tikunova
State Research Center of Virology and Biotechnology VECTOR, Koltsovo, Novosibirsk, Russia

86. Phage Display Immune Library of Human scFv Against Orthopoxviruses
Viktoria V. Voronina, Evgeny F. Belanov, Nina V. Tikunova
State Research Center of Virusology and Biotechnology VECTOR, Institute of Bioengineering, Koltsovo, Novosibirsk Region, Russia; State Research Center of Virusology and Biotechnology VECTOR, Institute of Molecular Biology, Koltsovo, Novosibirsk Region, Russia

88. Full-Size Human Antibodies Against Orthopoxviruses
Tanya Yun, Ludmila Shingarova, Tanya Batanova, Nina Tikunova
State Research Center of Virology & Biotechnology Vector, Koltsovo, Novosibirsk Region, 630559, Russia; Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences Ul. Miklukho-Maklaya, 16/10, 117997 GSP, Moscow V-437, Russia; State Research Center of Virology & Biotechnology Vector, Koltsovo, Novosibirsk Region, 630559, Russia; State Research Center of Virology & Biotechnology Vector, Koltsovo, Novosibirsk Region, 630559, Russia

90. Evaluation of New Cell Culture Inhibitors of Protease-resistant Prion Protein Against Scrapie Infection in Mice
John D. Morrey, David A. Kocisko, Richard E. Race, Jiancao Chen, Byron Caughey
Utah State University, Institute for Antiviral Research, Logan, UT, USA; NIAID, NIH, Laboratory of Persistent Viral Diseases, Hamilton, MT, USA; Chengdu Jinniu Institute, Food Bureau of Sichuan Province, Chengdu Sichuan, China

92. Mouse Adenovirus Type 1-Infected SCID Mice: a Unique Model for the Evaluation of Antiviral Compounds against Systemic Adenovirus Infections
Lieve Naesens, Liesbeth Lenaerts, Eric Verbeken, Erik De Clercq
Rega Institute for Medical Research, K.U. Leuven, Leuven, Belgium; Department of Morphology and Molecular Pathology, K.U. Leuven, Leuven, Belgium

94. Compounds Reactive against the Arenavirus RING Finger Z Protein Induce Z Oligomerization and Block its Interaction with the PRH Cellular Protein
Cybele C. García, Mahmoud Djavani, Maria S. Salvato, Elsa B. Damonte
Laboratory of Virology, Department of Biological Chemistry, Faculty of Sciences, University of Buenos Aires, Buenos Aires, Argentina; Institute of Human Virology, University of Maryland Biotechnology Center, Baltimore, MD, USA

96. Antiviral Activity of Cyclooxygenase Inhibitors Against Bovine Viral Diarrhea Virus (BVDV) Replication
Chiaki Baba, Koichiro Yanagida, Tamotsu Kanzaki, Masanori Baba
Department of Dermatology, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan; Division of Antiviral Chemotherapy, Center for Chronic Viral Diseases, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan; Planova Division, Asahi Kasei Pharma Corporation, Nobeoka, Japan

98. Substituted 5-Benzyl-2-phenyl-5H-imidazo[4,5-c]pyridines: Synthesis and Anti-BVDV Evaluation
Gerhard Pürstinger, Jan Paeshuyse, Robert Vrancken, Frank Koenen, Pierre Kerkhofs, Carine Letellier, Erik De Clercq, Johan Neyts
University of Innsbruck, Institute of Pharmacy, Innsbruck, Austria; Katholieke Universiteit Leuven, Rega Institute for Medical Research, Leuven, Belgium; Veterinary and Agrochemical Research Centre, Ukkel, Belgium

100. Antiviral Strategies Against Bunyaviruses using Antisense Morpholino Oligonucleotides
Anna Overby, Laure Deflube, Pramila Walpita, Kerstin Angner, Patrick Iversen, David Stein, Ramon Flick
University of Texas Medical Branch, Department of Pathology, Galveston, TX 77555; AVI BioPharma, Inc., Corvallis, OR 97333

102. Inhibition of Coxsackievirus B3 PD by Specifically Sulfated Heparin and Lysosomotropic Agents
Andreas E. Zautner, Birgit Jahn, Peter Wutzler, Michaela Schmidtke
Institut für Virologie und Antivirale Therapie, Friedrich-Schiller-Universiteit, Jena, Germany,
E-mail: andreas.zautner@web.de

104. Combined Effect of Oxoglaucin and Other Inhibitors of the Enteroviral Replication in Experimental Coxsackievirus B Infection in Newborn Mice
Ralitsa K. Vassileva-Pencheva, Angel S. Galabov
Bulgarian Academy of Sciences, Institute of Microbiology, Department of Virology, Sofia, Bulgaria

106. Anti-Coxsackievirus B Activity of 2-(3,4-dichlorophenoxy)-5-Nitrobenzonitrile Analogues
Armando M. De Palma, Gerhard Pürstinger, Erik De Clercq, Johan Neyts
Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium; Department of Pharmaceutical Chemistry, Institute of Pharmacy, University of Innsbruck, Austria

108. Inhibitory Action of Sulfated Polysaccharides on Dengue Virus Infection of Human Cells
Laura B. Talarico, Elsa B. Damonte
Laboratory of Virology, Department of Biological Chemistry, Faculty of Sciences, University of Buenos Aires, Buenos Aires, Argentina

110. Inhibition of Dengue Virus Serotypes 1–4 in Cell Culture with Morpholino Oligomers
Richard Kinney, Claire Huang, Becky Rose, Andrew Kroeker, Patrick Iversen, David Stein
CDC, Ft. Collins, CO, USA; AVI BioPharma Inc., Corvallis, OR, USA

112. Single Chain Antibodies Against Ebola Virus from Naive Phage Display Library
Tatiana A. Batanova, Elena V. Gzhirakovskaya, Alexander A. Chepurnov, Nina V. Tikunova
State Research Center of Virology and Biotechnology Vector, Koltsovo, Novosibirsk Region, 630559, Russia

114. Generation of Human scFv Against Guinea Pig-Adapted Variant of Ebola Virus
Elena V. Zhirakovskaya, Tatiana A. Batanova, Aleksandr A. Chepurnov, Nina V. Tikunova
Institute of Bioengineering, State Research Center of Virology and Biotechnology VECTOR, Koltsovo, Novosibirsk Region, Russia; Institute of Molecular Biology, State Research Center of Virology and Biotechnology VECTOR, Koltsovo, Novosibirsk Region, Russia

116. Oxoglaucine: a Selective Inhibitor of Enterovirus Replication
Lubomira Nikolaeva-Glomb, Irina Zhecheva, Ani Nikolova, Stephan Filipov, Angel S. Galabov
The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, 26 G. Bonchev St., 1113 Sofia, Bulgaria; Institute of Organic Chemistry, Bulgarian Academy of Sciences, 9 Bonchev St., 1113 Sofia, Bulgaria

118. Degradation Of Japanese Encephalitis Virus By Neutrophils
Shailendra K. Saxena, Sonilika Srivastava, Nivedita Khanna, Asha Mathur
Postgraduate Department of Microbiology, King George's Medical College, Lucknow, UP, India; Microbiology & Immunology, College of Medicine, The University of Arizona HSC, Tucson, AZ, USA

120. Antiviral Strategies against Nipah Virus: Exploring Gene Silencing Mechanisms to Identify Potential Antiviral Targets
Pramila Walpita, Allison Groseth, Heinz Feldmann, Ramon Flick
University of Texas Medical Branch, Department of Pathology, Galveston, TX 77555-0609, USA; Canadian Science Center for Human and Animal Health, Special Pathogens Program, Winnipeg, Man., Canada R3E 3R2

122. Effect of Various 2′,5′-Oligoadenylates with Antipapillomavirus Activities on DNA-Polymerases and DNA-Topoisomerases
Arman D. Pivazyan

Yale University School of Medicine, Department of Pharmacology, 333 Cedar St. New Haven, CT 06520, USA

124. Inhibition of Sendai Virus by a Natural Cinnamon Extract
Keren Gueta, Michael Ovadia
Department of Zoology, Tel Aviv University, Tel Aviv, Israel

126. Affinity of (a-*P*-borano)-NDPs to a Transition-State Analogue Complex of Rabbit Muscle Pyruvate Kinase
Mikhail I. Dobrikov, Ping Li, Barbara Ramsay Shaw
Department of Chemistry, P.M. Gross Chemical Laboratory, Duke University, Durham, NC 27708-0346, USA

128. In-Vitro Analysis of Iododeoxyuridine Ester Prodrugs for Activity Against Orthopoxviruses
S.L.J. Husband, K.A. Keith, E.R. Kern, P.F. Torrence
Northern Arizona University, Department of Chemistry and Biochemistry, Flagstaff, AZ, USA; University of Alabama School of Medicine, Birmingham, AL, USA

130. Synthesis and Antiviral Activity of Alkoxyalkylesters of Cidofovir Monophosphate
Jacqueline C. Ruiz, James R. Beadle, Julissa Trahan, Kathy A. Aldern, Kathy A. Keith, Caroll B. Hartline, Earl R. Kern, Karl Y. Hostetler
VA San Diego Heathcare System, San Diego, CA 92161, USA; University of California San Diego, Department of Medicine, La Jolla, CA 92093, USA; University of Alabama Birminghan, Department of Pediatrics, AL 35294, USA

132. Novel 5-Phosphono-pent-2-en-1-yl Nucleosides (PPen-Ns) and their Alkoxyalkyl Phosphonoesters: Synthesis and Antiviral Evaluation
Hyunah Choo, James R. Beadle, Julissa Trahan, Kathy A. Aldern, Karl Y. Hostetler
VA San Diego Healthcare System and University of California, San Diego, La Jolla, CA 92093, USA

134. Lung Targeted Antivirals: Studies with 1-*O*-Octadecyl-2-*O*-benzyl-sn-glycero-3-cidofovir
Julissa Trahan, James R. Beadle, Karl Y. Hostetler
Department of Medicine, VA San Diego Healthcare System and the University of California, San Diego, La Jolla, CA 92093-0676, USA

136. Activity of Alkoxyalkyl and Alkyl Esters of (*S*)-3-Hydroxy-2-Phosphonylmethoxypropyl Derivatives of Cytosine (HPMPC, Cidofovir) and Adenine (HPMPA) and Cyclic Cidofovir Against Orthopoxviruses
G. Andrei, J. Van den Oord, K.Y. Hostetler, J.R. Beadle, D. Geypens, E. De Clercq, R. Snoeck
Rega Institute for Medical Research, K.U. Leuven, Leuven, Belgium; Pathology Department, U.Z. Leuven, Leuven, Belgium; San Diego VAMC and the University of California, San Diego, USA

138. Amino Acid Ester Prodrugs of 2-Bromo-5,6-dichloro-1-(β-D-ribofuranosyl)benzimidazole Enhance Metabolic Stability In Vitro and In Vivo
Philip L. Lorenzi, Xueqin Song, Katherine Z. Borysko, Julie M. Breitenbach, Jae Seung Kim, John M Hilfinger, Leroy B. Townsend, John C. Drach, Gordon L. Amidon
Department of Pharmaceutical Sciences, College of Pharmacy, University of Michigan, Ann Arbor, MI, USA; Department of Medicinal Chemistry, College of Pharmacy, University of Michigan, Ann Arbor, MI, USA; Department of Biologic and Materials Sciences, School of Dentistry, University of Michigan, Ann Arbor, MI, USA; TSRL, Inc., Ann Arbor, MI, USA

140. A Possible Synthetic Strategy to Diastereomerically Pure *cyclo*Sal Prodrugs
Jens O. Thomann, Katharina B. Wallach, Edwin H. Rios-Morales, Chris Meier
University of Hamburg, Institute of Organic Chemistry, Hamburg, Germany

142. Novel “Lock-in” Modified *cyclo*Sal Nucleotides (I): Variations of the Linker Moiety
Dalibor Vukadinovic, Chris Meier, Jan Balzarini
Institute of Organic Chemistry, University of Hamburg, Martin-Luther-King-Platz 6, 20146 Hamburg, Germany; Rega-Institute for Medical Research, K.U. Leuven, Minderbroederstraat 10, B-3000 Leuven, Belgium

144. *Cyclo*Amb Nucleoside Phosphonates: Nucleoside Phosphonate Prodrugs Based on the *cyclo*Sal Concept
Ulf Görbig, Jan Balzarini, Chris Meier
University of Hamburg, Institute of Organic Chemistry, Hamburg, Germany; Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium

146. Use of Biolabile Constructs for Mononucleotide Delivery
Christian Périgaud, Suzanne Peyrottes, David Egron, Isabelle Lefebvre, Gilles Gosselin
UMR 5625 CNRS-UM II, Université Montpellier II, Montpellier, France

148. tBuSATE (dipeptidyl) Phosphotriesters as Potential Pronucleotides
Suzanne Peyrottes, Isabelle Lefevbre, Gaelle Coussot, Gilles Gosselin, Christian Périgaud
UMR 5625 CNRS — UMII, Université Montpellier II, Montpellier, France

150. Phosphoramidate Prodrugs of the Most Potent and Selective Anti-VZV Bicyclic Pyrimidine Nucleosides
Marco D. Migliore, Christopher McGuigan, Robert Snoeck, Gabriela Andrei, Jan Balzarini, Erik De Clercq
Cardiff University, Cardiff, Wales, UK; Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium

152. Synthesis of Nucleoside Boranophosphoramidates Conjugated with Amino Acids as A New Class of Promising Prodrugs
Ping Li, Barbara R. Shaw
Chemistry Department, Duke University, Durham, NC, USA

154. An Overview of Antimicrobial Peptides and Their Therapeutic Potential as Antiviral Drugs
Jerold Gordon, Eric Romanowski, Kathleen Yates, Alison McDermott
University of Pittsburgh, The Charles T. Campbell Laboratory, Pittsburgh, PA, USA; University of Houston, College of Optometry, Houston, TX, USA

156. Antiviral Activity of 1,2-Dithiol-3-Propylsulfonat Sodium In Vitro and In Vivo
Tatyana L. Gridina, Victor P. Lozitsky, Yuri A. Boschenko, Alla S. Fedchuk
I.I. Mechnikov Ukrainian Research Anti-Plague Institute, Odessa, Ukraine

158. Structurally Unrelated Pharmacological CDK Inhibitors (PCIs) Target Initiation of Transcription from Viral Genomes, A Novel Target for Antiviral Drugs
Jonathan J. Lacasse, Ersilia Coccaro, Véronic M.I. Provencher, Luis M. Schang
University of Alberta, Department of Biochemistry, Edmonton, Alta., Canada; University of Alberta, Department of Medical Microbiology and Immunology, Edmonton, Alta., Canada

160. Synthesis and Study of New Conformationally Restricted Nucleoside Analogues
Julien Gagneron, Gilles Gosselin, Christophe Mathé
University Montpellier II, UMR 5625 CNRS — University Montpellier II, Montpellier, France

162. Effects of Interferon Alpha on Human Hepatoma Cell Lines: DNA Microarrays Analysis and Evaluation of Cell Proliferation
Karina Fincati, Marta Trevisan, Giulia Masi, Francesca Sessa, Francesca Favaretto, Luisa Barzon, Giorgio Pal
Department of Histology, Microbiology and Medical Biotechnologies, University of Padua, Italy

164. Development of Highly Potent Pyrimidinedione Inhibitors as Topical Microbicides
Karen M. Watson, Robert W. Buckheit Jr.
ImQuest BioSciences, Inc., Frederick, MD, USA

Thursday, April 14, 2005

Oral Session V: Hepacivirus and Human Immunodeficiency Virus

Catalunya Room

Chairs: Joseph M. Colacino and Victor Buckwold

09:00 Plenary Speaker
Rafael Esteban, Servicio de Hepatologia, Hospital General Universitario Valle de Hebron, Barcelona, Spain
"Recent Advances and Future of Hepatitis C Virus Infection Treatment"

09:30 26. Differential Binding of Two Anti-E2 Human Monoclonal Antibodies to HCV Quasispecies Population in Liver Transplant Patients
Arie Zauberman, Ofer Nussbaum, Dorit Landstein, Shai Shahar, Tal Waisman, Rachel Eren, Ehud Ilan, Shlomo Dagan
XTL Biopharmaceuticals Ltd., Rehovot, Israel

09:45 27. The Predominant Mechanism by which Ribavirin Exerts its Antiviral Activity In Vitro Against Flaviviruses and Paramyxoviruses is Mediated by Inhibition of Inosine Monophosphate Dehydrogenase
Pieter Leyssen, Jan Balzarini, Erik De Clercq, Johan Neyts
K.U. Leuven, Rega Institute for Medical Research, Laboratory for Virology and Experimental Chemotherapy, Minderbroedersstraat 10, 3000 Leuven, Belgium

10:00 28. Potent and Selective Inhibition of Hepatitis C Virus Replication by the Non-Immunosuppressive Cyclosporin Analogue DEBIO-025
Jan Paeshuyse, Jean-Maurice Dumont, Brigitte Rosenwirth, Erik De Clercq, Johan Neyts
Rega Institute for Medical Research, K.U. Leuven, B-3000 Leuven, Belgium; Debiopharm, CP211-Lausanne, Switzerland; Biomedical Primate Research Centre, Rijswijk, The Netherlands

10:15 29. Inhibitors of Alpha Glucosidases Impair HCV Pseudoparticles Morphogenesis, Prevent Viral Secretion and Entry into Hepatoma Cells
Cynthia Chapel, Isabelle Vuillermoz, Birke Bartosch, Francois-Loôc Cosset, Nicole Zitzmann, Jean Dubuisson, Raymond D. Dwek, Christian Trèpo, Fabien Zoulim, David Durantel
Inserm U271, Lyon, France; Inserm U412, IFR 128, Lyon, France; Glycobiology Institute, Oxford, UK; CNRS-UPR2511, Lille, France

10:30 *Break*

11:00 30. 4′-*C*-Ethynyl-2′-Deoxy-2-Fluoroadenosine, A Nucleoside Derivative Potent Against HIV-1 with no Acute Mouse Toxicity: Highlights of the Role of 3′-OH for Biological Activity
Hiroshi Ohrui, Satoru Kohgo, Kenji Kitano, Noriyuki Ashida, Hiroyuki Hayakawa, Eiichi Kodama, Masao Matsuoka, Hiroaki Mitsuya
Tohoku University, Graduate School of Life Sciences, Sendai, Miyagi, Japan; Yamasa Corporation, Choshi, Chiba, Japan; Kyoto University, Institute for Virus Research, Kyoto, Kyoto, Japan; Kumamoto University, School of Medicine, Kumamoto, Kumamoto, Japan; National Cancer Institute/National Institute of Health, Bethesda, MD, USA

11:15 31. Inhibition of CD4-gp120 Binding Blocks Coreceptor Independent Cell to Cell HIV-1 Transmission
Berta Bosch, Julia Blanco, Maria T. Fernandez-Figueras, Bonaventura Clotet, José A. Esté
Retrovirology Laboratory irsiCaixa, Hosp. Germans Trias i Pujol, Badalona, Spain; Department of Pathology, Hosp. Germans Trias i Pujol, Badalona, Spain

11:30 32. Debio-025, A Novel Non-Immunosuppressive Cyclosporine Analog with Potent Anti-Human Immunodeficiency Virus Type 1 Activity: Pharmacological Properties and Mode of Action
B. Rosenwirth, M.P. De Bethune, R.G. Ptak, L.A. Pallansch, C.A. Stoddart, P.A. Gallay, M.P. Simonin, J. Marfurt, F. Philippoz, K. Besseghir, J.M. Dumont, P. Scalfaro, U.T. Ruegg, M. Mutter, R. Wenger

BPRC, Rijswijk, The Netherlands; Tibotec, Mechelen, Belgium; SRI, Frederick, USA; Gladstone Institute of Virology and Immunology, San Francisco, USA; Scripps, La Jolla, USA; Debiopharm, Lausanne, Switzerland; School of Pharmacy, Geneve, Switzerland; EPFL, Lausanne, Switzerland

11:45 33. HIV-1 Strains Resistant to Mannose- and *N*-Acetylglucosamine-Binding Proteins Show Mutations at Glycosylation Sites of gp120
Jan Balzarini, Kristel Van Laethem, Sigrid Hatse, Kurt Vermeire, Erik De Clercq, Willy Peumans, Els Van Damme, Anne-Mieke Vandamme, Dominique Schols
Rega Institute for Medical Research, K.U. Leuven, Leuven, Belgium; University Hospitals St. Rafel, Leuven, Belgium; Department of Molecular Biotechnology, University of Gent, Belgium

12:00 34. Resistance Profile of Human Immunodeficiency Virus to CADA, a Novel HIV Inhibitor that Targets the Cellular CD4 Receptor
Kurt Vermeire, Kristel Van Laethem, Anne-Mieke Vandamme, Thomas W. Bell, Erik De Clercq, Dominique Schols
Rega Institute for Medical Research, Katholieke Universiteit Leuven, Belgium; Department of Chemistry, University of Nevada, Reno, USA

12:30 *Lunch*
Mitre Room, Lobby Level
Princesa Sofia Hotel

Thursday, April 14, 2005

Oral Session VI: Other Viruses and Late Breaker Presentations
Catalunya Room
Chairs: Colleen B. Jonsson and Heather Greenstone

14:00 Plenary Speaker
Mike Bray, Biodefense Clinical Research Branch, NIAID, National Institutes of Health, Bethesda, MD, USA
"RNA Viruses that Present a Bioterror Threat"

14:30 35. Development of a Phosphorodiamidate Morpholino Oligomer Antisense to Ebola Zaire
Kelly Warfield, Dana Swenson, Patrick Iversen, Andrew Kroeker, David Stein, Sina Bavari
USAMRIID, Fort Dietrich, Fredrick, MD, USA; AVI BioPharma Inc., Corvallis, OR, USA

14:45 36. Identification and Characterization of Potent Small Molecule Inhibitor of Category A Hemorrhagic Fever New World Arenaviruses
Tové C. Bolken, Sylvie Laquerre, Tom Bailey, Shirley S. Kickner, Lindsey E. Sperzel, Kevin F. Jones, Travis K. Waren, S.A. Lund, Dana L. Kirkwood-Watts, David S. King, Amy C. Shurtleff, Mary C. Guttieri, Dennis E. Hruby
SIGA Technologies, Inc., 4575 SW Research Way, Corvallis, OR 97333, USA; MRIID, Dept. of Mol. Virol., Bldg. 1301, Fort Detrick, Frederick, MD, USA

15:00 37. Treatment of Acute Arenaviral Disease with Consensus Interferon-Alpha
Brian B. Gowen, Dale L. Barnard, Donald F. Smee, Min-Hui Wong, Anne M. Pace, Kie-Hoon Jung, Scott G. Winslow, Kevin W. Bailey, Lawrence M. Blatt, Robert W. Sidwell
Institute for Antiviral Research, Utah State University, Logan, UT, USA; InterMune, Brisbane, CA, USA

15:15 38. Inhibiting Effects of PMEG [9-(2-Phosphonylmethoxyethyl)guanine] on the Growth of Human Cervical Carcinoma Xenografts in Athymic Nude Mice
G. Andrei, G. Wolfgang, B. Lee, I. Lebeau, E. De Clercq, R. Snoeck
Rega Institute for Medical Research, K.U. Leuven, Leuven, Belgium; Gilead Sciences, Foster City, CA, USA

15:30 Late Breaker Presentation
15:45 Late Breaker Presentation
16:00 Adjournment of 18th I.C.A.R.

Oral Session I: Retroviruses

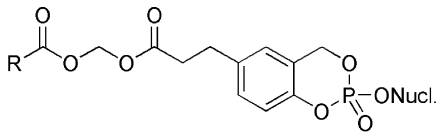
1

Novel "Lock-In" Modified *cyclo*Sal Nucleotides (II): Application of the AM- and the POM-Group

Chris Meier[1], Christian Ducho[1], Henning J. Jessen[1], Jan Balzarini[2]

[1]University of Hamburg, Institute of Organic Chemistry, Hamburg, Germany; [2]Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium

The *cyclo*Sal pronucleotide system has been designed for an intracellular delivery of therapeutically active nucleoside monophosphates. Recently, *cyclo*Sal nucleotides bearing esterase-cleavable side chains have been described. These "lock-in" modified derivatives are designed for an intracellular trapping of the prodrug due to carboxyesterase activity. In contrast of esters releasing moderately polar *cyclo*Sal alcohols, most compounds designed for the release of highly polar *cyclo*Sal carboxylates were poor substrates for carboxyesterase. Hence, different ways to achieve the intracellular release of the carboxylate had to be developed. Therefore, we synthesized *cyclo*Sal derivatives bearing an AM- or POM-protected carboxylate moiety in the side chain (see figure).



R = Methyl (AM), *tert*Butyl (POM) Nucl. = nucleoside analogue

Two different synthetic pathways to yield the target nucleotides have been developed. Both the AM- and the POM-modified *cyclo*Sal derivatives of d4T released the desired carboxylate derivative in CEM cell extracts within minutes while displaying satisfying hydrolytic stability in enzyme-free phosphate buffer (several hours half-life). Additional investigations have been carried out concerning the stability of the prodrugs at different pH values and in cell culture medium. Furthermore, POM-modified *cyclo*Sal derivatives of fluorescent nucleoside analogues have been synthesized for model studies on membrane penetration. The syntheses and hydrolysis studies as well as anti-HIV in vitro evaluation of the novel "lock-in" modified *cyclo*Sal nucleotides will be presented.

2

Deoxythreosyl Phosphonate Nucleosides as Selective Anti-HIV Agents

Tongfei Wu[1], Matheus Froeyen[1], Veerle Kempeneers[1], Christophe Pannecouque[2], Roger Busson[1], Erik De Clercq[2], Piet Herdewijn[1]

[1]Laboratory of Medicinal Chemistry, Rega Institute for Medical Research, Minderbroedersstraat 10, B-3000 Leuven, Belgium; [2]Laboratory of Virology, Rega Institute for Medical Research, Minderbroedersstraat 10, B-3000 Leuven, Belgium

Out of a series of eight new phosphonate nucleosides with a L-threose and a L-2-deoxythreose sugar moiety, two new compounds were identified (PMDTA and PMDTT) that showed potent anti-HIV-1 (HIV-2) activity [EC 50: 1 μg/mL (PMDTA) and 2.4 μg/mL (PMDTT) in MT-4 cells], while no cytoxicity was observed at the highest concentration tested (125 μg/mL). The kinetics of incorporation of PMDTA in DNA (using the diphosphate of PMDTA as substrate and HIV-1 reverse transcriptase as catalyst) was similar to the kinetics observed for dATP, while the diphosphate of PMDTA was a very poor substrate for DNA polymerase α. The incorporated PMDTA fits very well in the active pocket site of the HIV-1 reverse transcriptase.

3

Comparative Evaluation of Twelve Pyrimidinedione Inhibitors of HIV-1 For Further Preclinical and Clinical Development

Robert W. Buckheit Jr., Tracy L. Hartman, Karen M. Watson

ImQuest BioSciences, Inc., Frederick, MD, USA

We have previously reported the anti-HIV activity of SJ-3366 [1-(3-cyclopenten-1-yl)methyl-6-(3,5-dimethylbenzoyl)-5-ethyl-2,4-pyrimidinedione], which is a highly potent inhibitor of both HIV-1 and HIV-2. SJ-3366 inhibits the replication of HIV-1 by two mechanisms, acting as a non-nucleoside RT inhibitor and an entry inhibitor recognizing a conformational target formed upon association of virus with target cells. SJ-3366 inhibits HIV-1 at sub-nanomolar concentrations and HIV-2 at sub-nanomolar to micromolar concentrations. Comparative evaluation of 78 congeners of SJ-3366 resulted in the definition of 12 highly active inhibitors with therapeutic indices ranging from 0.5 to 4 million. Additional studies have been performed to define the antiviral properties of the 12 molecules in order to define the optimal clinical candidate for further development. The rank order of each of these 12 molecules in inhibitory bioassays measuring activity against HIV-1, HIV-2, virus attachment and reverse transcription was defined. Four of the molecules were highly inhibitory against viruses and RT possessing the K103N mutation in the RT. Further evaluation of the series was performed by defining the relative oral bioavailability of the candidate compounds in mice dosed with equivalent

amounts of material solubilized as a colloidal suspension, defining those compounds which would be expected to achieve the highest plasma concentrations. Further evaluations focused on the relative ability of the most active of the 12 test molecules to prevent the emergence of resistant virus strains. The concentration of each compound which was required to sterilize virus infected cultures was defined. Finally, highly sensitive comparative resistance selection assays were performed to quantitatively compare the kinetics of resistant virus emergence under identical selection conditions. The fold-resistance of the virus obtained at each passage as well as the resistance engendering mutations in the RT and env were defined. The complete preclinical characterization of the 12 molecules will be presented along with results of initial pharmacokinetic profile for SJ-3366 in mice.

4

Selective Removal of Superoxide Anions is Crucial for HIV Replication in Human Primary Macrophages and Prevents Peroxynitrite Mediated Apoptosis in Neurons

S. Aquaro[1], C. Muscoli[2], M. Pollicita[1], A. Ranazzi[1], T. Granato[2], M.C. Bellocchi[1], A. Modesti[1], D. Salvemini[2], V. Mollace[2], C.F. Perno[1]

[1]Department of Experimental Medicine University of Tor Vergata Rome, Italy; [2]Faculty of Pharmacy University of Catanzaro Magna Grecia, Roccelletta di Borgia Catanzaro, Italy; [3]MetaPhore Pharmaceuticals, Inc., 1910 Innerbelt Business Center Drive, St. Louis, MO 63114, USA

HIV-1 infection induces a heavy perturbation of oxidative status with increased production of superoxide anions during HIV-1 replication in macrophages (M/M). HIV-1 chronically and productively infected cells are represented in the body mainly by M/M, either in the systemic compartment or in the central nervous system. The role of superoxide anions in HIV-1 replication in M/M was assessed by using nitrotyrosine staining. Virus production was assessed by p24 ELISA, western blot, virus titration, and electron microscopy during treatment with M40401, a SOD mimetic compound. Gene expression in HIV-1-infected M/M was determined by microarray analysis. Apoptosis in two human neuronal cell lines (SK-N-SH and CHP-100), was evaluated by FACS analysis. Nitrotyrosine overproduction in HIV-infected M/M was dramatically decreased in presence of M40401 (6 uM). Microarray analysis of M/M showed that SOD and SOD1 soluble genes are both upregulated by HIV-1. Maturation of p55 and p24 was strongly inhibited in both acutely and chronically infected M/M by M40401 (30 uM). This result was confirmed by electron microscopy which showed a strongly reduction of HIV-1 particles in infected M/M treated with M40401. Consequently, HIV-1 infectivity was reduced of about 1 log compared to control by drug treatment. M40401 treatment showed a reduction of HIV-1 replication in both acutely and chronically infected M/M: 99% and 90% inhibition of p24 released in sups compared to controls, respectively. Moreover, treatment with M40401 (20 uM) of SK-N-SH and CHP-100 incubated with supernatants from HIV-1-infected M/M strongly antagonized the apoptosis related to superoxide anions generation. Results support the role of superoxide anions production in both HIV-1 replication in M/M and its related neurodegeneration, and suggest that SOD mimetic compounds may counteract both HIV-1 replication and HIV-related neural damages in combination with other antiretroviral treatments.

5

Substrate Dependence of HIV RNase H Activity and Inhibition by Active Site and Allosteric Site Binding Compounds

Julie Qi Hang, Yu Li, Yanli Yang, Stan Tsing, Jim Barnett, Nick Cammack, Joseph A. Martin, Klaus Klumpp

Roche Palo Alto LLC, Palo Alto, CA, USA

HIV RNase H activity is essential for the synthesis of viral DNA by HIV reverse transcriptase (HIV-RT). Differences in interaction and enzymatic activity of HIV-RT to substrates with recessed DNA 3′-ends (D-mode) as compared to substrates with recessed RNA 5′-ends (R-mode) have been described previously. Mutations in the allosteric non-nucleoside reverse transcriptase inhibitor (NNRTI) binding site can also influence RNase H activity of HIV-RT. A fluorescent RNase H assay was developed to allow rapid comparative enzyme activity measurements and active site concentration determination of HIV RNase H using HIV-RT wild-type and mutant proteins. Substrate dependence and mode of binding on RNase H activities were also assessed. HIV-RT wild-type, single mutant K103N, Y181C, Y188L and double mutant K103N/Y181C enzymes showed similar RNase H specific activities and mean active site concentrations between 74 and 96% when measured in R-mode. Apparent active site concentrations were reduced by 40–50% relative to R-mode, when measured in D-mode, suggesting that HIV-RT may bind to substrates with recessed DNA 3′-ends in two different conformations. Reference compounds from the recently identified *N*-hydroxyimide series of RNase H inhibitors showed similar inhibitory potencies with either substrate, consistent with their binding interaction in the RNase H active site. Non-active site binding compounds could also interfere with HIV RNase H activity. The NNRTI capravirine was found to partially inhibit HIV RNase H activity of HIV-RT in the R-mode, but not in the D mode. The potency of inhibition was significantly reduced with the Y188L variant HIV-RT enzyme, consistent with the binding of Capravirine to the allosteric NNRTI binding site of HIV-RT.

6

Homology Modeling of HIV-1 gp120 and Docking of Molecules on its Surface Agree with Experimental Data

Mercedes Armand-Ugûn[1], Imma Clotet[1], Cristina Tintori[2], Fabrizio Manetti[2], Bonaventura Clotet[1], Maurizio Botta[2], José A. Esté[1]

[1]Retrovirology Laboratory irsiCaixa, Hospital Universitari Germans Trias i Pujol, 08916 Badalona, Spain; [2]Dep. Farmaco Chimico Tecnologico, Università degli Studi di Siena, Via Aldo Moro, I-53100 Siena, Italy

We present a modeled HIV-1 gp120 on which the docking of molecules was applied showing accordance with in vitro experiments.

Molecular modeling simulations were performed to build the structure of gp120 starting from the X-ray crystallographic coordinates of the gp120 core, to which the V3 and V4 loops were computationally added. Appropriate mutations were included in the model to obtain three sequences that were in turn submitted to structural optimization through molecular dynamics simulations. The three different gp120 were modeled based on the gp120 amino acid sequence from three HIV-1 strains. One was from the wild type NL4-3 laboratory adapted strain, the second from a NL4-3-derived strain resistant to AR177 (NL4-3/AR177res) and the third from a NL4-3/AR177res resistant to ADS-J1 (NL4-3/ADS-J1res). AR177 and ADS-J1 are compounds that interfere with virus binding to the CD4 lymphoid cell line MT-4 and the resistant viruses show mutations on gp120.

Molecular docking calculations were applied to explore the binding mode of the negatively charged molecule ADS-J1 and to evaluate its binding conformations onto the gp120 structures to which the compound was supposed to bind with different affinity. As a result, physicochemical properties of the theoretical complexes between ADS-J1 and the three gp120 structures were in agreement with in vitro data. First, the inhibitor preferentially bound at the level of the V3 loop, in agreement with the fact that most of the NL4-3/ADS-J1res were found in the V3 loop. Moreover, the decreased positive charge of the NL4-3/ADS-J1res V3 loop caused a deep change of the stereoelectronic properties of the protein surface, justifying the marked drop in affinity of ADS-J1 toward NL4-3/ADS-J1res, mainly due to missed profitable electrostatic interactions and hydrogen bond contacts. In fact, the electrostatic contribution to the ADS-J1/NL4-3/ADS-J1res binding was reduced to about 10%, while it was about 23% and 31% in the complexes ADS-J1/NL4-3wt and ADS-J1/NL4-3/AR177res, respectively.

7

Dioxolane-Thymine Nucleoside is Active Against a Variety of Clinically Relevant NRTI Drug Resistant HIV-1 Strains

Chung K. Chu[1], Vikas Yadav[1], K.L. Rapp[2], Mervi Detorio[2], Raymond F. Schinazi[2]

[1]The University of Georgia, College of Pharmacy, Athens, GA 30602, USA; [2]Emory University/VA Medical Center, Decatur, GA 30033, USA

Nucleosides are an integral part of combination therapy used by many HIV-infected individuals because of their potency and relative safety. The development of nucleoside-resistant mutants of HIV-1 is a serious problem for the management of these infections. Since DAPD (Amdoxovir) was found to be active against AZT- and 3TC-resistant mutants, several other nucleosides with a dioxolane moiety have been synthesized in our laboratories and their anti-HIV activity against drug sensitive and drug-resistant mutants determined. Their molecular mechanisms have been studied by molecular modeling.

Among the series of dioxolane nucleosides, 1-(β-D-dioxolane)thymine (DOT) showed significant and promising anti-HIV activity without cytotoxicity ($IC_{50} > 100$ μg M, in human PBM cells) against variety of clinically relevant nucleoside-resistant mutants, as shown below. It was found from the molecular modeling studies that the dioxolane moiety plays a significant role in stabilizing the binding between the mutant HIV-RT and the nucleoside triphosphate.

HIV-1 strains	EC_{50} (μM)	Fold increase
Wild type	0.43	–
K65R	0.21	0.5
L74V	0.33	0.8
M184V	0.20	0.5
T215Y	0.27	0.6
T215Y/M184V	0.23	0.5
M41L/D67N/K70R/T215Y	0.49	1.1
T69SSG/L210W/V108I/T215D	0.19	0.1

DOT was markedly effective against many clinically relevant drug resistant mutants including those containing K65R, M184V, TAMs and the 69-insert in the HIV-RT. Thus, additional biological studies are warranted to determine the full potential of DOT as a clinical candidate.

Acknowledgement: Supported by NIH AI32351, AI25899 and veterans affairs.

8

Reduced Susceptibility to Lopinavir due to V32I/I47A Mutations in HIV-1 Protease

Kirsten Stray, Andrew Mulato, Holly MacArthur, Stephanie Leavitt, Christopher Baer, Xiaohong Liu, Christian Callebaut, Gong-Xin He, Martin McDermott, Tomas Cihlar

Gilead Sciences, Foster City, CA, USA

Background: Lopinavir (LPV) in combination with low dose ritonavir is a frequently prescribed HIV protease inhibitor (PI) with rare emergence of in vivo primary resistance. When LPV resistance arises either in vitro or in vivo, the selected mutations in protease (PR) typically include I84V or I50V. Multiple PR mutations are often necessary for high-level resistance to LPV. Here we report the characterization of a novel combination of PR mutations selected in vitro by LPV.

Methods: HIV-1 IIIB and 89.6 were passaged in MT-2 cells in the presence of LPV. Selected viruses were characterized both genotypically and phenotypically. Recombinant viruses with PR mutations were generated from proviral DNA clones. Enzyme kinetics and isothermic titration calorimetry (ITC) were used to assess interactions of PIs with WT and mutant PR.

Results: The exposure of the IIIB strain to LPV for >6 months selected for mutations L10F/M46I/I84V in PR. The virus was 21-fold less susceptible to LPV than WT and showed similar resistance to several other PIs. In contrast, parallel selection with the 89.6 strain resulted in V32I/I47A mutations in PR. The selected virus showed 120-fold resistance to LPV and 25-fold resistance to amprenavir (APV), but remained susceptible to saquinavir (SQV). Recombinant I47A virus replicated extremely inefficiently, which precluded its phenotypic characterization. Recombinant V32I remained fit and susceptible to LPV. In contrast, V32I/I47A virus showed reduced susceptibility to LPV and APV, but was hypersensitive to SQV. The V32I/I47A enzyme exhibited 20-fold lower specific activity and 10-fold elevated IC_{50} for LPV and APV relative to the WT enzyme. However, no difference in SQV IC_{50} was found between the WT and mutant PR. Data from ITC indicate that V32I/I47A affects both the enthalpic (ΔH) and entropic ($-T\Delta S$) contributions to the binding of LPV to PR, resulting in >100-fold reduction in binding affinity.

Conclusions: V32I/I47A is a novel combination of PR mutations selected by LPV in vitro. Both of these residues are localized to the PR active site, however, our results indicate that I47A should be considered a primary LPV resistance mutation while V32I is likely to have a compensatory effect.

Oral Session II: Hepadnaviruses

9

Analysis of the Evolution of the HBV Quasi-Species During Sequential Therapy Shows the Emergence of Multiple Drug Resistant Virus

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Sequential anti-HBV therapy may lead to the selection of complex mutants. We analyzed the genetic and phenotypic evolution of viral quasispecies of a patient who received successively lamivudine (3TC), add-on adefovir (ADV) + 3TC, followed by a 3TC + ADV + Hepatitis B immunoglobulins (HBIg) after liver transplantation. For each sample, a 1142 bp region of the polymerase gene encompassing the RT domain and overlapping the S gene was amplified by PCR and sequenced. At baseline, all HBV genomes carried a wild-type (wt) RT gene but 36% harbored the P120S mutation within the S gene associated with vaccine escape. Following viral breakthrough to 3TC monotherapy, a complex mixture of 3TC-resistant HBV strains (rtL180M + M204V, rtV173L + L180M + M204V and rtM204I mutants) prevailed. After addition of ADV to the ongoing treatment, the viral load dropped, the patient underwent a liver transplantation and received HBIg. As the viral load rose again, the rtN236T mutation emerged in combination with rtV173L + L180M + A181V or rtV173L + L180M + A181V + M204V mutations, and 100% of the HBV genomes harbored the P120S S gene mutation escaping to HBIg. In further samples, HBV strains with the rtV173L + L180M + A181V + N236T mutations prevailed and rtM204I/V mutation disappeared. To get some insights about the combination of rtL180M + M204V and rtN236T mutations, we constructed a rtL180M + M204V + N236T HBV mutant by site-directed mutagenesis from HBV replication-competent plasmid. Hepatoma cell lines were transfected to compare the replication fitness of this strain to wt HBV and their susceptibility to a panel of nucleos(t)ide analogs. The phenotypic analysis of the triple mutant in hepatoma cell lines indicated that it replicates weakly its genome but exhibits resistance to pyrimidine analogs including the combination of 3TC + ADV. This mutant was sensitive in vitro to tenofovir and entecavir. In conclusion, our genetic and phenotypic analysis shows the evolution of the viral quasispecies towards the selection of mutants escaping to multiple selective pressures. This clearly indicates that sequential therapy may select for multiple drug resistant mutants and that de novo combination therapy should be further evaluated to prevent or delay viral drug resistance.

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Synthesis and Properties of Novel Types of Chiral Open-Ring Acyclic Nucleoside Phosphonates

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New generation of acyclic nucleoside phosphonates (ANP) is based on replacement of purine heterocycle bearing the chain with the phosphonate residue at the N9 position by 2,4-diamino-6-hydroxy- or 2-amino-4,6-dihydroxypyrimidine wherein the substituent containing phosphonomethyl ether group is linked to the oxygen atom at the position 6. In the 2-(phosphonomethoxy)propyl series where the substituent contains a chiral center, the antiviral activity is enantiospecific and parallel to that of the corresponding ANP with the complete purine ring. Also the (*R*)-[3-hydroxy-2-phosphonomethoxy)propyl derivative exhibits very high activity against HBV and poxviruses.

In an effort to elucidate the effect of chirality of the phosphonate-bearing side-chain on the biological activity in this series we have synthesized the both enantiomers of the latter mentioned compound as well as those of their guanine congeners by multistep procedure from the optically active 2,2-dimethyl-4-hydroxymethyl-1,3-dioxolane and 4-chloro-2,4-diamino- or 2-amino-4,6-dichloropyrimidine, respectively.

The dichloro derivative was also used for preparation of novel types of chiral ANPs containing at the positions 4 and 6 two identical or opposite enantiomers of the same or different chiral phosphonate-bearing substituents.

Details of synthesis, chiroptical properties and biological activities will be discussed.

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Anti-HBV Activity and Intracellular Metabolism of Tenofovir In Vitro

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Background: Tenofovir (TFV) shares significant structural homology with adefovir (AFV), a nucleotide approved for the treatment of chronic hepatitis B (as the prodrug adefovir dipivoxil (ADV)). Tenofovir disoproxil fumarate (TDF), a prodrug of TFV, is approved for the treatment of HIV and has also demonstrated potent suppression of serum HBV DNA in co-infected patients.

Aims: (1) To evaluate the in vitro activity of TFV against wild-type, lamivudine-resistant (LAM-R) and ADV-resistant (ADV-R) HBV. (2) To compare the intracellular metabolism of TFV and AFV.

Methods: The Ki of TFV diphosphate (TFVpp) was measured using recombinant HBV polymerase. Cell-based

antiviral activity of TFV was measured HepG2 cells transiently or stably-transfected with HBV. Susceptibilities of LAM-R and ADV-R HBV to TFVpp and TFV were analyzed in enzymatic and cell culture assays, respectively. Intracellular phosphorylation and the half-life of TFVpp and AFVpp were measured in HepG2 cells and primary human hepatocytes by mass spectroscopy.

Results: TFVpp was a competitive inhibitor ($Ki = 0.18\ \mu M$) of HBV polymerase with regard to dATP. EC50 values for TFV ranged from 0.17 to 1.14 μM in transiently or stably-transfected HepG2 cells. TFV had additive anti-HBV activity with AFV or FTC in cell-based combination assays. TFV demonstrated a $\pm 0.4\ \mu M$) was 2.4-fold higher than that of AFVpp ($2.1 \pm 1.0\ \mu M$) in HepG2 cells. Both TFVpp and AFVpp displayed a half-life of >40 h in primary human hepatocytes.

Conclusions: Tenofovir alone or in combination with adefovir or FTC demonstrated potent anti-HBV activity. Tenofovir was active against all major patterns of LAM-R HBV, but had a 3–4.6-fold increase in EC50 against ADV-R HBV in vitro. The clinical efficacy of TDF in patients with ADV-R HBV is unknown. Tenofovir achieved a higher intracellular diphosphate concentration than adefovir in HepG2 cells. The half-life of tenofovir diphosphate was >40 h in primary hepatocytes.

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Characterisation of Hepatitis B Virus Adefovir Resistance Mutations Outside the Polymerase Active Site Background/Aims: Resistance to ADV was Originally Found in the D Domain of the HBV Polymerase at rtN236T

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Background/aims: Resistance to ADV was originally found in the D domain of the HBV polymerase at rtN236T. Mutations in the B domain at rtA181V/T have also been associated with ADV resistance. We have now detected a further class of HBV polymerase mutations from patients during ADV therapy. The aim of this study was to analyse the mechanism of ADV resistance and cross-sensitivity profiles using molecular modeling together with in vitro functional analysis of these newly described mutations.

Methods: The HBV polymerase gene was amplified by PCR and sequenced from patients who failed ADV therapy. A three dimensional model of the HBV polymerase was developed based on the HIV reverse transcriptase crystal structure. In vitro phenotypic analysis of specific HBV mutations was performed using transient transfection of infectious clones using standard techniques.

Results: A new cluster of ADV resistance mutations outside the active site were detected in patients who failed ADV therapy. These mutations are located in the C–D inter-domain at rtV214A and rtQ215S. Molecular modeling of these in the C–D inter-domain reveal that they are not directly interacting with the nucleotide binding pocket nor the DNA and may be involved in conformational interactions within the polymerase. Using the SeqHepB database the mutation at rtQ215S was also detected in patients on LMV treatment. In vitro antiviral testing showed that this mutation was associated with both ADV resistance and also a 10-fold increase in lamivudine resistance.

Conclusion: We have identified a new class of ADV resistance mutations outside the active site of the polymerase. These new class of mutations have the potential to be multidrug resistance mutations. Molecular modeling in association with in vitro testing assist in determining the significance of mutations and may aid in the choice of the next therapeutic agent.

Mini Symposium: Biodefense and Emerging Infections

No Abstracts.

Oral Session III: Herpesviruses and Poxviruses

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Maribavir Induces the Formation of Tegument Aggregates in Cells Infected with Human Cytomegalovirus

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Maribavir has good antiviral activity against human cytomegalovirus replication in vitro and is in clinical trials for the treatment of infections with this virus. Inhibition of UL97 protein kinase activity is thought to be important in the mechanism of action of this drug. One characteristic of infected cells treated with this inhibitor, is the formation large nuclear inclusions that appear late in infection. These structures were also observed in cells infected with a recombinant virus that does not express the UL97 kinase. We hypothesized that viral proteins aggregated inappropriately in the absence of kinase activity and investigated the nature of these structures to help understand the mechanism of action of Maribavir as well as the function of the kinase. Nuclear inclusions were purified to near homogeneity and the constituent proteins were identified by MALDI-TOF mass spectrometry. This analysis demonstrated that the aggregates were formed principally of the tegument protein, pp65, as well as other virion proteins. Immunoblotting experiments confirmed these results and identified a number of additional viral proteins present in the purified tegument aggregates. Interestingly, the formation of these structures appeared to be dependent on pp65, since they did not occur in drug treated cells infected with a recombinant virus that does not express this protein. The pp65 negative virus also unexpectedly exhibited significant resistance to the antiviral effects of this inhibitor, suggesting that this tegument protein was involved in the mechanism of action of the drug. We suggest that tegument aggregates form as a consequence of reduced kinase activity by a process that is dependent on pp65. These data are consistent with a model in which the inhibition of the UL97 kinase by Maribavir results in the aberrant function of tegument phosphoproteins in the nucleus.

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ST-246: A Potent and Specific Inhibitor of Orthopoxvirus Replication

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Recent concerns over the use of variola (smallpox) virus as a biological weapon have prompted new interest in development of small molecule therapeutics that target variola virus replication. ST-246 is a small molecule compound (MW = 376), that is potent (EC_{50} = 0.010 μM), selective (CC_{50} > 40 μM), and active against monkeypox, camelpox, cowpox, ectromelia (mousepox), and variola viruses. ST-246 was also active against a cidofovir-resistant strain of cowpox virus. Drug resistant variants were isolated and the drug resistance phenotype was mapped to a single amino acid change within the vaccinia virus F13L gene. The F13L gene encodes a major envelope protein (p37) required for production of extracellular enveloped virus (EEV). In virus yield assays, ST-246 at 5 μ M reduced formation of EEV by 158-fold and intracellular mature virus (IMV) by 11-fold. In vivo, ST-246 delivered orally at 50 mg/kg b.i.d. protected ANC/R mice from lethal infection following intranasal inoculation with 40,000 × LD_{50} of ectromelia virus. Infectious virus titers at day 8 post-infection in liver, spleen, and lung from ST-246-treated animals were below the limits of detection 5.2×10^7, and 1.8×10^5 PFU/ml, respectively. ST-246 protected BALB/c mice from lethal infection following intranasal inoculation with 10 × LD_{50} of vaccinia virus. ST-246-treated mice that survived infection acquired protective immunity and were resistant to subsequent challenge with a lethal dose (10 × LD_{50}) of vaccinia virus. When administered orally, ST-246 inhibited vaccinia virus-induced tail lesion in NMRI mice inoculated via the tail vein. In immunocompromised (*nu*/*nu*) NMRI mice, ST-246 delivered orally or topically inhibited progressive vaccinia virus infection. Taken together, these results indicate that F13L is a valid antiviral target and demonstrate that ST-246 delivered orally can be used to treat orthopoxvirus infections.

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Identification and Proposed Mechanism of Antiviral Nucleoside Metabolism by DNA Repair Enzymes

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The rapid degradation in mice and monkeys of the potent HCMV inhibitor 2-bromo-5,6-dichloro-1-(β-D-ribofuranosyl)benzimidazole (BDCRB) compared to a structural L-analog, maribavir, has been attributed to selective glycosidic bond cleavage. An enzyme responsible for this selective BDCRB degradation, however, has not been identified. Here, we report the identification of two enzymes, 8-oxoguanine DNA glycosylase (OGG1) and

N-methylpurine DNA glycosylase (MPG), that catalyze *N*-glycosidic bond cleavage of BDCRB but not maribavir. Exploration of the substrate specificity of OGG1 revealed another nucleoside substrate, the 2-chloro homolog of BDCRB. To our knowledge, this is the first demonstration that free nucleosides are substrates of OGG1 and MPG, whose endogenous functions are excision of damaged bases from DNA via *N*-glycosidic bond cleavage. To elucidate this unique catalytic mechanism, docking simulations were performed using the native hOGG1 crystal coordinates. The results of these simulations suggested that nucleoside *N*-glycosidic bond cleavage involves protonation of the imidazole N-3 by Cys^{253} and subsequent nucleophilic attack of C1' by Lys^{249}. Although nucleophilic attack by Lys^{249} was previously reported in the catalysis of DNA substrates by OGG1, Cys^{253} was not considered to be directly involved in that mechanism. The minimum requirement for catalysis of free nucleosides by OGG1 was therefore a relatively basic imidazole nitrogen located opposite the glycosidic bond (pK_a roughly greater than 5), and this requirement may explain the selective metabolism of BDCRB compared to maribavir in vivo. These findings suggest DNA repair enzymes pose a previously unidentified obstacle to the effective delivery of antiviral and anticancer nucleoside drugs.

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Cyclic HPMPC is a Highly Effective Therapy for CMV-Induced Deafness in a Guinea Pig Model

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The objective of this study was to evaluate the utility of therapy with the cyclic cogener of the anti-CMV agent, cidofovir (cHPMPC), for efficacy against CMV-induced hearing loss in a guinea pig model. Thirty-six guinea pigs were randomly divided into four groups ($n = 9$). The first group underwent auditory brainstem response (ABR) testing with click stimuli and no intervention. The second group underwent ABR testing, followed by sham surgery consisting of unilateral round window injection of 25 μl of sterile viral media on day 0. The third group and fourth groups underwent ABR testing followed by round window injection of 1.7×10^5 pfu of guinea pig cytomegalovirus (GPCMV) on day 0. Group 4 received antiviral treatment with intraperitoneal injection of cidofovir (40 mg/kg) in two divided doses on days 1 and 5 post-inoculation. All animals challenged with GPCMV seroconverted, although antibody titers were significantly lower in treated animals. Systemic viral load was monitored with a real-time PCR assay. One animal in the untreated group developed high-grade DNAemia, but none in the cidofovir treatment group. All animals had ABR's performed on days 0, 4, and 7. Four of nine (day 4) and five of nine (day 7) animals who received GPCMV and no cidofovir treatment demonstrated a hearing loss of at least 30 dB. In contrast, none of the animals in the untreated, sham surgery, or cidofovir-treated groups had a hearing loss of >20 dB. This difference was statistically significant for both day 4 ($p = 0.04$, one-tailed Fisher's exact test) and day 7 ($p = 0.01$, one-tailed Fisher's exact test). Histologic evaluation of hearing-impaired animals revealed inflammatory infiltrates (predominately mononuclear cells), particularly in the scala tympani. We conclude that cidofovir is an effective antiviral intervention against cytomegalovirus-induced hearing loss in guinea pigs. These insights support further experimental evaluation of the pathogenesis and treatment of GPCMV labyrinthitis, and of antiviral therapies for HCMV-associated deafness in infants.

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Cyclic HPMPC Therapy Improves the Outcome of Guinea Pig Cytomegalovirus Congenital Infection and Decreases the Viral Load in the Placenta and Fetus

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Congenital CMV Infection occurs in 0.5–1.5% of births in the United States and is a common cause of hearing loss and mental retardation. We have previously shown that cyclic HPMPC (cHPMPC) is safe and effective in an immunocompromised model of guinea pig CMV (GPCMV) infection when given in a single dose [Antiviral Res. 47, 103, 2000]. In this study, we evaluated the effects of cHPMPC treatment of GPCMV infected dams on infection and outcome in the dams and pups. Pregnant Hartley guinea pigs were infected with virulent GPCMV ($\sim 10^5$ pfu) during the second/third trimester. Guinea pigs received either cHPMPC (35 mg/kg, IP, once) or placebo. In the first experiment animals ($n = 12$/group) were followed through delivery and the number of liveborn and stillborn pups were evaluated. Tissues were harvested from liveborn and stillborn pups and evaluated by cell culture for GPCMV infection. In the placebo group 28.2% of pups were liveborn, whereas 83.7% were liveborn in the cHPMPC treated animals, ($P < 0.01$) in placentas of cHPMPC treated animals. Similarly treatment reduced the viral load in fetal liver and spleen. Thus, antiviral therapy decreased the mortality of congenital CMV infection and the magnitude of viral replication in both the dam and fetus but did not prevent infection of the pups. This is the first evidence that antiviral therapy can modify the outcome of congenital CMV infection.

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Development of an Aerosol Model of Rabbitpox: Experimental Infection and Comparative Pathogenesis

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Smallpox is a viral threat agent that, if disseminated as an aerosol, would cause significant morbidity and mortality in immunologically naÔve populations. There is a need for robust animal models of aerosol infection to both study viral pathogenesis and accelerate medical product development. The underlying mechanisms of poxvirus pathogenesis are only partially understood; even less is known about aerosol-acquired poxviral disease. Rabbitpox represents a potentially useful model; early studies suggest that this model produces a definable dose-response relationship between route-specific exposure and disease, development of a clinical prodome, and disease onset and progression similar to human smallpox. Initially, lethality was established by exposing groups of NZ rabbits ($n = 40$) to rabbitpox virus (*Utretch*) by aerosol (MMAD = 1.0 μm, GSD = 1.4) to rabbitpox virus in escalating log doses (1.0E+01 to 1.0E+04 PFU). A dose-related clinical prodome was evident, with a significant increase in clinical signs of disease +72 h postexposure. Probit analysis indicated a LD_{50} of 154 PFU and a dose-related mean time to death of +168 (1.0E+04) to +216 h (1.0E+01 to 1.0E+03). These data, when compared with similar studies of different routes of experimental infection (ID, IN), indicated significant differences in clinical route-specific progression of disease. In order to further define viral dissemination, rabbits receiving the equivalent of 100 aerosol LD_{50}s were killed serially. Tissue-specific viral plaques and histopathological analysis indicated a unique viral progression that contrasted starkly with other routes of exposure. Further study of aerosol rabbitpox infection demonstrated a definable dose-response infection characterized by clinical symptoms characteristic of classic poxviral disease. Based on these results, future studies will include further defining host–pathogen interaction, characterization of initial quantal host response in the respiratory system, and comparability with other poxviral animal models.

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Progressive Outer Retinal Necrosis in an AIDS Patient During the Era of Highly Active Anti-Retroviral Therapy (HAART): Successful outcome with Intravitreal Drugs and Monitoring with Quantitative PCR

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Progressive outer retinal necrosis (PORN) is a unique form of herpetic retinitis that occurs most commonly in HIV infected individuals with AIDS. Varicella zoster virus (VZV) is often the etiologic agent. Prior to HAART, patients with PORN often succumbed to other opportunistic infections soon after diagnosis. There is little evidence-based literature regarding the long term management of these patients. Since the advent of HAART, many patients diagnosed with AIDS have immune recovery that has led to increase in life expectancy. Therefore, understanding the diagnosis, treatment, and monitoring of this disease is critical to decreasing morbidity in AIDS patients with a response to HAART.

We report a case of a 32-year-old woman with AIDS who was diagnosed with PORN by clinical exam and real time PCR amplification of VZV from the aqueous humor. The disease occurred initially in the right eye but rapidly became bilateral. Although the right eye was unsalvageable, the vision in the left eye has been maintained (>11 months duration) 20/20. We describe the extended clinical course of this patient and how the use of 58 intravitreal injections in the left eye, HAART, and quantitative PCR were temporally correlated in obtaining a successful outcome with the tandem approach by ophthalmology and medicine.

Key elements in the long-term management of PORN can be inferred from this case. Recurrence occurred in our patient despite continuous intravenous antivirals and responded only to aggressive intravitreal injections with dual antivirals. This supports the use of intravitreal injections as the main mode of therapy and highlights the inadequacy of systemic antivirals alone in managing this disease. Second, PORN remission appeared to occur only after CD4 count improvement to >50.

This emphasizes the importance of immune recovery in controlling disease progression, and suggests that there may be a CD4 threshold at which antivirals may ultimately be withdrawn. The use of quantitative PCR appears to be a useful tool for monitoring the long-term progression of PORN and may serve as a useful clinical surrogate marker.

Oral Session IV: Respiratory and West Nile Viruses

20

A Novel Broad-Spectrum Inhibitor of Influenza Virus Infections

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Influenza is a highly infectious acute respiratory disease characterized by recurrent annual epidemics and periodic major worldwide pandemics. Outbreaks are caused by the highly diverse and mutable influenza viruses A and B (IFV A and IFV B). Ongoing human infections by the highly pathogenic avian H5N1 strain have set off the alarm for a pandemic. Influenza vaccines require annual updates; the supply is unreliable and would be inadequate during a pandemic. The neuraminidase inhibitor Oseltamivir, which is the only effective antiviral chemical compound, was recently linked to a surprisingly high frequency of drug resistant viruses in children. To provide the urgently needed alternative treatment modalities for influenza, we have generated a recombinant fusion protein which are referred to as Fludaseé. Fludaseé works by eliminating the receptors for human and animal influenza viruses. Fludaseé also has built-in features to enable long drug retention time on the airway surface and low immunogenicity to humans. Distinct from conventional vaccines, Fludaseé potentially confers broad-spectrum protection against all subtypes and strains of influenza viruses without the need for annual updates. Fludaseé can be produced inexpensively in bacteria and is to be applied topically as an inhalant to prevent and treat influenza. We will present data demonstrating potent protective effect of Fludaseé against a spectrum of human and animal influenza viruses in vitro, as well as in vivo data from a ferret study.

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Effect of Hemagglutinin Glycosylation on Influenza A Virus Susceptibility to Neuraminidase Inhibitors: a Reverse Genetics Study

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Influenza virus infections pose a serious threat to public health and economy. Inhibitors of viral neuraminidase (NAIs), zanamivir (inhaled) and oseltamivir (oral), are approved for clinical use. Influenza NA destroys sialic-acid bearing receptors that bind to viral hemagglutinin (HA); the NAIs exert antiviral effects by inhibiting this NA enzymatic function and preventing the release of progeny virions. In cell culture, mutations in the HA can confer resistance to this class of drugs by allowing viral release through reduced affinity of HA for its receptors and associated dependence on NA activity. Inhibition of NA activity is accompanied by sialylation of *N*-glycans attached to the newly synthesized HA molecules. Consequently, we investigated the effect of individual *N*-glycans attached in the vicinity of the HA receptor-binding site (94a, 129, and 163) on the oseltamivir-susceptibility of influenza A (H1N1) viruses. With the use of site-directed mutagenesis and reverse genetics technique, we generated a series of HA glycosylation-site mutant viruses and assessed their drug-susceptibility in a standard MTT assay in MDCK cell culture. Glycosylation at 94A was accompanied by >5-fold increase in the virus susceptibility to oseltamivir, whereas glycan at 129 reduced it by ∼20-fold. Glycan at 163 on the tip of the HA reduced drug-susceptibility greatly (∼2000-fold). A dominant role of glycan at 163 (regardless of the HA amino acid backbone, A/WSN/33 or A/Bayern/7/95) was also supported by successful rescue of the recombinant mutants completely lacking the NA activity due to a deletion in the NA gene. Nonetheless, despite the compensatory role of HA glycosylation in vitro, the NA activity-lacking mutants were severely attenuated when tested in a ferret model of influenza virus infection based on the significant reductions in nasal wash viral titers (>3 log $10TCID_{50}$/ml), cell counts (three-fold) and protein concentrations (10-fold) compared to wild-type virus. Our results demonstrate that the influenza A virus dependence on NA function is much greater in vivo than in cell culture and thus reinforce the utilization of NA as a target for antiviral design.

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Development, Validation and Optimization of a Luminescence-Based High Throughput Screen for Inhibitors of Severe Acute Respiratory Syndrome-Associated Coronavirus

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We have developed a high-throughput, cell-based assay to address the critical need for antiviral drugs for treatment of severe acute respiratory syndrome-associated coronavirus (SARS-CoV). In this assay, the inhibition of SARS-CoV induced cytopathic effect (CPE) in Vero E6 cells was assessed using CellTiter-Glo Luminescent Cell Viability Assay by Promega. This reagent measures the amount of ATP present in cells such that the signal is directly proportional to the number of metabolically active cells. Validation studies were executed to establish optimal cell density, viral concentration, DMSO concentration for compound solubilization, incubation time for virus-induced CPE and control drug concentration. Compounds exhibiting CPE inhibition greater than 50% were considered a "hit". Calpain IV was chosen as the drug control for performing with consistency and reproducibility. Data variability and thus assay quality were assessed by the calculation of the estimated Z'-factor that is test library independent. Prestwick and MicroSource, two different FDA-approved diversity sets of compounds, were screened in triplicate at the concentrations of 1 ug/ml and 5 ug/ml. The "hit" rate for both libraries was determined to be 1.42%. The developed assay identified several compounds that effectively inhibited the induced CPE of SARS-CoV in vitro, providing candidates for further evaluation.

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Inhibition, Escape and Attenuation of SARS Coronavirus Treated with Antisense Morpholino Oligomers

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Coronaviruses evolve rapidly and are associated with severe disease in birds and mammals including humans. Peptide-conjugated antisense morpholino oligomers (P-PMO) were used to inhibit production of infectious SARS coronavirus (Tor2 strain) and to probe the function of conserved viral RNA motifs and secondary structures. P-PMO reduced virus-associated cytopathology and spread as a consequence of decreasing viral growth. P-PMO were active when administered at any time prior to peak viral synthesis, and exerted sustained antiviral effects while present in culture medium. P-PMO showed low non-specific activity against non-target RNA and an unrelated arenavirus. A random-sequence control P-PMO also showed low activity against SARS coronavirus. Two P-PMO targeting the viral transcription-associated sequence in the 5'-untranslated region were most effective. SARS coronavirus developed point mutations at the binding site of the most effective antiviral P-PMO, resulting in development of attenuated, P-PMO-resistant virus clones. We also report complementary results using P-PMO against the related coronavirus, murine hepatitis virus. These results suggest PMO compounds have powerful therapeutic and investigative potential toward coronavirus infection.

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Terminal Differentiation of Trophoblast Cells Serves as a Barrier to West Nile Virus Infection of the Fetus in Mice

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The placenta often serves as a protective barrier to viral infection of the fetus. Our objective was to determine the role of the placenta in fetal West Nile virus (WNV) infection. Subcutaneous challenge of timed-pregnant mice with $10^{5.3}$ cell culture infectious doses of WNV on 5.5, 7.5, and 9.5 days post-coitus (dpc) resulted in fetal infection where infectious virus was detected 6 days after maternal challenge. Viral challenge on 11.5 and 14.5 dpc resulted in low fetal infection rate and no fetal infection, respectively. The murine placental barrier between maternal and fetal blood is functional around 10.5 dpc, which correlates with the reduction or inhibition of fetal WNV infection observed. The placenta was susceptible to viral infection at relatively high titers regardless of the gestational time-point of maternal infection. The placenta had detectable WNV titers as early as 3 days after maternal viral challenge. Murine trophoblast stem (TS) cells were used as an in vitro model of placental cells and were maintained in a proliferative state in media containing fibroblast growth factor-4 (FGF-4). Upon removal of FGF-4, TS cells will terminally differentiate into giant cells (GC). TS cells and differentiated GC were challenged with a WNV construct expressing green fluorescent protein (GFP-WNV). GFP fluorescence was observed 2, 4, and 6 days post-viral challenge in TS cells, but GC were not susceptible to

WNV infection and no fluorescence was observed. If GFP-WNV-infected TS cells were allowed to differentiate after viral infection, GFP fluorescence was observed in differentiated GC. These results suggest that the formation of a functional placenta including the differentiation of syncytiotrophoblast cells prevents fetal infection across the mouse placenta.

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Presumptive Identification of a Protein Associated with West Nile Virus Encephalitis in CSF of Hamsters

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Antemortem markers for benchmark pathological events have not been adequately identified for West Nile virus (WNV) encephalitis. The only biological samples reasonably available for antemortem clinical analysis in humans are serum and cerebrospinal fluid (CSF). Among the proteins in CSF that may be markers of CNS disease, are 14–3–3 seen in multiple sclerosis and CJD and ferritin as a marker for non-specific acute neurological episodes of HIV-1. Cerebrospinal fluid can be collected from hamsters for antemortem analysis in a minimally invasive procedure that does not adversely affect the animal. In the current study we used a proteomics approach to identify proteins expressed in WNV-infected hamsters. CSF collected from WNV infected and non-infected hamsters was labeled with different fluorochromes and superimposed over each other using 2D DIGE. As many as 150 separate spots were identified, with 16 of them being differentially expressed in infected animals. One major spot from infected animals was picked and identified by mass spectrophotometer analysis to putatively be apolipoprotein E (ApoE), which modulates cholesterol and phospholipids homeostasis in selective subcellular membrane compartments in brain cells. Genetic variants of ApoE are also markers for Alzheimer's disease. This provides a proof-of-principle that the hamster model can be used to study WNV disease markers in the CSF. As additional proteins associated with WNV infection are identified and their roles characterized they can be evaluated for their ability to indicate clinical outcome of the disease.

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Oral Session V: Hepacivirus and Human Immunodeficiency Virus

26

Differential Binding of Two Anti-E2 Human Monoclonal Antibodies to HCV Quasispecies Population in Liver Transplant Patients

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Background: Hepatitis C virus (HCV) related cirrhosis is the leading indication for liver transplantation. Currently, there is no available therapy to prevent re-infection of the liver graft that occurs shortly after transplantation in almost all patients. Neutralizing antibodies could be considered as passive immunotherapy in preventing re-infection of liver transplant patients. The high mutation rate in the HCV genome causes genetic heterogeneity leading to the evolution of quasispecies, a complex population of genetically distinct, but closely related viral variants in a given host. Following liver transplantation, changes in quasispecies composition occur. A single monoclonal antibody (mAb) will not recognize all variants in the heterogeneous quasispecies population.

Objective: We propose to use a mixture of two different human mAbs that bind to distinct epitopes on the envelope protein (E2) of HCV to obtain broad reactivity and prevent re-infection in liver transplant patients.

Methods: Quasispecies analysis was performed in sera of five liver transplant patients. Sequences were analyzed and compared prior and 85 days post transplantation. In addition, we tested the ability of two neutralizing human mAbs, HCV-AB 68 and HCV-AB 65, to immunoprecipitate viral particles from sera samples taken prior and post transplantation.

Results: Changes were detected in the binding characteristics of each mAb to HCV particles before and after re-infection of the liver graft. Immunoprecipitated viral particles were sequenced and analyzed by sequence alignment indicating that the evolved quasispecies after liver transplantation bind differently to each mAb. Sequence analysis indicates that the quasispecies population in all patients varies post transplantation and the variations are observed in the entire E2 amino acid sequence, not only on the HVR1.

Conclusion: This analysis implies that more than one mAb may be needed to bind a wide range of viral quasispecies. Our antibodies that are directed against different epitopes may have potential broad reactivity against evolved quasispecies.

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The Predominant Mechanism by which Ribavirin Exerts its Antiviral Activity In Vitro Against Flaviviruses and Paramyxoviruses is Mediated by Inhibition of Inosine Monophosphate Dehydrogenase

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It is as yet debated to which extent depletion of intracellular GTP pools contributes to the antiviral activity of ribavirin. Therefore, the inhibitory effects of (i) ribavirin, (ii) its 5-ethynyl analogue EICAR, and (iii) mycophenolic acid (MPA, a compound that solely inhibits cellular inosine monophosphate dehydrogenase activity) were evaluated on the replication of the flavivirus yellow fever (YFV, 17D vaccine strain) and the human parainfluenza type 3 (hPIV3), a paramyxovirus, in Vero cells. MPA proved to be the most, and ribavirin the least potent (EC_{50} against YFV for ribavirin, EICAR and MPA: 12.3; 0.35 and 0.02 μg/ml, respectively; EC_{50} against hPIV3: 9.4; 0.27 and 0.015 μg/ml, respectively). MPA proved also the most potent in reducing intracellular GTP pools (EC_{50} in Vero cells: 12.8; 0.48 and 0.023 μg/ml for ribavirin, EICAR and MPA). A linear correlation was observed over a broad concentration range between the antiviral activity of ribavirin, EICAR and MPA, and their effects on GTP pool depletion (for YFV 17D; $R \leq$ ribavirin = 0.966; EICAR = 0.954; MPA = 0.974; for hPIV3; $R \leq$ ribavirin = 0.885; EICAR =0.943; MPA = 0.982). When the EC_{50} values for antiviral activity of ribavirin, EICAR and MPA were plotted against their respective EC_{50} values for GTP pool depletion, a linear correlation was obtained (for YFV, $R \leq = 0.982$; for hPIV3, $R \leq = 0.997$). Similar correlations were also calculated between GTP depletion and the EC_{50} for inhibition of the replication of three other flaviviruses (dengue virus type 2, Modoc virus and Montana myotis leukoencephalitis virus) in Vero cells. Similarly, a strong correlation was observed between inhibition of respiratory syncytial virus replication in Hela cells (EC_{50}: 3.74 and 0.095 μg/ml for ribavirin and MPA) and GTP pool depletion (EC_{50} of: 3.80 and 0.14 μg/ml, respectively). These data provide compelling evidence that the predominant mechanism of action of ribavirin in vitro against flavi- and paramyxoviruses is based on inhibition of cellular inosine monophosphate dehydrogenase.

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Potent and Selective Inhibition of Hepatitis C Virus Replication by the Non-Immunosuppressive Cyclosporin Analogue DEBIO-025

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Cyclosporin A (CsA) has recently been shown to inhibit in vitro the replication of HCV [Watashi et al., 2003. Hepatology 38, 1282–1288; Nakagawa et al., 2004. Biochem. Biophys. Res. Commun. 313, 42–47]. Here we report on the potent anti-HCV activity of the non-immunosuppressive cyclosporin analogue DEBIO-025. The antiviral activity was assessed by monitoring the effect on HCV (genotype 1b) subgenomic replicon replication either by (i) determination of luciferase activity in Huh5-2 cells (in which the HCV replicon contains the firefly luciferase gene) or (ii) by q (quantitative) RT-PCR on viral RNA isolated from Huh5-2 cells. The 50% effective concentration (EC50) for inhibition of viral replication in Huh5-2 cells by DEBIO-025 was 0.03 ± 0.04 μg/ml and for CsA 0.28 ± 0.13 μg/ml when luciferase activity was monitored. The concentration that reduced the growth of exponentially proliferating Huh5-2 cells was >27 μg/ml for DEBIO-025 and 11.6 ± 5.7 μg/ml for CsA thus resulting in a selectivity index of ∼900 for DEBIO-025 as compared to ∼40 for CsA. The superior anti-HCV activity of UNIL25 was corroborated by Q-RT-PCR. The EC50 for inhibition of subgenomic replicon replication was 0.27 ± 0.05 μg/ml and 1.2 ± 0.2 μg/ml, respectively for DEBIO-025 and CsA. Our data support the findings of Watashi (see supra) that the anti-HCV activity of CsA is independent from its immunosuppressive function. DEBIO-025 and CsA had no inhibitory effect on the replication of other members of the family of the Flaviviridae, i.e. the bovine viral diarrhea virus (genus pestivirus) in MDBK cells and the yellow fever virus (17D) (genus Flavivirus) in Vero cells or HepG2 cells. DEBIO-025, which exhibits also potent anti-HIV activity, is currently being evaluated in phase I clinical trials. DEBIO-025 may be an interesting candidate for the treatment of chronic HCV infections including HIV infected patients coinfected with HCV.

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Inhibitors of Alpha Glucosidases Impair HCV Pseudoparticles Morphogenesis, Prevent Viral Secretion and Entry into Hepatoma Cells

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The morphogenesis of HCV belongs to these steps of the viral cycle that have not yet been targeted by antiviral strategies. Using the bovine viral diarrhea virus, a pestivirus related to HCV; as a model, we have previously shown iminosugar (IS) which are inhibitors of alpha glucosidases could inhibit viral morphogenesis in cellulo via the perturbation of the *N*-glycosylation pathway and the folding of envelope glycoprotein. Due to the heavy *N*-glycosylation of HCV glycoproteins, it was anticipated that such inhibitors would also affect HCV morphogenesis. With the lack of an efficient and reliable culture system able to produce and secrete HCV virions, we used two complementary approaches to study the effect of IS on HCV morphogenesis, secretion and entry. First, we used baculoviruses carrying HCV structural genes to produce virus like particles (VLPs) in Sf-9 cells in order to study the effect of IS on the folding and assembly of HCV glycoproteins, on VLPs production and binding/internalisation properties. We have shown that, in presence of IS, gpE1 and gpE2 synthesised and retained in the ER (i) contained unprocessed triglycosylated *N*-glycans, (ii) were impaired in their interaction with calnexin and (iii) were at least partially misfolded. Moreover, we observed that VLPs produced in the presence of IS had modified binding and internalisation properties to hepatoma cells. Second, we used infectious HCV pseudotyped retroviral particles (HCVpp) harbouring unmodified gpE1 and gpE2 to measure and demonstrate the effect of IS on viral secretion and entry. Using this model we found (i) that the production and the secretion of HCVpp was impaired in presence of inhibitors of alpha glucosidases, (ii) that HCVpp contained misfolded and misassembled viral glycoprotein, and (iii) that HCVpp entry into target cells was impaired after treatment with IS. These two approaches have allowed us to demonstrate the antiviral effect of IS on HCV structural glycoprotein folding and assembly, morphogenesis, viral secretion, and viral infectivity.

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4′-*C*-Ethynyl-2′-Deoxy-2-Fluoroadenosine, A Nucleoside Derivative Potent Against HIV-1 with no Acute Mouse Toxicity: Highlights of the Role of 3′-OH for Biological Activity

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Despite of the initial success of therapy for HIV infection, we have encountered a number of challenges such as drug-related toxicities and the emergence of drug-resistant HIV variants. Thus, the identification of new anti-HIV agents remains an important therapeutic objective.

Resistance to reverse-transcriptase (RT)-inhibitory 2′,3′-dideoxynucleoside (ddN) indicates that HIVs can acquire the ability to discriminate between ddN and physiologic 2′-deoxynucleoside(dN) and does not incorporate ddN into the growing proviral DNA and/or eliminate the already incorporated ddN from the proviral DNA terminus.

By taking the above mechanism into account and based on the following rationale (1) and (2), we have designed 4′-*C*-substituted-2′-deoxynucleoside (4′SdN) as the one that could be highly active against various HIVs and delay or does not allow the emergence of resistant HIVs.

(1) 4′SdN is very much like dN because it has all the functional groups of dN and therefore it could be very difficult for HIV to discriminate between them.
(2) Quaternarization of the 4′-carbon makes the 3′-OH into a very unreactive neopentyl type secondary alcohol, therefore DNA biosynthesis would stop at 4′SdN and thus 4′SdN could be the chain-terminator of RT reaction.

We have synthesized various kind of 4′SdNs. Some of them are highly active against a wide spectrum of HIVs but they were very toxic, too. Recently, we designed and synthesized 4′-*C*-ethynyl-2′-deoxy-2-fluoroadenosine, which turned out to be potent against HIV-1; e.g. EC_{50} (IIIB) = 0.2 nM, (M184V) = 3 nM, (MDR) = 0.15 nM), S.I. = 110,000. No acute mouse toxicity up to 100 mg/kg has been seen. The role of the 3′-OH for the biological activity (Sliddiqui et al., 2004) will be discussed at the presentation.

Reference

Sliddiqui, M.A., Huhhes, S.H., Boyer, P.L., Mitsuya, H., Van, Q.N., George, C., Sarafinanos, S.G., Marquez, V.E., 2004. J. Med. Chem. 47, 5041–5048.

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Inhibition of CD4-gp120 Binding Blocks Coreceptor Independent Cell to Cell HIV-1 Transmission

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The contact between HIV producing cells and primary CD4 T cells may induce the uptake of HIV particles by target cells in the absence of membrane fusion or productive HIV replication. HIV uptake by CD4 T cells depended on cellular contacts mediated by the binding of gp120 to CD4 but was independent on the expression of the appropriate HIV coreceptor CCR5 or CXCR4. Later steps of HIV viral cycle were also not required. Thus, anti-HIV agents targeting HIV CCR5 or CXCR4, gp41-dependent fusion or reverse transcriptase did not block the transfer of HIV particles to intracellular compartments and that were later released as infectious viral particles. A recombinant CD4-based protein (CD4IgG2) completely inhibited the uptake of HIV particles by CD4 + T cells from persistently infected cells expressing R5, X4 or T-20 resistant HIV envelope glycoproteins. Consequently, the subsequent release of virus particles from endocytic vesicles and infection of reporter U87-CD4 cells was also prevented. Polyanionic agents such as dextran sulfate, not only did not prevent the intracellular uptake of virions by CD4 + T cells but also increased the HIV uptake in a dose dependent manner, suggesting functional differences between the specific gp120-targeting CD4IgG2 agent and non-specific HIV binding inhibitors. This novel mechanism of HIV transmission converts CD4 T cells lacking the appropriate coreceptor in HIV carriers that could favor the spreading of HIV to compartments where antiretroviral drugs could not reach. Further studies of gp120 targeting agents in the coreceptor independent virus transmission will provide new tools to understand the relevance of this novel mechanism.

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Debio-025, A Novel Non-Immunosuppressive Cyclosporine Analog with Potent Anti-Human Immunodeficiency Virus Type 1 Activity: Pharmacological Properties and Mode of Action

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Debio-025 is a synthetic Cyclosporin A (CsA) analog selected for its absence of immunosuppressive capacity but high affinity for binding to cyclophilin A (CypA). Lack of immunosuppressive activity as compared to CsA was demonstrated in vitro and in vivo. Debio-025 selectively inhibits replication of laboratory strains of HIV-1 in T4 lymphocyte cell lines and in PBMC. Potent activity against most of 40 tested clinical isolates of different subclades was demonstrated in PBMC. Debio-025 was also a potent inhibitor of clinical isolates with multiple resistance to reverse transcriptase and protease inhibitors. In drug combination studies synergistic or additive inhibitory effects with clinically used drugs were found. In vivo activity was evaluated in the SCID-hu Thy/Liv mouse model: mice infected with an X4 and an R5X4 virus strain were treated with Debio-025 by once-daily oral gavage, which reduced viral load dose dependently and protected thymocytes from virus-mediated depletion. By competing with HIV-1 Gag for CypA binding Debio-025 inhibits incorporation of CypA in virus particles. CypA is known to be essential for early steps in HIV-1 replication. The most plausible mechanism is that it counteracts the effects of the innate primate restriction factor Ref-1. In support of this, clinical isolates were identified that are naturally resistant to Debio-025 and that do not depend on CypA for infection. By sequence comparison a motif was identified in the CypA binding region of capsid protein that confers this resistance. Safety, pharmacokinetics and toxicology were investigated in rats, monkeys and man. Good oral bioavailability, long half-life and a low toxicity profile were found. A proof of concept study in humans was started recently.

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HIV-1 Strains Resistant to Mannose- and N-Acetylglucosamine-Binding Proteins Show Mutations at Glycosylation Sites of gp120

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Several sugar-binding proteins with selectivity for mannose such as the plant lectins derived from Hyppeastrum hybrid (HHA) and Galanthus nivalis (GNA), and cyanovirin, a mannose-specific lectin from the blue–green algae Nostoc ellipsosporum, and the plant lectin derived from Urtica dioica (UDA) which is specific for *N*-acetylglucosamine, were shown to be potent inhibitors of HIV replication in cell culture and to prevent transmission of the virus between persistently HIV-infected T-cells and uninfected T-lymphocytes. They prevent entry of HIV in the target cells and therefore, may qualify as potential microbicide drugs. When HIV-1 (IIIB) was exposed to escalating HHA, GNA and UDA concentrations in CEM cell cultures, virus strains could be isolated at several stages during the selection process. The HIV-1 strains showed decreased susceptibility to the mannose- and *N*-acetylglucosamine-specific plant lectins. Amino acid changes in the envelope glycoprotein gp120, but not gp41, were observed. The majority of amino acid changes occurred at *N*-glycosylation sites and at S or T residues that are part of the *N*-glycosylation motif in gp120. The degree of drug resistance closely correlated with an increased number of mutated glycosylation sites. Several other HIV entry inhibitors retained full sensitivity to the plant lectin-resistant virus strains. The virulence of several mutant virus strains was altered. Those virus strains with the highest degree of drug resistance contained mutations in at least 6 or 7 out of a total of 22 glycosylation sites in gp120. The resistance profile (mutation of glycosylation sites in gp120) of HIV-1 to mannose- and *N*-acetylglucosamine-binding proteins is entirely different from that of other anti-HIV drugs, including other viral entry inhibitors such as the CXCR4 antagonist bicyclam AMD3100, the virus adsorption inhibitor dextran sulfate and the virus-cell fusion inhibitor enfuvirtide T-20.

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Resistance Profile of Human Immunodeficiency Virus to CADA, a Novel HIV Inhibitor that Targets the Cellular CD4 Receptor

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The infection of target cells by human immunodeficiency virus (HIV) is mainly dependent on the presence of the CD4 surface molecule, which serves as the primary virus receptor. Therefore, drugs that target the CD4 receptor, and thus inhibit viral entry, may be promising agents for the treatment of AIDS. Here, we report on the resistance profile of HIV to the CD4 receptor down-regulator CADA, a novel potent anti-HIV agent. The prototype compound, cyclotriazadisulfonamide (CADA), inhibited HIV infection in T-cell lines, PHA-stimulated PBMCs and monocytes/macrophages (EC50: 0.3–3.2 μM). The anti-HIV activity was markedly enhanced after pre-treatment of the cells with CADA for 24 h. CADA showed synergy when evaluated in combination with various other known HIV inhibitors, such as reverse transcriptase inhibitors, protease inhibitors and virus

entry inhibitors. Flow cytometric analysis revealed a significant decrease in the cell surface and intracellular expression of the CD4 receptor at the CADA-treated cells. Moreover, the antiviral activity of CADA correlated with its ability to down-modulate the CD4 receptor. Interestingly, CADA did not affect the expression of at least 20 other surface molecules that were examined, including the HIV co-receptors CXCR4 and CCR5. When exposed to escalating concentrations of CADA, a HIV-1 (NL4.3) strain was isolated after 40 subcultivations which showed a decreased sensitivity to the compound. The CADA-resistant virus strain was investigated for its sensitivity-resistance profile against a variety of HIV inhibitors and showed a significant reduction (six-fold) in sensitivity to the anti-CD4 monoclonal antibody RPA-T4 as compared to the wild-type counterpart. Determination of amino acid changes in the viral envelope revealed several mutations in the envelope glycoprotein gp120. Interestingly, two amino acid changes occurred at the CD4-binding domain of gp120 and are now subject of further mutagenesis experiments.

Oral Session VI: Other Viruses and Late Breaker Presentations

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Development of a Phosphorodiamidate Morpholino Oligomer Antisense to Ebola Zaire

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Antisense phosphorodiamidate morpholnio oligomers (PMO) were designed to inhibit Ebola Zaire expression of NP, VP35, VP40, VP30, VP24 and L gene products. Reporter plasmids containing a portion of the viral sequences flanking the translation start site for VP35, VP24 and L fused to luciferase were constructed. In vitro translation of RNA from the VP35 reporter construct identified an effective antisense sequence, AVI-4539, with an IC_{50} of approximately 100 nM, which was a noncompetitive inhibitor of VP35 translation. Single dose pharmacokinetic studies indicate AVI-4539 can be administered by either IV or subcutaneous route and is effectively distributed to tissues including liver, kidney and spleen. These tissues accumulate the oligomer at concentrations greater than 500 nM for over 24 h. Analyses of plasma and tissue samples indicate AVI-4539 is not degraded and is cleared primarily in the urine as unchanged oligomer. Survival efficacy has been observed against the mouse adapted Ebola Zaire at doses of 500 ug by either IP or SQ route of administration. The greatest anti-viral efficacy was observed following administration of PMO targeting VP35 and VP24, efficacy targeting L was intermediate and nominal efficacy was observed targeting VP40, VP30 and NP. In vivo survival efficacy was both dose and oligomer sequence dependent. These studies indicate continued evaluation of (PMO) for prophylaxis and treatment of Ebola infection is reasonable.

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Identification and Characterization of Potent Small Molecule Inhibitor of Category A Hemorrhagic Fever New World Arenaviruses

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Among the high threat Category A viruses are four New World Arenaviruses (JunÌn, Machupo, Guanarito and Sabi· viruses) capable of causing severe and often fatal hemorrhagic fever disease in humans. No arenavirus-specific antiviral drugs are currently approved for use in humans. The availability of antiviral drugs directed at these viruses would provide both a strong deterrent against their use as biowarfare agents. Antiviral drugs could be readily stockpiled and can be rapidly deployed in the event of an arenavirus outbreak. Since antiviral drugs are easily administered (oral pill or liquid) and exert their antiviral effect within hours of administration, they will serve to effectively treat diseased patients, protect those suspected of being exposed to the pathogen (post-exposure prophylaxis), and assist in the timely containment of an outbreak. As work with the Category A arenaviruses requires biosafety level-4 (BSL-4) containment, we used a related BSL-2 New World Arenavirus, Tacaribe virus, to develop a high throughput screening (HTS) assay for virus replication. Tacaribe virus is 67–78% identical to JunÌn virus at the amino acid level for all four viral proteins. Approximately 400,000 small molecule compounds were screened using the Tacaribe virus-induced cytopathic effect (CPE) assay. Compounds identified in this screen showed antiviral activity and specificity against not only Tacaribe virus, but also the Category A New World Arenaviruses. Drug resistant variants were isolated, suggesting that these compounds target viral gene products and do not act through cellular toxicity mechanisms. A lead compound, ST-294, has been chosen for drug development. The in vitro antiviral potency and selectivity, mechanism of action, as well as animal pharmacokinetics and efficacy of ST-294 will be presented. This inhibitor represents an important step toward the development of small molecule antiviral drugs for New World Arenaviruses.

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Treatment of Acute Arenaviral Disease with Consensus Interferon-Alpha

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Infection with a growing number of arenaviruses can lead to frequently fatal hemorrhagic fever. Due to the severity of disease and potential for intentional release, identifying effective treatments for infectious diseases of arenaviral origin is of considerable priority to the biodefense mission. In the past, the treatment of arenaviral infections with type I interferons have not yielded favorable results. Here we present evidence that interferon alfacon-1 (Infergen™), a non-naturally bioengineered consensus interferon that contains the most frequently occurring amino acids among the non-allelic interferon-alpha subtypes, is active against Pichinde and Tacaribe arenaviruses in cell culture. In the hamster model of Pichinde virus (PCV) infection, interferon alfacon-1 treatment significantly protected animals from death, prolonged

the survival of those that eventually died, reduced virus titers, and limited liver damage characteristic of PCV-induced disease. Further, treatment was still effective when initiated as late as 2 days post-virus challenge, demonstrating potential applicability as a therapeutic agent. Despite the observed beneficial activity of interferon alfacon-1 in the PCV hamster model, efforts to stimulate protective immunity with the known interferon-inducer, Ampligen® (poly I: poly C(12)U), offered only limited protection. These data are the first report demonstrating efficacious treatment of acute arenaviral disease with interferon-based drugs and suggest that the increased potency of the bio-optimized interferon alfacon-1 molecule may be essential to the observed antiviral effects.

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Inhibiting Effects of PMEG [9-(2-Phosphonylmethoxyethyl)guanine] on the Growth of Human Cervical Carcinoma Xenografts in Athymic Nude Mice

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At present more than 100 human papillomavirus (HPV) genotypes have been described and each shows a cutaneous or mucosal tropism. There is a strong association between infection with specific genital viruses (i.e. types 16 and 18) and the development of cervical cancer. In vitro studies have shown that, as has been noted for cidofovir (CDV), the mechanism of cell death following treatment with PMEG appeared to be induction of apoptosis, with accumulation of cells in the *S*-phase of the cell cycle. Here we describe the effects of PMEG and CDV on the growth of cervical carcinoma [SiHa (HPV-16 positive) and HeLa (HPV-18 positive)] xenografts in athymic-nude mice. Six-week old mice were injected subcutaneously with $2–5 \times 10^7$ SiHa or HeLa cells. Once tumors were established, the mice were injected with 50 μl of PBS (placebo) or a solution of 10 mg/ml CDV, 0.1 mg/ml or 0.5 mg/ml PMEG or 10 mg/ml AraC (cytarabine) at the tumor site five times per week for a total of five weeks. Tumor size was recorded weekly in each group of mice (five animals per group). After five weeks of treatment, a significant reduction in tumor size was observed in PMEG- and CDV-treated groups, compared to either untreated or placebo-treated animals, without signs of toxicity. Tumor size increased by 6.2- and 5.4-fold, respectively, in untreated and placebo-treated SiHa xenografts, compared to 0.89-, 0.64- and 3.59-fold for CDV-treated, 0.5 mg/ml PMEG-, and 0.1 mg/ml PMEG-treated mice, respectively. Similarly, a striking decrease in tumor growth was noted in PMEG- and CDV-treated HeLa xenografts: tumor size increased by 3.5-, 2.9-, 0.75-, 0.13-, 1.55-fold for control, placebo-, CDV-, 0.5 mg/ml PMEG- and 0.1 mg/ml PMEG-treated groups, respectively. In contrast, treatment with AraC did not reduce tumor growth; the tumor size increased 5.64- and 3.22-fold, respectively, for SiHa and HeLa xenografts in mice treated with a solution 10 mg/ml AraC. In conclusion, we have shown that in athymic-nude mice both CDV and PMEG inhibited the growth of human cervical carcinoma xenografts (which harbor integrated HPV-16 or HPV-18).

Poster Session I: Retroviruses, Hepatitis Viruses, Respiratory Viruses, West Nile Virus, Virological Methods

Retroviruses

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Multi-targeting the Entrance Door to Block HIV-1 by Aminoglycoside–Arginine Conjugates (AACs)

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The multi-step nature of HIV-1 entry provides multi-site targeting at the entrance door of HIV-1 to cells. Using one drug that can target multiple sites and/or steps in the viral life cycle will have obvious advantages in clinical use. The AACs represent a new class of compounds that may serve as lead compounds for the development of multi-target HIV-1 entry inhibitors (Litovchick et al., 2000; Litovchick et al., 2001; Borkow et al., 2003; Borkow et al., 2003; Lapidot et al., in press), as they serve as CXCR4 antagonists and as fusion inhibitors. The most potent AACs is hexa-arginine neomycin B (NeoR6). NeoR6 competes with gp120 and SDF-1É—binding to CXCR4. AACs interact with CXCR4 selectively without affecting SDF-1É—VCXCR4 natural activity, in contrast to other coreceptor inhibitors. NeoR6 resistant (NeoR6res) viral isolates contain mutations in the gp120 (in C3, C4 and V4) and gp41 (in HR2) envelope subunits. Mutations in C4 and in HR2 are associated with changes of non-polar to polar amino acid residues, which apparently are important to the antiviral activity of NeoR6. The mutations in gp120 and gp41 in NeoR6res isolates are found in amino acid residues that differ from those described in viral isolates resistant to other HIV-1 inhibitors of gp120–CXCR4 interaction. This, taken together with (i) the competition of AACs with monoclonal antibodies to CXCR4, (ii) competition of AACs with gp120 binding to CXCR4, and (iii) being the NeoR6res isolates as sensitive as the wt virus to SDF-1é—én, the natural ligand of CXCR4, strongly suggest that AACs obstruct HIV-1 replication by interfering with the fusion step. The fusion step is dependant of both conformational changes in gp120 following CD4 and CXCR4 interaction, as well as by conformational changes in gp41 induced by HR1 and HR2 interaction. NeoR6 also inhibits gp120-induced death of human neuroblastoma cells in vitro (Catani et al., 2003). The plausible mechanism of NeoR6 neuroprotection is via blockade of gp120–CXCR4 interaction. Taken together, our data support the notion that different AACs exert their antiviral activity via different mechanisms.

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Alkoxyalkyl Esters of (*S*)-HPMPA are Potent Inhibitors of HIV-1 In Vitro

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(*S*)-9-[3-hydroxy-2-(phosphonomethoxy)propyl]adenine, (*S*)-HPMPA, is an effective broad spectrum antiviral against many DNA viruses including herpes simplex virus, human cytomegalovirus, human adenovirus and various orthopoxviruses. However, (*S*)-HPMPA has been reported to be essentially inactive against HIV-1 and retroviruses. We reported previously that long chain alkoxyalkyl esters of nucleoside phosphonates, such as cidofovir, show significantly increased antiviral activity against double stranded DNA viruses. We prepared alkoxyalkyl esters of (*S*)-HPMPA and (*R*)-HPMPA and now report their activity in MT-2 cells infected with HIV-1_{LAI} using a p24 reduction assay. To compare the increased antiviral effect with (*S*)-HPMPA or (*R*)-HPMPA, we synthesized the two enantiomers and prepared lipid esters, hexadecyloxypropyl-HPMPA (HDP-HPMPA), octadecyloxyethyl-HPMPA (ODE-HPMPA), oleyloxyethyl-HPMPA (OLE-HPMPA) and oleyloxypropyl-HPMPA (OLP-HPMPA). Both unmodified (*S*)-HPMPA and (*R*)-HPMPA were essentially inactive as reported previously with EC_{50} values >10 μM. However, the alkoxyalkyl esters of the (*S*) enantiomer were highly active against HIV-1 and all exhibited submicromolar EC_{50}s. Although the alkoxyalkyl esters of the (*R*) enantiomers of HPMPA were considerably less active, they still exhibited substantially increased antiviral activity compared with unmodified (*R*)-HPMPA. Alkoxyalkyl esters of (*S*)-HPMPA are orally active against orthopoxviruses in mice and are worthy of further evaluation as a possible therapy for HIV-1 infection in man.

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External Qi and Qi Water of Yan Xin Life Science Technology (YXLST) Potently Inhibit HIV-1 Replication

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Background: Case-based studies have shown that external Qi (Qi) and external Qi Water (Qi Water) of Yan Xin Life Science Technology (YXLST) exhibited significant effects on reducing viral load and improving immune functions of AIDS patients but had no observed side effects. To explore the potential application of Qi and Qi Water of YXLST as new therapeutic agents for HIV-1 infection, we examined the effects of Qi and Qi Water on HIV-1 replication and the underlying molecular mechanisms.

Methods: Human PBMC were infected with X4 (HIVNL4-3), R5 (HIV-ADA) and X4R5 (HIV-89.6) viruses, respectively, and cultured in the presence or absence of the Qi or Qi Water. For HIV-1 latently infected U1 cells, PMA treated cells were cultured in the presence or absence of Qi or Qi Water. HIV-1 production in cell culture supernatants was measured by reverse transcriptase (RT) assay. To test possible effect of Qi and Qi Water on HIV-1 LTR activity, Jurkat cells co-transfected with pHIV-LTR-Luc and pCMV-Tat plasmids were cultured in the presence or absence of the Qi or Qi Water, and cell lysate was prepared for luciferase assay at 48 h after transfection.

Results: The Qi or Qi Water of YXLST can potently inhibit viral replication of X4 and R5 HIV-1 in PBMC, and dramatically suppress HIV-1 production from PMA treated U1 cells. Moreover, the Qi or Qi Water potently inhibited Tat-mediated HIV-1 LTR activity in Jurkat cells. However, there was no significant cytotoxicity to the cells treated either with the Qi or Qi Water.

Conclusions: Our data suggest that the external Qi and the Qi Water of YXLST may have potential to become novel therapeutic agents for HIV-1 infection.

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Potent and Selective Inhibition of HIV-1 Transcription by a Novel Naphthalene Derivative

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In search for effective HIV-1 transcription inhibitors, we have evaluated more than 100,000 compounds for their inhibitory effects on TNF-α-induced HIV-1 long terminal repeat (LTR)-driven reporter gene expression and identified JTK-101, a novel naphthalene derivative. This compound could suppress HI1-1 production from proviral DNA-integrated latently and chronically infected cells at very lower concentration. Its 50% effective concentration (EC_{50}) for the TNF-α-induced HIV-1 expression in latently infected cells (OM-10.1) and constitutive viral production in chronically infected cells (MOLT-4/IIIB) were 0.0014 and 0.0057 μM, respectively. JTK-101 did not affect the viability and proliferation of these cells at these concentrations, and its 50% cytotoxic concentrations (CC50) were 3.8 μM for OM-10.1 and 1.3 μM for MOLT-4/IIIB cells. JTK-101 selectively suppressed TNF-α-induced-HIV-1 mRNA synthesis in OM-10.1 cells in a dose-dependent fashion, as determined by quantitative RT-PCR analysis. More than 50% of viral mRNA synthesis was achieved with JTK-101 even at 0.001 μM. However, in acutely infected cell cultures, such as peripheral blood mononuclear cells (PBMCs), the antiviral activity of JTK-101 was found to be diminished. These results suggest that JTK-101 inhibits HIV-1 replication through the suppression of viral gene expression, especially at the transcription level.

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Exploring a New Approach in AIDS Therapy. Design, Synthesis and Biological Evaluation of Potential Dimerization Inhibitors of HIV-1 Reverse Transcriptase

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Reverse transcriptase (RT) represents one of the main targets in the development of chemotherapy against HIV. The active form of the enzyme is a heterodimer formed by two subunits (p66 and p51). Dimerization of RT is a prerequisite for enzymatic activity, therefore, interference with this process could constitute an additional interesting strategy to the conventional RT inhibition.

TSAO derivatives (a peculiar NNRTIs family discovered in our group) are the first example of a small non-peptidic molecule that can interfere with the dimerization process upon binding to the β7–β8 loop region of the p51 subunit. Two residues of this loop seem particularly important for enzyme stability (and thereby enzyme activity), the highly conserved Asn-B136 and the Glu-B138 (key interaction residue of TSAO derivatives with the enzyme).

Here, we present the design, synthesis and biological evaluation of tripeptide derivatives that may mimick the key interactions of Asn-B136 and Glu-B138 with the p66 subunit. As N-terminal residue an Asn residue was chosen. As C-terminal residue an amino acid of aromatic nature (Phe, Tyr) to interact by stacking with the Tyr-A181 of the p66 subunit, was used. As central residue a D-Pro or more flexible residues such as Gly or Ala have been introduced. The designed peptides were prepared with different N-/C-terminal capping groups in order to increase their stability and lipophylicity. The compounds synthesized were evaluated for their inhibitory effect against HIV-1 and HIV-2 replication in cell-culture. Preliminary results showed that some derivatives were endowed with moderate anti-HIV-1 activity in the micromolar range. Our studies suggest that

both the Asn and the D-Pro residues are important structural components for the observed antiviral activity.

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"Borano-nucleotides" as Molecular Tools to Circumvent Nucleosidic Drugs Resistance of HIV-1 RT

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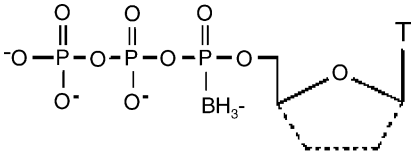
Reverse transcriptase (RT) of HIV-1 virus is a critical target for antiviral chemotherapies because of its essential rule in the replication process. Nucleosidic inhibitors are the first class of antiviral compounds that have shown efficacy against HIV-1 RT. Unfortunately, anti-HIV chemotherapy is limited by the appearance of drug-resistant viruses (mutations map to the viral pol gene encoding the RT). The abundance of 'multi-drug-resistant' (MDR) viruses is one of the major problem of many treated patients for which therapy is ineffective. A new generation of drugs is urgently needed.

Therapeutic research is actively ongoing to design and synthesize new analogues active against MDR viruses, showed better antiviral efficacy and better pharmacologic properties. In 2000, we have found new analogues showing excellent properties of resistance and multi-resistance suppression: alpha-borano-phosphates (Figure).

Enzymatic studies realized on several alpha-borano derivatives and several RTs have shown a better efficacy of phosphorylation by cellular kinases (NDPK) of these compounds than unmodified analogues, a better inhibition of RT than unmodified analogues due to the increase of efficiency of incorporation into DNA, no discrimination compared to natural nucleotides and a decrease or complete suppression of resistance. More recently, alpha-borano-nucleotide analogues have been found to increase the catalytic rate of phosphodiester bond formation independently from substitutions in RT and the nucleotide analogue used.

At this time, it is the first example of HIV-1 RT resistance suppression found with nucleotide analogues. Following up this work, we have synthesized two new and original series of 'borano-nucleotides'.



AZT, d4T, 3TC, dT, ddA

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Dimerization Inhibitors of Wild-type and Mutated HIV-1 Proteases: A Pathway to Circumvent Resistances to Classical Antiproteases

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Cross resistances to current antiproteases seriously compromise their efficiency in AIDS treatments. Remarkably, the antiparallel β-sheet formed by interdigitation of N- and C-terminal strands of each protease monomer which contributes over 75% to the stabilizing force of the dimer, is found relatively free of mutations. By targeting this highly conserved dimerization interface, we demonstrated that HIV-1 protease dimer is disrupted with loss of activity by lipopeptides (K_{id} = 5 nM; Dumond et al., 2003, guanidinium-based molecules (K_{id} = 150 nM; Brecciaet al., 2003) and constrained molecular tongs (K_{id} = 80 nM; Merabet et al., 2004). For the latter molecules, two peptidic strands are cross-linked by a rigid naphthalene or quinoline spacer (Figure). In order to create proteolysis resistant inhibitors, one peptidic strand has been replaced by a pseudopeptide. The activity of several molecules against recombinant proteases displaying mutations found in resistant variants was also analyzed. The results indicate that the antidimer strategy using low-molecular-weight molecules may provide a novel therapeutic approach.

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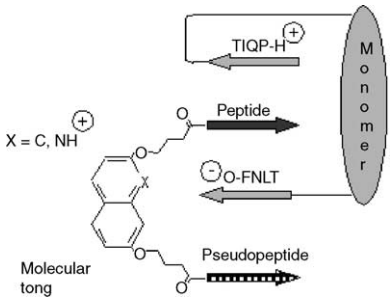

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Inhibitors of HIV Integrase: New Diketo Structures with Heterocyclic Scaffolds

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The pol gene of HIV encodes three key viral enzymes that are essential for the replication of this virus. The discovery and development of clinically useful inhibitors of two of these enzymes, HIV reverse transcriptase and HIV protease, for the treatment of AIDS over the last two decades have been remarkable. However, the third enzyme of the pol gene, HIV integrase, has received much less attention. There are no drugs in clinical use for HIV/AIDS where the mechanism of action is inhibition of HIV integrase. The biochemical mechanism of integration of HIV DNA into the host cell genome occurs by a specifically ordered sequence of DNA tailoring (3ÑS-processing) and coupling (strand transfer and integration) reactions. A number of compounds with considerable structural diversity have been reported in recent years as inhibitors of integrase. Among them are some nuclease-stable dinucleotides reported by us and non-nucleoside compounds that bear the 2,4-dioxobutanoic acid functionality. With respect to the latter class, almost all of the compounds investigated appear to show selectivity of inhibition for the 3ÑS-processing step. We have designed and synthesized diketo structures with heterocyclic scaffolds that are potent inhibitors of both the 3ÑS-processing and the strand transfer steps of HIV-1 integrase. This presentation will discuss our approach to designing these compounds, the methodology used for their synthesis, details of their structure and conformation and their inhibitory data involving HIV-1 integrase.

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Unusual Tricyclic Nucleosides Derived From TSAO-T With Activity Against Human Immunodeficiency Virus Type-1

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Recently, we reported the efficient and stereoselective synthesis of new bicyclic nucleosides using a common cyclic enamine **1** as the starting material. This compound was easily prepared by reaction of 5′-*O*-Tosyl TSAO-T under basic nonnucleophilic conditions. In addition, the reaction of **1** with ethanol gave a tricyclic nucleoside **2** resulting from the nucleophilic attack of the alcohol to the C-4′ carbon atom. Compounds **1** and **2** were tested for their in vitro inhibitory effects on HIV replication. Compound **2** was found to exhibit a highly specific anti-HIV-1 activity being inactive against HIV-2 and other (retro)viruses.

To establish the structural-activity relationships required for anti-HIV-1 activity, different types of modifications were carried out in the prototype compound **2**. These modifications included the replacement of the ethoxy group at the C-4′ position by hydrogen or some other (thio)alkoxy side-chains of different length and flexibility. We also studied the effect of terminal functionalization in the alkoxy side chain and the role of the TBDMS group at the 2′ position.

The best anti HIV-1 activity was achieved with those compounds bearing flexible alkoxy side-chains of medium length that contained a TBDMS group at the 2′ position. The tricyclic nucleosides herein described represent an entirely new class of NNRTI's.

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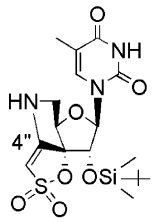

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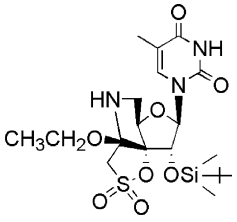

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Synthesis and Quantitative Structure–Activity Relationships of CADA Compounds Having Anti-HIV and CD4 Down-modulation Activities

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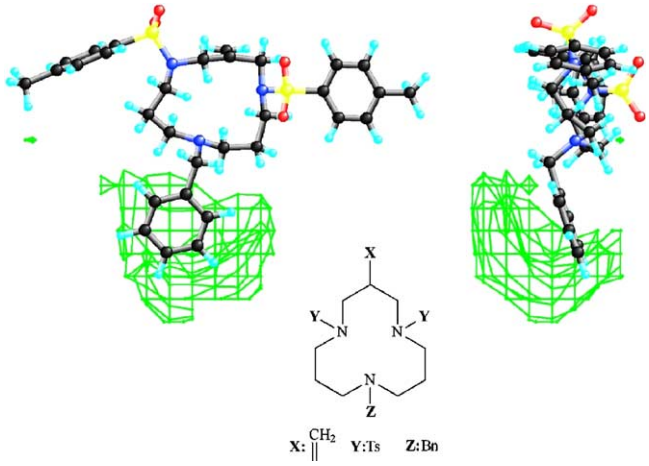
CADA (9-benzyl-3-methylene-1,5-di-*p*-toluenesulfonyl-1,5,9-triazacyclododecane) specifically down-modulates the CD4 receptor on the surface of lymphocytes and monocytes/macrophages, the principal host cell utilized by HIV for replication. Structural modifications of CADA were made to increase potency and reduce cytotoxicity. We report a 3-dimensional quantitative structure–activity relationship (QSAR) study based on the comparative molecular field analysis (CoMFA) of derivatives of CADA. Specifically, a partial-least squares (PLS) analysis was performed with the 50% inhibitory concentrations (IC_{50}) for CD4 down-modulation for CADA analogues to generate a computer model correlating important structural features with potency. For example, an increase in steric bulk in the tail (Z) region of CADA (indicated by the mesh in the Figure, top right, top

left) correlates with higher CD4 down-modulation activity. We also report the synthesis of CADA compounds, including side-arm (Y), tail (Z), and head group (X) analogues (Figure, center). Side-arm variations include benzenesulfonyl groups bearing electron donating and electron withdrawing substituents. Tails consist of various aromatic and aliphatic groups. Head groups include polar substituents and good leaving groups. An alternate synthetic route to CADA analogues involving 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) as an intermediate is also reported. Current studies are aimed at refining the QSAR as well as determining the molecular target of CADA.



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Synthesis and Study of 1-(2′-Deoxy-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide as an Anti-HIV-1 Mutagenic Agent

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In the search of more potent anti-HIV agents, attention has been given to mutagenic nucleoside analogues [L.A. Loeb et al., Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 1492–1497]. By producing a modest increase in the error frequency in the genome of RNA viruses, this class of mutagen agents may drive virus population in an “error catastrophe area” where infectivity and viability are highly reduced.

In order to limit cross-resistance as well as additive toxicity, our group is interested in the study of compounds inhibiting HIV-1 proliferation by original mechanisms. As a part of our current work, we prepared 1-(2′-deoxy-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide **1** (the 2′-deoxygenated analogue of ribavirine) and its 5′-triphosphate derivative **2**. The 1,2,4-triazole-3-carboxamide base was chosen because it is proposed to enable base pairing with the four naturally occurring nucleobases through simple bond rotations. The mutagenic activity of compound 1 on HIV-1 NL4.3 was examined in CEMx174 cell culture and the elongation of several primer/template complexes by HIV-1 RT in the presence of triphosphate **2** was evaluated in vitro.

The chemical synthesis of the title compound and its 5′-triphosphate derivative will be described. The results of biological studies concerning the ability of theses compounds to create mismatches and lethal errors during HIV-1 reverse transcription will be discussed.

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Effect of Non-nucleoside Reverse Transcriptase Inhibitors on the HIV-1 Reverse Transcriptase Associated Ribonuclease H Activity

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Nevirapine and TIBO derivatives are non-nucleoside reverse transcriptase inhibitors (NNRTIs) that had been previously shown to activate the ribonuclease H (RNase H) activity associated to the HIV-1 RT [Gopalakrishnan and Benkovic, J. Biol. Chem. (1994); Palaniappan et al., J. Biol. Chem. (1996)]. In order to explore the physiological correlation between the NNRTI binding pocket and the RNase H active site, we wanted to verify whether this effect was generic for all NNRTIs, irrespectively of their chemical structure and interaction with the NNRTI binding pocket, or whether different NNRTIs could affect the RNase H activity with compound-specific patterns. Therefore, we assessed the effect on the HIV-1 polymerase-independent RNase H activity shown by nevirapine, efavirenz and MC1220, a NNRTI with potential microbicide activity currently under development, which inhibits the HIV-1 RT associated RNA-dependent DNA polymerase activity non-competitively. Results showed that all three NNRTIs activated the polymerase-independent RNase H activity, however the potency of stimulation varied according the NNRTI studied. In particular, a 200% increase in RNase H activity was obtained with 10 μM Efavirenz and MC1220 and with 100 μM nevirapine. Steady-state kinetic studies showed that the presence of the NNRTIs did not vary the K_m values for the RNase H reaction homopolymeric RNA:DNA hybrid substrate whereas they determined a twofold increase in the k_{cat} value for the hydrolysis reaction. When a heteropolymeric hybrid substrate was used in the reaction, results showed that the NNRTIs alter the cleavage specificity by RNase H determining a compound-specific substrate cleavage pattern. These results confirm that the two RT active sites are structurally correlated and indicate that the allosteric interaction between the NNRTIs and the HIV-1 RT determines an inhibitor-specific effect on the HIV-1 RT RNase H associated activity.

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ddNTP Resistance and Fidelity of DNA Synthesis of Ala-114 Mutants of HIV-1 Reverse Transcriptase

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HIV-1 reverse transcriptase (RT) plays a key role in the virus life cycle, as the enzyme responsible for the conversion of the viral genomic RNA into double-stranded DNA that is then integrated into the host cell genome. It lacks proofreading activity and is highly error prone, therefore contributing to the high degree of genetic variability of HIV.

Ala-114 is highly conserved and forms, together with Asp-113, Tyr-115 and Gln-151, the pocket that accommodates the ribose moiety of the incoming dNTP. Amino acid substitutions at these positions are known to be deleterious for RT function (i.e. Asp-113), or influence fidelity of DNA synthesis (i.e. Tyr-115 and Gln-151). In addition, changes at some of those positions confer resistance to dideoxynucleoside analogues used for treatment of HIV infection (i.e. Q151M).

We have obtained a series of mutant RTs with substitutions at position 114 (A114G, A114S, A114V and A114T) in order to define its role in ddNTP resistance as well as in nucleotide recognition and fidelity of DNA synthesis. We found that increasing the volume of the side-chain at position 114 had a deleterious effect on the DNA polymerase activity. Thus, mutants A114V and A114T showed reduced primer extension efficiency.

In comparison with the wild-type RT, mutants A114G and A114S showed decreased susceptibility to AZTTP in DNA polymerization assays with poly(rA)/oligo(dT). However, all enzymes showed similar AZTTP versus dTTP selectivity in assays carried out with heteropolymeric template/primers. Furthermore, all of them showed similar selectivity values for rNTP versus dNTP incorporation, and no differences were found in fidelity of DNA synthesis, as measured by misinsertion and mispair extension fidelity assays or by using *lacZ*-based genetic assays.

The loss of interactions between the methyl group of Ala-114 and the ribose moiety of the incoming dNTP emerge as a major determinant of the predicted resistance to zalcitabine and didanosine displayed by A114G.

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Molecular Mechanism for Suppression of Drug-resistant MMLV Reverse Transcriptase by (α-*P*-borano)-2′,3′-dideoxycytidine-5′-triphosphate

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2′,3′-Dideoxynucleosides (ddNs) are widely used in the clinical treatment of AIDS. They are metabolically activated to the corresponding 2′,3′-dideoxynucleoside triphosphates (ddNTPs), which inhibit the viral DNA synthesis. However, the emergence of HIV-1 reverse transcriptase-dependent drug resistance limits the effectiveness of treatment by ddNs. Enzymatic studies showed that α-*P*-borano-ddNDPs are better substrates for cellular NDP kinase than parent ddNDPs. Further, the α-*P*-borano-ddNTPs are better inhibitors of viral reverse transcriptases and are more resistant to ATP-dependent removal from viral DNA than parent ddNTPs.

In order to obtain structure–activity relationships for discrimination of α-phosphate modified ddNTPs over dNTPs by bacterial DNA polymerases and viral reverse transcriptases, we examined steady-state and pre-steady-state kinetics for incorporation of α-*P*-borano- and α-*P*-thio-ddCTP analogues by two ddNTP-resistant enzymes: Murine Moloney leukemia virus reverse transcriptase (MMLV RT) and *Taq* DNA polymerase.

In pre-steady-state conditions, the α-boranophosphate substitution in ddCTP increases by 33-fold the efficiency for incorporation of the Rp-ddCTPαB isomer by MMLV RT, but slightly decreases the efficiency by *Taq* polymerase. In contrast, the α-thiophosphate modification decreases by 2-fold and 123-fold the efficiency for incorporation by MMLV RT and *Taq* DNA polymerase, respectively. Both modifications alter only rate constant (k_{pol}) values and do not effect the affinity constant (K_d) values. Based on these data we propose a chemical mechanism to explain the differential influence of α-*P*-borano- and α-*P*-thio-phosphate substitution on the drug resistance of MMLV RT and *Taq* DNA polymerase. Selective suppression of drug-resistant viral RT by the α-boranophosphate ddCTP analogue suggests an approach to the design of new antiviral agents.

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A Multi-parametric Assay to Screen and Dissect the Mode of Action of Anti HIV Envelope Drugs

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Most of the assays for HIV envelope (Env) function are based on the measure of post-fusion events. However, prior to induce complete fusion, Env requires the binding of the gp120 subunit to CD4 and coreceptor and gp41 mediated membrane hemifusion. Using flow cytometry, we have simultaneously quantified some of the pre-fusion events associated to Env function. We co-cultured X4 (NL4-3) and R5 (BaL) chronically infected cell lines with unstimulated primary CD4 T cells for 24 h. Primary cells were chosen by its sensitivity to cell surface expressed Env-induced cell death and its inability to replicate HIV. Cellular contacts mediated by gp120 and CD4 were sufficient to induce the transfer of HIV particles from infected to target cells that were measured by staining p24 HIV antigen. Coreceptor expression was necessary to allow for target cell death and cell-to-cell fusion. Death of

single target cells (associated to hemifusion) was measured by changes in cell morphology and cell-to cell fusion was evaluated by calculating the absolute number of single CD4 T cells that disappeared from the culture.

Morphological measure of cell death was validated by comparison to standard techniques (DiOC6 staining) showing a high correlation: $r = 0.95$, $p < 0.001$. The measure of lost cells correlated to the microscopic evaluation of syncytium formation ($r = 0.8$, $p < 0.01$). The analysis of several inhibitors (Leu3a, AMD3100, TAK779 or C34) revealed particular inhibitory profiles for drugs acting at different steps of HIV Env function. All anti HIV Env compounds tested blocked cell-to-cell fusion. However, only drugs targeting the binding of gp120 to CD4 blocked HIV transfer and cell death; while gp41 inhibitors blocked cell death but increased HIV transfer. Coreceptor inhibitors acting after CD4 engagement selectively blocked cell death induced by the appropriate Env and failed to inhibit transfer of HIV particles from infected to uninfected cells.

By combining the use of X4 and R5 HIV envelopes and a multi-parametric analysis, we provide a rapid method to simultaneously evaluate the binding of gp120 to CD4/coreceptor and hemifusion/fusion events mediated by gp41. This analysis may be useful to screen anti HIV envelope drugs and to rapidly identify the mode of action of active compounds.

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RNA Interference of p53 Blocks HIV Replication

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It has been shown that HIV infection leads to the activation of p53 and a cascade of events leading to cell death. P53 expression and activation have been associated to faster HIV disease progression most probably by inducing CD4+ T cell death but also through its cooperative effect in the control of viral gene transcription by viral regulatory proteins. Activation of p53 has also been associated to HIV envelope-dependent death of HIV-induced syncytia. We have generated a p53 negative lymphoid CD4+/CXCR4+ cell line (SUP-T1p53−) by the stable expression of a short hairpin RNA (shRNA) targeting the p53 gene. Infection of SUP-T1p53− or cells expressing a shRNA targeting HIV Rev by the HIV-1 NL4-3 strain was significantly reduced as compared to control SUP-T1 cells. Conversely, interference of p53 or expression of the shRNA targeting Rev did not alter cell viability of proliferation. The effect of silencing p53 on HIV replication was confirmed in single round replication experiments in which SUP-T1p53− are infected with a luciferase-expressing HIV chimera.

Syncytium formation in co-cultures of persistently infected HIV-1 cells with SUP-T1p53− cells was not significantly altered as compared to co-cultures with control SUP-T1 cells. Pseudotype HIV expressing the VSV envelope (VSVenv-HIV) were used to assess the role of HIV entry in the p53-dependent block of virus replication. Contrary to HIV expressing the HIV envelope (HIVenv-HIV) VSVenv-HIV infection could not be blocked by inhibitors of HIV entry but could be blocked by shRNA targeting Rev. Similarly, replication of both HIVenv-HIV and VSVenv-HIV was blocked in SUP-T1p53− suggesting that p53 plays a role in a post-entry event of virus replication.

In conclusion, our results suggest that p53 expression significantly alters HIV replication in lymphoid cells but it role appears to be independent on HIV-envelope and the HIV entry process.

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Compounds Acting as Virostatic Agents Inhibit Lymphocyte Activation When Tested in the Murine Model of Immunodeficiency Disease (MAIDS)

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Recent clinical data indicates regimens containing drugs that inhibit ribonucleotide reductase (RR) such as hydroxyurea (HU) are effective therapeutic options for HIV-infected patients. Immune activation is responsible for the altered immune pathology present in AIDS; therefore, compounds that inhibit immune activation are now considered as a "novel" approach to treat HIV-infection. Compounds such as HU are now considered as a member of new family of antiviral compounds referred to as "virostatic" which function by inhibiting immune activation in addition to viral replication. In this study we compared antiviral and immune activation effects of HU and two novel polyphenol inhibitors of RR (RRIs), trimidox (3,4,5-trihydroxybenzamidoxime) and didox (3,4-dihydroxyhydroxamic acid) in the presence or absence of didanosine (ddI) in the LP BM5 MuLV retrovirus model (murine AIDS). We evaluated treated viral infected animals for the following parameters: splenomegaly, hypergammaglobulinemia, activated B-splenocytes (CD43+, CD45+), loss of splenic architecture, viral *def* expression, femoral cellularity and hematopoietic progenitor stem cells. The combination of RR inhibitors and ddI were extremely effective (DX > TX > HU) in inhibiting retrovirus-induced disease including viral replication. Toxicity results showed that TX and DX combined with ddI was very well tolerated; however, HU + ddI was associated with myelosuppression. Inhibitors of RR in combination with ddI provided significant protection against retroviral disease in murine AIDS with TX and DX more effective with less myelosuppression than HU when tested with ddI in this model.

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Second Generation Anti-HIV Short Hairpin RNA (Vif shRNAs and Decoy TAR RNAs) to Avoid RNAi-mediated Escape Mutant Phenomenon

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RNA interference (RNAi) silences gene expression through short interfering 19–27 mer double-stranded RNA segments that guide cognate mRNA degradation in sequence-specific fashion (RNA–RNA interaction). However, our and other groups reported that shRNA directed against the viral nef and vif genes induced HIV-1 escape variants. In order to overcome this problem, one should use dual HIV-1 anti-genes to avoid the evolution of escape variants. We hereby report that employing the two mechanistic strategies in a vector system resulted in a novel construct that simultaneously expressed decoy TAR and shRNAs as a single molecule, latter cleaved by the endogenous dicer in the cells into their separate components. The HIV-1 decoy TAR RNA competitively interacts with the tat protein (RNA–protein interaction), to down-regulate the level of HIV-1 gene expression from the LTR promoters. To achieve sustainable suppression of escape mutants, the 3′ end of the shRNA was linked to the 5′ end of the decoy TAR RNA by a (UU) signal for cleavage to warrant the TAR RNA to act as a complement factor (protein–RNA interaction). Sequence analysis revealed mutations in vif-gene of both the vif-shRNA–TAR-RNA and control vif-shRNA alone. On the other hand, the strongly enhanced the synergistic inhibition efficacy on HIV-1 replication ($\geq$98%) and suppresses the shRNA-related escape mutant phenomenon in a long-term culture assay.

This multiple HIV-1 anti-genes could lead to an effective gene therapy strategy to avoid RNAi-mediated escape variants.

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Comparison of Methodology to Assess Fitness and Replication Capacity of Reverse Transcriptase Inhibitor and Protease Inhibitor Resistant Viruses

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The rapid selection of drug-resistant virus strains in HIV-infected patients during antiviral therapy is a primary reason for treatment failure with both nucleoside and nonnucleoside RT inhibitors. Although resistance would appear to be a detrimental outcome of antiviral therapy, it may be possible to select for resistant viruses with reduced replication capacity and/or fitness. Resistance-engendering mutations may yield beneficial therapeutic effects if the selected mutations cause reductions in the rate and/or extent of virus replication. Reduced virus replication may effectively prolong the interval between initial HIV infection and AIDS, allow the immune system to more effectively deal with the virus, and allow more effective therapeutic intervention with other HIV inhibitory agents. Our laboratory has developed assays which allow us to evaluate the relative rate of resistance selection between antiviral compounds, and to compare wild-type and resistant virus strains with regard to their replication kinetics and capacity and their relative fitness, as well as identifying the potential contribution of compensatory changes in the virus which result in enhanced replication of low fitness viruses. These assays employ NL4-3 virus with specific amino acid changes introduced into the reverse transcriptase or protease by site directed mutagenesis. Using competitive viral replication assays, we have shown that virus containing point mutations that confer resistance to nucleoside and nonnucleoside RT inhibitors and protease inhibitors may exhibit reduced or increased rates and extents of virus replication or may exhibit no change in replication. The relative growth potential of the mutant virus was also compared to wild-type virus in the absence and presence of drug selective pressure. The assay compares relative replication capacity in both CEM-SS cells and fresh human primary mononuclear cells. These results demonstrated that drug-induced single and multiple mutations in the reverse transcriptase and protease have both positive and negative effects on the ability of HIV to replicate in human cells. Further evaluation of each mutation, alone or in combination, may prove valuable in designing therapeutic strategies for HIV-infected patients.

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Advanced Preclinical Development of Cyanovirin-N as an Anti-HIV Vaginal Microbicide

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Cyanovirin-N (CV-N) is an 11 kDa protein with potent anti-HIV activity. CV-N was originally discovered in and purified from extracts of the cyanobacterium (blue–green alga) Nostoc ellipsosporum. It has been reported that CV-N is extremely resistant to methods used for physicochemical degradation. CV-N can withstand treatments with denaturants, detergents, organic solvents, and extreme temperatures without significant loss of antiviral activity. Low nanomolar concentrations of CV-N inhibit the replication of all clinical HIV strains evaluated and laboratory strains of HIV-2, SIV and FIV. Moreover, CV-N is active against all subtypes of HIV-1. CV-N binds irreversibly to the HIV surface envelope glyco-

protein gp120, specifically interacting with the high mannose groups. This binding blocks interactions between gp120 and receptors on various target cells, leading to inhibition of attachment and fusion of the virus particle to the target cell. We have expressed recombinant CV-N as inclusion bodies in the cytoplasm of *E. coli*, resulting in monomeric CV-N which binds to gp120 with nanomolar affinity and retains its potent anti-HIV activities in cell-based assays. We have evaluated the antiviral activity of a variety of excipients, alone and in combination with recombinant CV-N, as well as the in vitro activity of formulated drug product. Specifically, recombinant CV-N was formulated in hydroxyethylcellulose (HEC) or a co-polymer and tested in Depo-provera-treated Chinese rhesus macaques. In animals treated with 0.5% CV-N in HEC, no infections were noted up to 4 weeks post-infection as measured by plasma viral RNA. The results suggest that CV-N formulated gel will be an effective vaginal microbicide. In addition, HEC placebo gel was analyzed for in vitro and in vivo effects on safety and efficacy, demonstrating appropriate physical properties, stability as a vaginal gel formulation, and safety for use in the clinical study of investigational microbicides. Finally, the unique properties of CV-N and its potent inactivation of HIV make this protein relevant for development as a vaginal anti-HIV microbicide. Advanced preclinical development is underway.

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Cell-dependent Interference with Viral Transactivation by 6-Aminoquinolone Derivatives

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Quinolone derivatives have been shown to inhibit human immunodeficiency virus (HIV) replication at the transcriptional level. Recently, we published a series of new 6-aminoquinolones that are endowed with stronger anti-HIV activities compared to the formerly reported (fluoro)quinolone derivatives [Tabarrini et al., J. Med. Chem. 47 (2004) 5567–5578]. The compounds completely suppressed tumor necrosis factor alpha (TNF-α)- and phorbol 12-myristate 13-acetate (PMA)-induced HIV-1 expression in the latently HIV-1-infected cell lines OM-10.1 and U1 at subtoxic concentrations. In addition, not only HIV-1 expression but also HIV-1 mRNA production in both cell lines was markedly inhibited in a dose-dependent manner. In the same concentration range, the compounds were able to inhibit TNF-α release from PMA-induced OM-10.1 cells but some induction of the TNF-α production was observed in U1 cells at cytotoxic concentrations. The 6-amino-quinolone derivatives were not only inhibitory to the Tat-mediated transactivation of the HIV-1 LTR promotor, but were also found to interfere in a cell-dependent way with the transactivation process mediated from the CMVIE and the human EF1-α promotor. These findings suggest that the mechanism of action of the 6-aminoquinolone derivatives is not attributable to a specific inhibition of HIV replication. The inhibitory effects of the 6-aminoquinolone derivatives on the transcription transactivation process may explain their broad-spectrum antiviral activities (such as anti-HCMV activity). The wide therapeutic spectrum and the remarkably low cytotoxicity may provide interesting perspectives for the antivirally active quinolones.

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Inhibition of DHBV and HBV Replication by Chlorophyllin

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Despite the existence of efficient vaccines, chronic hepatitis B virus infection continues to be a major public health problem worldwide. Current treatment for chronic hepatitis B relies mainly on the use of interferon alpha and nucleoside analogs such as lamivudine and adefovir dipivoxil. However, limited response, relapse upon withdrawal of treatment and emergence of viral resistance are still the major shortcomings of these treatments. Therefore, the discovery of safe and effective antiviral drugs continues to present considerable challenges. Chlorophyllin is a mixture of sodium–copper salts of a chlorophyll derivative that has been found to be an effective anticarcinogen in both experimental models and clinical trials. Those data suggest that chlorophyllin may serve as a chemopreventive agent for aflatoxin-induced hepatocellular carcinoma. Besides, it lacks any known toxicities. These have prompted us to evaluate the potential of chlorophyllin as an antiviral. In the present study, we used primary duck hepatocyte culture postnatally infected with duck hepatitis B virus (DHBV) and a HBV-producing cell line Hep G2 2.2.15 to study the in vitro antiviral activity of chlorophyllin on duck hepatitis B virus (DHBV) and hepatitis B virus (HBV), respectively. The results revealed that chlorophyllin induced a reduction in extracellular DHBV DNA level with an IC_{50} value (50% inhibitory concentration) of 280 μg/mL. In addition, chlorophyllin was also found to strongly inhibit the secretion of hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg) in Hep G2 2.2.15 cells with IC_{50} of 69.5 μg/mL and 48 μg/mL, respectively. These data indicate that chlorophyllin is a potent antihepadnaviral agent and it is worth to study its efficacy in in vivo animal models.

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A TaqMan PCR Assay Using Degenerate Primers for the Quantitative Detection of Woodchuck Hepatitis Virus DNA of Multiple Genotypes

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Woodchuck hepatitis virus (WHV) is a valuable model system for studies of hepatitis B virus infection. Accurate assessments of WHV viral load are necessary in these studies; however, the sequence variation in WHV isolates generally necessitates the use of blotting-based detection methods. To overcome this problem we have created a real-time TaqMan PCR assay for WHV using degenerate primers with inosine residues employed at the locations of known sequence heterogeneity. This TaqMan assay has a dynamic range of 10 to 10^8 of genomic equivalents (ge) WHV DNA per reaction and the assay is robust and reproducible in the 10^2 to 10^7 ge WHV DNA per reaction range (intra-assay coefficient of variation = <2.0%, inter-assay CV = <2.9%). During our assay validation we cloned and analyzed a series of six naturally occurring virus variants which contained sequence heterogeneity in the TaqMan primer sequence region. We showed that the presence of some of these sequence variations prevented the PCR amplification of the target when regular primer sequences were used, while degenerate primer sequences were able to efficiently amplify all tested sequences equally well.

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New Class of Small Molecule Inhibitors of Hepatitis B Virus Surface Antigen Secretion

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The high levels of hepatitis B surface antigen (HBsAg)-bearing non-infectious particles in the serum of infected individuals are thought to play a role in suppressing hepatitis B virus (HBV)-specific immune response. Current HBV therapeutics do not directly reduce viral antigenemia. Our group has been pursuing strategies and compounds that can reduce HBsAg secretion to either complement existing therapies or serve as research tools. High-throughput screening (HTS) of our own small molecule library of 80,000 drug-like compounds was undertaken to discover novel inhibitors of HBsAg secretion. Using the stably HBV-transfected, human hepatocyte (HepG2)-derived cell line 2.2.15, we developed an HTS-compatible ELISA protocol for the detection of HBsAg in the culture media. As of early December 2004, approximately 30,000 compounds have been screened yielding a primary hit rate of 1.3%. Of these, ~30% have been determined to be toxic. From the remaining hits, a closely-related series of pyridines has emerged, with EC_{50} measurements ranging from 5.0 to 0.5 μM and CC50 measurements >50.0 μM. Molecular weights range from 320 to 360 Da, and clog *P* ranges from 5.8 to 6.2. Nascent structure–activity relationship suggests that a central moiety of the molecules is essential to activity, with an aromatic side group contributing to potency. The series is currently being investigated further for SAR and determination of the mechanism of action.

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Glucosidase Inhibitors Cause the Specific and Prolonged Proteasomal Degradation of the Hepatitis B Virus M and L Glycoproteins

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The secretion of the hepatitis B virus (HBV) large "L" and middle "M" envelope glycoproteins requires proper protein folding and is prevented by inhibitors of the endoplasmic reticular (ER) alpha glucosidases. Using competitive inhibitors of the ER glucosidases, here it is shown that the amount of glycosylated and unglycosylated forms of HBV "L" and "M" proteins are reduced dramatically, in tissue cultures producing HBV envelope glycoproteins. In contrast, the glucosidase inhibitors do not affect the secretion or stability of the HBV "S" protein. Proteasomal degradation pathways mediate the reduction of the "L" and "M" glycoproteins, since lactacystin or epoxomicin, two inhibitors of the proteasome, prevent the degradation. Importantly, this specific degradation occurs for up to 5 days after the removal of glucosidase inhibitor from culture, suggesting a prolonged antiviral affect. Since there is no detectable proteasomal degradation of "L" and "M" in cells with functional glucosidase, the implications of the nearly identical sensitivity of glycosylated and unglycosylated forms of "L" and "M" proteins in cells in which glucosidase is inhibited is surprising and its implications in possible immunotherapy will be discussed.

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Synthesis and In Vitro Anti-HBV and Anti-HCV Activities of Ring-Expanded ("Fat") Nucleobases and Nucleosides Containing the Imidazo[4,5-*e*][1,3]diazepine-4,8-dione Ring System

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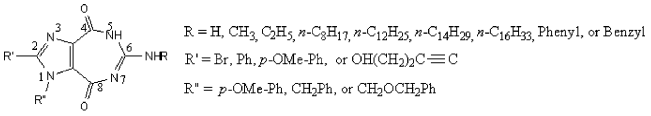
In view of the recent discovery of promising antiviral activities of the ring-expanded ("Fat") nucleobases and nu-

cleosides containing the imidazo[4,5-*e*][1,3]diazepine-4,8-dione ring system (**I**), we have now synthesized and biologically screened a number of heterocyclic and nucleoside derivatives and analogues of **I** as part of our extended structure–activity relationship studies. A number of compounds showed potent activity against the Hepatitis B (HBV) and/or the Hepatitis C (HCV) virus. Synthesis and biological screening results of these compounds will be presented.



I

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Determination of the Precise Mode of Action of Nucleotide Analog Inhibitors of the HCV-NS5B Polymerase

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Several nucleotide analogues have been described as inhibitors of NS5B, the essential viral RNA dependent RNA polymerase of the Hepatitis C virus. However, their precise mode of action remains poorly defined at the molecular level, much like the different steps of de novo initiation of viral RNA synthesis. Here, we show that before elongation, de novo RNA synthesis is made of at least two distinct kinetic phases, the creation of the first phosphodiester bond being the most efficient nucleotide incorporation event. By using different RNA templates, we were able to separate and to biochemically characterize the different steps of RNA replication, namely the initiation and the elongation. This experimental procedure allows the identification of the precise mode of action of nucleotide analogs at a molecular level. As a first example, we have studied 2′-*O*-methyl GTP as an inhibitor of NS5B-directed RNA synthesis. As a nucleotide competitor of GTP in RNA synthesis, 2′-*O*-methyl GTP is able to act as a chain terminator and inhibit RNA synthesis. Relative to GTP, we find that this analogue is strongly discriminated against at the initiation step (∼150-fold) compared to ∼2-fold at the elongation step. Interestingly, discrimination of the 2′-*O*-methyl GTP at initiation is suppressed in a variant NS5B deleted in a sub-domain critical for initiation (the "flap", encompassing aa 443–454), but not in P495L NS5B which shows a selective alteration of transition from initiation to elongation. Our results demonstrate that the conformational change occurring between initiation and elongation is dependent on the allosteric GTP-binding site, and relaxes nucleotide selectivity. RNA elongation may represent the most probable target of 2′-modified nucleotide analogues as it is more permissive to inhibition than initiation.

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Activity of 2′-C-Me-Cytidine Against Hepatitis C Virus Subgenomic Replicons of Different Genotypes

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Worldwide there are more than 170 million people infected with Hepatitis C virus (HCV) resulting in at least 100,000 cases of liver cancer annually. Despite the magnitude of this problem treatment options remain limited. The development of replication competent subgenomic replicon RNAs has been a huge impetus to antiviral drug development. However, the majority of replicons used in antiviral drug discovery efforts are of genotype 1b. While this genotype is one of the most important clinically, worldwide a number of other genotypes contribute significantly to disease burden. Since there can be significant differences between genotype in susceptibility to current treatment regimens we are seeking to improve the utility of our antiviral screening program by employing replicons of genotype 1a and 2a in addition to those of genotype 1b. Here we report the results of studies with the nucleoside analog 2′-C-Me-Cytidine. This compound was previously found to have activity in an HCV 1b subgenomic replicon [Stuyver et al., J. Virol. 77 (2003) 10689–10694]. We evaluated the compound using secreted alkaline phosphatase expressing replicons of multiple genotypes. 2′-C-Me-Cytidine showed excellent activity against all genotypes reducing SEAP production with EC_{50} values in the 1–10 ug/ml range. Activity was confirmed by direct quantification of the effect of treatment on viral RNA abundance by RT-PCR. Studies designed to examine the effect of combination treatments showed a clear additive activity between 2′-C-Me-Cytidine and interferon alpha 2b. Further studies to explore therapeutic interactions with other classes of compounds are underway.

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The Impact of Serum Levels and Gene Polymorphism of Cytokines on Chronic Hepatitis C Infection and Response to Interferon-Ribavirin Therapy

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Chronic Hepatitis C infection has been an important health topic in Taiwan since no effective vaccine are available for protection, and therefore, an early and effective treatment becomes the practicable way for blocking the disease progression to cirrhosis or hepatocellular carcinoma. The current treatment for HCV, a combination therapy with interferon and ribavirin, is often costly and time-consuming but with low efficiency. Numerous predictive factors, including viral and host factors, have been studied for predicting the efficiency. In previous studies, genetic polymorphisms of certain cytokines have been proved to involve in the regulation of cytokine production and additionally, the inappropriate production of certain cytokines appeared to contribute to viral persistence and to affect the antiviral therapy response for Hepatitis C infection. In this study, serum levels and genetic polymorphism of several cytokines in 72 Hepatitis C patients and 180 healthy controls were analyzed by ELISA and PCR-RFLP, respectively. Furthermore, the associations between these parameters and Hepatitis C chronic infection, as well as therapy response were statistically analyzed.. These analyzed SNP of cytokines included TNF-α G238A, TNF-α G308A, IL-4 C589T, IL-10 A1082G, IL-10 T819C and IL-10 A592C. The results showed that the serum levels of certain cytokines, including TNF-α, IL-4 and IL-10, of chronic HCV patients were higher than those of health controls. However, there was no correlation between cytokine serum levels and treatment response. Distribution of TNF-α G308A allele was statistically different between patients and healthy controls ($P = 0.015$), but no significant difference between health controls and patients in genotype or allele distribution of IL-10 and IL-4. In addition, there was no significant correlation between SNP of these cytokine genes and the long-term response to antiviral therapy. Furthermore, we found was no significant correlation between SNP of these cytokine genes and their serum levels.

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Preclinical Evaluation of Two Neutralizing Human Monoclonal Antibodies against HCV: A Potential Treatment to Prevent HCV Re-infection in Liver Transplant Patients

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Background: There is an urgent need to develop effective therapies to prevent re-infection in Hepatitis C virus (HCV) associated liver transplant patients. HCV re-infection post-liver transplantation could be prevented by passive immunotherapy. A combination of several monoclonal antibodies directed against different epitopes may be advantageous for a highly mutating virus such as HCV.

Objectives: To develop human monoclonal antibodies (HMAbs) against the E2 envelope protein of HCV and to test their ability to neutralize the virus and prevent human liver infection using unique in vitro and in vivo systems.

Methods: From a panel of HMAbs, two antibodies, HCV-AB^{XTL}68 and HCV-AB^{XTL}65, recognizing different epitopes on E2, were selected and assessed for their binding characteristics. Their viral neutralization potential was tested in a HCV cell culture system as well as in a mouse animal model for HCV infection (HCV Trimera® Model).

Results: The HMAbs were characterized in vitro biochemically and functionally. Both HMAbs are IgG1 and have affinity constants to E2 in the range of 10^{-11} M. They are able to immunoprecipitate HCV particles from infected patients' sera from diverse genotypes and to stain HCV infected human liver tissue.

The ability to prevent infection of liver cells was demonstrated in vitro in a cell-based assay system for HCV infection in which human hepatoma cell lines were infected with HCV high titer sera. Viral replication was demonstrated by the presence of (+) and (−) strand HCV-RNA in the infected cells. Adding HMAbs to the infectious sera prevented infection of the cell lines.

In vivo, both HMAbs were capable of inhibiting HCV infection of human liver fragments in the HCV Trimera® model as measured by reductions in the mean viral load and in the percentage of HCV-RNA positive mice.

Conclusion: The demonstrated neutralizing activities of two HMAbs against HCV E2, HCV-AB^{XTL}68 and HCV-AB^{XTL} 65, indicate their potential for prevention of re-infection in liver transplant patients.

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Pre-clinical Evaluation of Human Omega Interferon: A Potent Anti-Flaviviridae Virus Antiviral Agent

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Human omega interferon (IFN-ω) is a type I IFN which has been shown to be well-tolerated in man and to induce reductions in the Hepatitis C virus (HCV) RNA levels, which were dose-dependent for genotype 1 and effective at all tested doses for genotypes 2–4 in a series of human clinical trials. Here, we provide an overview of our pre-clinical evaluation of the human IFN-ω produced in CHO cells that is being evaluated clinically. IFN-ω was not associated with any biologically relevant adverse effects in a series of 10 safety pharmacology experiments, in the Ames mutagenicity test, in the micronucleus test or in intra-arterial, intravenous, paravenous or subcutaneous local tolerance studies conducted in rabbits or rats. Acute, subacute, subchronic and reproductive toxicity studies performed in cynomolgus monkeys and rats were unremarkable, with a toxicity profile similar to that of human IFN-α. Except for the acute (single-dose) toxicology study, all of the other toxicity studies also showed evidence for the formation of anti-IFN-ω antibodies over time in the animals. These antibodies were found to neutralize IFN-ω antiviral activity in vitro in a dose-dependent manner. The average pharmacokinetic parameters following a single dose of IFN- ω in rabbits, rats and monkeys were determined. The antiviral activity of human IFN-α, -β, -γ and -ω was assessed in a series of in vitro inhibition of cytopathic effects assays using bovine viral diarrhea virus (BVDV), yellow fever virus and West Nile virus. IFN-ω was the most potent of all the tested IFNs against these viruses. Drug–drug combination analysis was performed using IFN-ω and ribavirin against BVDV which is a commonly-employed surrogate model of HCV replication. A statistically significant synergy of antiviral effects between the two drugs was found, as well as a significant antagonism of the cytotoxic effects of ribavirin by IFN-ω. A clinical trial examining the effect of IFN-ω by daily subcutaneous injection and ribavirin has been initiated.

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Synergistic Inhibition of Flaviridae Virus by Celgosivir in Combination with Ribavirin or Interferon-α

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Celgosivir, 6-*O*-butanoyl castanospermine, is currently in clinical trial for the treatment of chronic Hepatitis C infections. Celgosivir targets the host α-glucosidase I that is essential for the replication of some Flaviviridae including Hepatitis C virus (HCV) and bovine viral diarrhea virus (BVDV). A cytopathic protection assay of MDBK (Madin-Darby Bovine Kidney) cells infected with BVDV was used to determine the therapeutic effect of celgosivir when combined with interferon-α or ribavirin. The synergistic levels of antiviral effects were calculated as the synergistic volumes derived from the 95% confidence interval of celgosivir in combination with ribavirin or interferon-α using the MacSynergy II software. In this model, ribavirin demonstrated synergism when combined with interferon-α with an average synergistic volume of 69 μM^2%. Celgosivir in combination with interferon-α or ribavirin demonstrated synergistic antiviral effect with average volumes of 122 μM^2% or 45 μM^2%, respectively. The synergistic interaction of celgosivir with ribavirin or interferon-α suggests that celgosivir can potentiate the efficacy of interferon-α or ribavirin in HCV infections.

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Pharmacokinetics of Celgosivir (MX-3253), a Novel α-Glucosidase-1 Inhibitor, in Loperamide-treated and Diarrhoea-induced Rats

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Celgosivir (MX-3253) is a novel antiviral agent currently in clinical development for the treatment of chronic Hepatitis C virus (HCV) infection. Celgosivir (6-*O*-butanoyl castanospermine) and its active metabolite castanospermine are potent inhibitors of α-glucosidase-1, an enzyme that alters processing of viral glycoproteins. Orally administered celgosivir is well-tolerated in humans producing mainly side effects of flatulence and mild to moderate diarrhea. The purpose of the present study was to determine the effect of the antidiarrhoeal drug loperamide hydrochloride on the pharmacokinetics (PK) of orally administered celgosivir. The effect of diarrhea was also investigated. Male CD rats were orally administered loperamide at 0.35 mg/kg followed by oral dosing of celgosivir at 35 mg/kg. The dosages of loperamide and celgosivir were based on human doses adjusted to total body surface area. Another group of rats was fasted overnight and then administered castor oil (5 mL/kg) to induce diarrhea. These animals were immediately given access to food followed by dosing with celgosivir one hour later. Blood samples were collected over 24 h. Celgosivir PK was investigated by following the plasma concentration of its primary metabolite, castanospermine, using LC/MS. Oral administration of a 35 mg/kg dose of celgosivir to normal rats yielded mean C_{max}, T_{max} and AUC values of 8.8 μg/mL, 0.44 h and 10.5 μg/h/mL, respectively. Comparable results were obtained for celgosivir in loperamide-treated animals, indicating that this antidiarrhoeal agent had no significant effect on the PK of celgosivir. The Cmax and AUC values in

diarrhoea-induced rats were reduced by 54% and 44%, respectively, compared with normal rats. The difference in the Cmax was determined to be statistically significant. In conclusion, the concomitant administration of loperamide had no effect on the PK of celgosivir in normal rats and could be considered a viable treatment option for reducing gastrointestinal effects that may be associated with celgosivir treatment. Since induced-induced rats showed a reduction in castanospermine Cmax and AUC, treatment with loperamide might prevent lowered systemic drug exposure in patients experiencing diarrhea.

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In Vitro Characterization of Celgosivir, a Clinical Stage Compound for the Treatment of Hepatitis C Viral Infections

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Celgosivir (MX-3253), a compound in phase II clinical trials for the treatment of chronic Hepatitis C viral (HCV) infections, targets intracellular α-glucosidase I, an endoplasmic reticulum (ER) enzyme that plays a critical role in viral maturation by initiating the processing of N-linked oligosaccharides of viral envelope glycoproteins. The inhibitory activities of celgosivir and its metabolite, castanospermine, were tested against the HCV-surrogate virus, bovine viral diarrhea virus (BVDV), maintained in Madin-Darby bovine kidney (MDBK) cells at various multiplicities of infection (MOI). Celgosivir and castanospermine had EC_{50} values of 2.1 and 13.0 μM, respectively, for blocking virus release in a single cycle assay at an MOI of ∼1. In a multiple cycle assay, celgosivir blocked BVDV's cytopathic effect with EC_{50} values of 7.2 and 17.2 μM at MOIs of 0.01 and 0.1, respectively. Similarly, castanospermine blocked BVDV's cytopathic effect, with EC_{50} values of 75 μM and 185 μM at MOIs of 0.01 and 0.1, respectively. When BVDV-infected cells were pretreated for 24 h, viral re-growth times (time for $1 \times \log_{10}$ viral growth) were 4, 4, and 8 h post-treatment for 11, 33 and 100 μM of celgosivir, respectively. With 11, 33 and 100 μM of ribavirin, re-growth times were 4, 8, and 16 h, respectively, compared with 2 h for untreated BVDV-infected cells. This suggests that celgosivir targets the late viral replication stage whereas ribavirin targets early replication. Celgosivir and castanospermine showed minimal cytotoxicity (CC50 >1000 μM) when tested against non-infected human hepatocytes. Celgosivir and its metabolite castanospermine exhibit potent anti-BVDV efficacy and low host cell toxicity. These findings confirm the potential of celgosivir as a Hepatitis C viral therapy in humans.

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An Assay for the Biological Testing of Potential Inhibitors for the HCV Helicase, Dengue Virus Helicase and Dengue Virus Helicase/Protease Complex (NS3 Domain)

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Hepatitis C and dengue are enveloped ñ positive-sense RNA viruses. Hepatitis C virus is the major etiological agent of post-transfusion hepatitis worldwide. An estimated 3% of the world's population is infected with HCV according to the World Health Organization. Infection with HCV will most regularly result in chronic hepatitis, which leads to liver cirrhosis, hepatocellular carcinoma and liver failure. Dengue is currently the most important viral disease, transmitted by mosquitoes and afflicting humans worldwide. Clinical symptoms range from mild fevers to a severe hemorrhagic disease. To date, no specific antiviral treatments exist nor are there any vaccines available for either infections. Thus there is an urgent need for new therapies.

The aim of this project is to design and establish an enzymatic assay that will be used to screen for potential inhibitors of the Helicases of the HCV and dengue viruses as well as the Helicase/protease complex of the dengue virus. Helicases are interesting targets for drug design, firstly for their vital function in the viral cell cycle and secondly for the fact that human cells lack helicases capable of unwinding positive sense double stranded RNA. The genes of the HCV Helicase, the dengue virus Helicase and the dengue virus NS3 domain (Helicase and Protease) were incorporated into a pET system expression vector. The vectors carrying the genes were then transformed into *E. coli* cells and the genes were expressed (BL21-pLysS strain). It was determined that both Helicases are produced in the cell without the need for induction. This was confirmed by an expression test with variable concentrations of inducer (0–1 mM IPTG). It was found that the protein was present under all expression systems. However the one induced at 1 mM showed max yield. After induction, the cell suspensions were harvested. SDS–PAGE and His-Tag Western blotting confirmed the existence of the various proteins. Protein isolation was based on the 6(His)-Tags of the three proteins. The proteins were tested for their functionality using specific enzymatic assays.

Respiratory Viruses

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Protective Action of Biologically Active Food Supplement Biotrit C during Experimental Influenza Infection

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Background: Influenza is the most mass acute infection. Influenza viruses also can cause global epidemics of disease, known as pandemics, during which rates of morbidity and mortality from influenza-related complications can increase dramatically. That is why elaboration of the methods and means for its prevention and therapy are extremely important and actual tasks. Some adaptogens, vitamins, biogenic stimulators may increase the resistance of organism to infections. The goal of this research was to study antiviral activity of biologically active food supplement Biotrit C in the experimental influenza infection model.

Methods: Mice from experimental and control groups were infected intranasally with highly pathogenic for mice influenza virus strain A/PR/8/34 (H1N1). The experiment was carried out using six mice for each virus dilution within the range of 10-1 to 10-8. Death of animals was monitored and recorded between day 2 and day 14 after modeling of infection. LD 50 was calculated by Kerber's method modified by I. Ashmarin.

Results: Biotrit C is the biologically active food supplement made of pulverized green sprouts of wheat. It also contains 10% of Vitamin C. It is elaborated and produced by Institute of Stomatology in collaboration with Odessa's Biotechnology Company. Biotrit C in daily dose of 7.2 mg per mouse has shown the protective action during experimental influenza in mice. Animals from experimental group were taking Biotrit C during 2 weeks before being infected, and continued to take it until the end of the experiment. This supplement decreased considerably the death rate of infected animals starting from the 9th day after mice got experimentally infected with influenza infection. Difference of LD50 between control and experimental groups was 1.5 log10 from day 10 till day 14 after the mice got infected.

Conclusions: The results of the present study show evidence on anti-influenza efficacy of Biotrit C. Therefore we believe that inclusion of this biologically active food supplement in a diet in healthy people during epidemic period and in patients with influenza infection is advisable.

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Activities of Oseltamivir and Ribavirin Used Alone or in Combination Against Infections Caused by Mouse-adapted Recent Isolates of Influenza A and B Viruses

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Mouse models have been extensively used for evaluating potential influenza virus inhibitors. The viral strains employed are fairly old, however, and do not represent currently circulating viruses in nature. We developed two new lethal infection models in mice using mouse-adapted influenza A/New Caledonia/20/99 (H1N1) and influenza B/Sichuan/379/99 viruses. Both virus infections were used to study the effects of oral treatment with oseltamivir and ribavirin, used alone or in combination. Oral treatment regimens were twice a day for 5 days starting 4 h before infection in our initial studies. Against influenza A, ribavirin was active at 80, 40, and 20 mg/(kg day), protecting 80–100% of mice from death and reducing lung consolidation. Oseltamivir was similarly effective at 40, 20, and 10 mg/(kg day). When treatments were initiated after virus challenge, delaying treatment with oseltamivir even 1 day caused it to be ineffective. Ribavirin prevented mortality by 50–80% when treatments were delayed 1–4 days after infection. The combination of the two drugs (ribavirin, 40 mg/(kg day); oseltamivir, 20 mg/(kg day)) was no better than ribavirin alone. Ribavirin and oseltamivir showed similar dose-related antiviral activities against influenza B virus infections in mice. Oseltamivir and ribavirin both increased survival significantly when treatments started out to 4 days after infection, but ribavirin was more active (50–80% survival compared to 30–40% survival, when starting treatments on days 2–4 after infection). By varying the doses of each drug that were used in combination (ribavirin, 20, 10, and 5 mg/(kg day); oseltamivir, 5, 2.5, and 1.25 mg/(kg day)) certain drug dosage combinations were superior to either compound used alone as assessed by decreased mortality, lung virus titer, lung score, and lung weight. The activities of the two drugs against these viruses differed from published results with older viruses (such as A/NWS/33 (H1N1) and B/Hong Kong/72) in that oseltamivir was less effective and ribavirin was more active than reported previously.

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Rimantadine Reduces "Oxidative Stress" in Influenza Virus Infected Mice: Is it an Antioxidant?

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The present study was designed to investigate some aspects of the effect of rimantadine on the "oxidative stress" in alveolocytes, isolated from influenza virus infected mice. It was established that supplementation of mice with rimantadine protected the alveolocytes against lipid peroxidation induction in mice experimentally infected with influenza virus A/Aichi/2/68 (H3N2). Two products of lipid peroxidation in cell suspension were determined: malondialdehyd (HPLC analysis), and lipofuscine-like products (spectrofluorimetric analysis). The levels of natural antioxidants Vitamin E and glutathione were detected by HPLC and spectrofluorimetry, respectively. The results showed that influenza virus infection A/Aichi/2/68 (H3N2) was accompanied with a significant increase of the endogenous lipid peroxidation products and a decrease of natural antioxidant Vitamin E and gluthatione in mouse alveolocytes. It was found that rimantadine treatment led to a decrease of the lipid peroxidation products as well as to an increase of the content of both endogenous Vitamin E and glutathione in influenza virus infected mice. In order to elucidate the mechanism of this effect, experiments were carried out with some model systems. The capability of rimantadine to scavenge superoxide radicals (scavenging properties) was studied in a system of xanthine-xanthine oxidase to generate superoxide. The amount of superoxide was measured spectrophotometrically by the NBT-test and by chemiluminesce. The potency of rimantadine to interact with hydroxyl radicals was studied in a system of iron ion-dependent hydroxyl radical formation and was detected by luminol-dependent chemiluminesce. The antioxidant properties of rimantadine were investigated by measurement of induced lipid peroxidation in a Fe2+ and (Fe2+-EDTA) system with an egg liposomal suspension. The results obtained showed that rimantadine does not exert superoxide and hydroxyl radical scavenging properties and its antioxidant-like effect observed in vivo is not a result of its direct action on the processes of lipid peroxidation and/or interaction with antioxidant enzymes. Our findings with model systems do not prove an antioxidant effect of the drug on the processes of lipid peroxidation. Apparently, the observed antioxidant-like effect of rimantadine in vivo is not connected directly with free radical processes in the infected organism.

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Investigation of Anti-influenza Activity Using Hierarchic QSAR Technology on the Base of Simplex Representation of Molecular Structure

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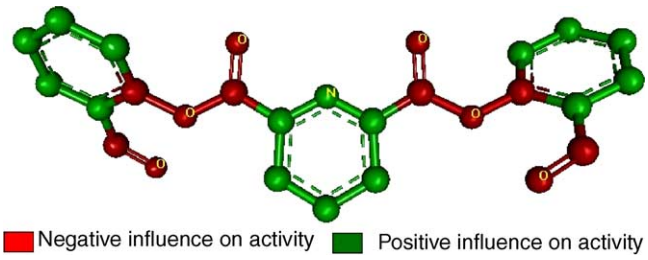
In this research we investigated the influence of molecular structure of macrocyclic pyridinophanes and their analogs on their anti-influenza activity. The hierarchical technology of QSAR models from 1D to 4D based on the simplex representation of molecular structure (SiRMS). The essence of this technology is that the QSAR problem is solved sequentially in a series of the improved models of the description of molecular structure. In the SiRMS approach, a molecule is represented as the system of different simplex descriptors (tetratomic fragments), the level of detailing increases consecutively from 1D (gross-formula) to 4D (ensemble of conformers) representation of molecular structure. The taking into account of different atom characteristics (for example, charge, and lipophilicity, etc) is the principle feature of offered approach.

Anti-influenza activity is expressed in $lgTID_{50}$ and reflected suppression of viral replication in "experimental" samples to "control".

The training set possesses by essential structural variety: different MCP and their acyclic analogues and well-known antiviral agents (deiteforin, remantadine, ribavirine, ambenum and other) of various classes of organic compounds have been represented. The popular 3D QSAR approaches such as CoMFA, HASL can not to be applied for this set. The main reason is that one-valued and optimal alignment of such heterogeneous structures for calculation of molecular fields parameters is impossibility. Such limitation does not exist for simplex representation of molecular structure.

Statistic characteristics for QSAR of Partial Least Squares models are satisfactory ($R^2 = 0.961$; $CVR^2 = 0.641$). The molecular fragments that increase and decrease antiviral activity were defined and will be demonstrated.



This information is useful for design and directed synthesis of novel antiviral agents. Several compounds with predicted high anti-influenza and antiherpetic activities were already synthesized and their activities were confirmed experimentally.

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A Novel Proteinaceous Protease Inhibitor from *Streptomyces chromophuscus* with Antiviral Activity

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A novel proteinaceous protease inhibitor (PISC-2002) was isolated from the culture supernatants of *Streptomyces chromofuscus*. Purification of PISC-2002 was achieved by a procedure including amonium sulfate precipitation, ion exchange chromatography on DEAE Sepharose, reversed phase liquid chromatography (RPLC) and analytical HPLC. Analysis by electrospray mass spectrometry estimated a protein with Mw 11 214 Da. Amino acid analyzes revealed a very rich contents of hydrophobic residues: Ala (16.9%), Gly (10.8%), Leu (9.5%), Pro (9%) and Val (10%). PISC-2002 exhibited a marked anti-influenza virus effect. The reproduction of a range of representative influenza viruses was inhibited significantly by PISC-2002; the virus-induced cytopathic effect, the production of hemagglutinin and infectious virus were all reduced. In a single cycle of viral growth, the penetration of viral particles and the late stages of viral replication were the most susceptible to inhibition. PISC-2002 protected mice from mortality in the experimental influenza A/Aichi/2/68 (H3N2) virus infection.

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Effect of a Plant Polyphenol Extract on Protease and Protease-Inhibitory Activities in Mice Lungs during Experimental Influenza Virus Infection

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Influenza virus infection with A/Aichi/2/68 (H3N2) triggered a reduction of protease activity in mice lungs at 5 and 48 h p.i. and a marked increase at 24 h and 6 days p.i. Protease inhibition in the lungs was reduced at 48 h and a slight rise was observed at 5 h and 6 days p.i. Nasal inoculation of a polyphenol-rich extract, obtained from *Geranium sanguineum* L. (PC) in the dose 10 mg/kg, 3 h before infection, brought both activities to normal levels. PC-treatment of virus-infected mice led to significant decrease of mortality rates (protection index = 77.8%) and distinct prolongation of MST (+5.2 days). Lung lesions were visibly ameliorated as shown by macroscopic and microscopic examination. Lung weights, scores and indices were all reduced. Lung virus titres were also decreased (δ log10 $TCID_{50}$/ml = 1.6–2.4). In intact mice PC caused slight reduction of both protease and antipotease activities 5 h after inoculation. Later no differences with control mice were observed. In vitro PC inhibited the activity of trypsin, pepsin, proteinase K and catepsin in a dose-dependent manner, but was not active toward subtilizin and chimotrypsin.

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The Features of Antiviral Action of Arbidol—Selection and Characterization of Arbidol-resistant Mutants

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An antiviral drug arbidol (1-metyl-2-phenyl-thiomethyl-3-carbotoxy-4-dimetylaminomethyl-5-hydroxy-6-bromo-indolehydrochloride monohydrate) is widely used for prophylaxis and therapy of influenza A and B in Russia. The study of effect of arbidol on viral replication showed that arbidol inhibited viral reproduction of all antigen subtype of human influenza A and B viruses, avian influenza viruses, possessing H5 and H9, and rimantadine-resistant strains of influenza A viruses. Arbidol demonstrated broad-spectrum antiviral activity against respiratory viruses inhibiting RSV and adenovirus type 3 viral replication in cell culture. Arbidol was previously shown to inhibit early stage of influenza A virus replication. The studies of the arbidol effect upon replication of panel of reassortants between A/Singapore/1/57(H2N2) and A/Chiken/Germany/27 (Weybridge strain, H7N7) showed that the greater sensitivity of the Weybridge virus to arbidol was determined by the HA gene; there was no correlation between sensitivity to arbidol and any other gene Arbidol-resistant mutants were obtained by passing viruses in MDCK cells in the presence of increasing drug concentrations. Mutants selected for resistance to arbidol promoted membrane fusion at higher pH (0.2–0.4) than wild-type virus. Arbidol inhibited haemolysis induced by the wild-type virus, but did not inhibit the haemolysis induced by arbidol-resistant mutants. To determine the molecular basic of the arbidol-resistance the HA genes of the wild-type and arbidol-resistant mutants were sequenced. All mutants had amino acid substitutions only in HA2 subunit, but at different positions. The study of the effect of arbidol on conformation of the HA using conformational antibodies showed that arbidol caused conformational change in the structure of HA of wild-type virus, but not in arbidol-resistant mutants. The data indicate that the target

of arbidol is the HA and that arbidol increases its stability to low pH-induced changes and as a consequence inhibits membrane fusion during virus infection.

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Inhibition of Experimentally Induced Influenza Virus Infections by Barrogen, a Potent New Immunostimulant

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Barrogen, a protein from an endemic gut protozoan, *Eimeria* spp., is a potent stimulator of IL-12 release from dendritic cells, and up-regulates IL-12, MCP-1, IL-6, TNF-α, and IFN-γ. It has demonstrable antitumor properties in mice and in man. The material was studied for efficacy against influenza A/NWS/33 (H1N1) virus infections in mice utilizing varying treatment schedules and intraperitoneal (i.p.) and intranasal (i.n.) treatment routes. Barrogen, administered i.p. 4 h post- and 3 days post-virus exposure utilizing dosages of 0.1, 1, and 10 μg per injection, was considered highly effective as seen by prevention of death, lessening arterial oxygen saturation (SaO_2) decline, and inhibiting lung virus titers, the latter occurring relatively late (days 6 and 9) after virus exposure. Treatments administered i.n. or i.n. combined with i.p. were less efficacious than using i.p. treatment only. Similar therapy of influenza A/Victoria/3/75 (H3N2) and B/Sichan/379/99 virus infections in mice indicated Barrogen to also inhibit these infections, although to a lesser extent, probably because of overwhelming viral challenge. No toxicity was seen in toxicity control mice using dosages as high as 500 μg per injection. Studies by others, from cancer trials in mice and in vitro, have indicated the combination treatment with Barrogen and granulated macrophage-colony stimulating factor (GM-CSF) resulted in 100% cures. Such studies prompted a similar study in mice infected with the influenza A (H1N1) virus. GM-CSF was used i.p. at dosages of 20 and 200 ng per injection with Barrogen at a dose of 10 μg per injection. The combination of the higher GM-CSF dose with Barrogen resulted in an increased number of survivors and prolonged mean day to death compared with Barrogen used alone. GM-CSF used alone was not considered inhibitory to the virus infection. These data suggest that the immunostimulator, Barrogen, has potential for the treatment of influenza virus infections.

Acknowledgement: Supported in part by Barros Research Institute and by Contract NO1-AI-15435 from the Virology Branch, NIAID, NIH.

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Inhibition of Influenza A Virus in Cell Culture with Morpholino Oligomers

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Influenza A remains a major worldwide human health problem. Current therapeutics have considerable side-effects and generate drug-resistant virus. Phophorodiamidate morpholino oligomers (PMOs) are nuclease resistant antisense compounds that act by steric blocking of target RNA sequence. Several PMO designed to hybridize to various regions of influenza A H1N1(PR8) RNA were evaluated for their ability to inhibit influenza virus production in Vero cell culture. The PMOs were conjugated to a short arginine-rich peptide in order to facilitate cell uptake. Vero cells were incubated with PMO compounds, inoculated with influenza virus, and viral titer determined by hemagglutinin assay and/or plaque-assay 1–2 days later. A survey of eight antisense sequences revealed that compounds targeting the AUG translation start site of the NP or PB1 segment mRNAs, or the 3′-terminus of their respective vRNAs, were highly effective, reducing the titer of influenza virus by 1–3 orders of magnitude, in a dose-dependent and sequence-specific manner over a period of 2 days. Some combinations of PMOs exhibited a synergistic antiviral effect. The effective anti-influenza A compounds did not alter the titer of the non-homologous influenza B virus grown in Vero cells. These data indicate that further evaluation of several of the PMOs tested in this study as potential influenza A therapeutics is warranted.

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Antiviral Action of the Bis-quarternary Ammonium Bases

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Background: Aethonium and decametoxin are the bis-quarternary ammonium-bases. They are approved in Ukraine as effective antiseptics with cationic surfactant action. They have demonstrated broad antibacterial and antimycotical activities spectrum. The aim of this research was to study antiviral activity of aethonium and decametoxin.

Methods: In vitro antiviral activity of aethonium and decametoxin was studied on the model of influenza virus strains A/Hong Kong/1/68 (H3N2), A/PR/8/34 (H1N1), ¬/Leningrad/17/86 and NDV strain La Sota replication in the tissue culture of 11–14 days chicken embryos' choryoallan-

toic membranes (CAM). Anti-influenza efficacy of intranasal applied preparations was tested in mice infected with virus A/PR/8/34 (H1N1). Action of aethonium and decametoxin on DNA replication of some viruses was researched on the basis of study of these preparations influence on polymerase chain reaction (PCR). Activities were expressed via minimal concentrations in mM of each preparation that inhibited polymerase chain reaction completely.

Results: It was determined that aethonium in dose 125 mkg/ml and decametoxin in dose 25 mkg/ml have inhibited the reproduction of influenza A and B viruses and NDV. Preparations in these doses have shown the virucidal action and have decreased of CAM cells ability to support of viruses replication. Prophylactic and therapeutic application of the preparations considerably decreased mortality of animals infected by influenza virus. Aethonium completely inhibited DNA replication of HSV 1 and 2, CMV, papilloma viruses 16 and 18 in dose 0.167 mM. Decametoxin completely inhibited polymerase chain reaction of the same infectious agents in such doses: HSV 1 and 2 −0.011 mM; CMV 0.167 mM; papilloma viruses 16 and 18 0.011 mM.

Conclusions: The results of the present study show evidence of antiviral efficacy of bis-quarternary ammonium-bases aethonium and decametoxin. Their usage for treatment of viral infections will be preventing bacterial complications thanks to their well-known antiseptic action.

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Natural Inhibitor of Influenza A-Pr8 Extracted from Cinnamon

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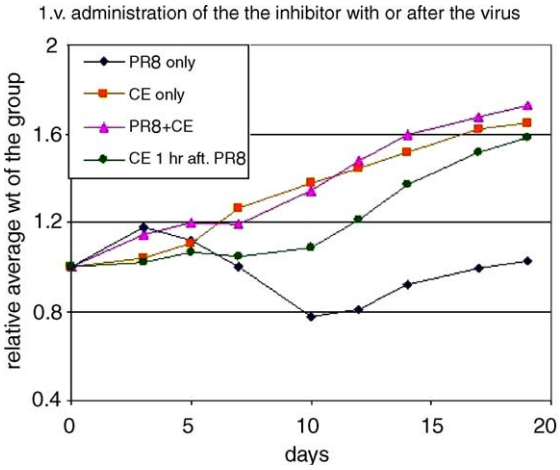
Influenza is one of the most prevalent and significant viral infections. Despite the availability of formalin-inactivated trivalent vaccines, influenza is still associated with significant morbidity and mortality worldwide. Recent estimates have put the cost of influenza epidemics to the economy of the United States alone at 71 and 167 billion dollars per year (W.H.O. Fact Sheet, March 2003). The aim of this research was to investigate cinnamon extract (CE) and its fractions as a novel source for an antiviral inhibitor and to explore its commercial potential. The ability of CE to neutralize the influenza virus was tested both in vitro on human erythrocytes and in vivo in mice. A dose of 125 μg/ml of the crude extract was sufficient to achieve total neutralization of 256 HAU of the virus within one minute. CE has a long shelf-life of at least 2 years in the refrigerator or at room temperature. It still retained its antiviral activity after dialysis in bags with a cut-off of 10 KD or after heating at various temperatures up to 121 °C. The antiviral activity was also stable at a wide range of pH between 1 and 12.5; and even heating at 70 °C at these extreme pHs only partially impaired the antiviral activity. The CE has also proved its ability to inhibit the virus in vivo. When CE was mixed with the virus prior to infection of the mice, or administrated 1 h after the viral infection, the mice did not develop the disease nor did they lose weight or die. In conclusion, CE has exhibited an effective antiviral activity against influenza virus both in vitro and in vivo. The cinnamon has been used in the human diet for thousands of years and should therefore not be an obstacle to introducing the isolated antiviral fraction for human and animal use.



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Comparative Anti-Influenza Rimantadine Efficacy After Oral and Transdermal Administrations

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The influenza infection possess special place among viral infections because it is the most widespread infectious decease. Influenza epidemics hurt human health in great degree, which is why one of the most important purposes of pharmaceutical science is development for its prophylaxis and treatment. One of the most effective anti-influenza drugs is rimantadine, but its oral administration often leads to such side effects as nausea, dizziness, abdominal pain, etc. Reducing of these effects can be approached using transdermal drug delivery. This method of administration provides many advantages, such as prolongation of drug's action, maintaining of its concentration in the therapeutic range and reducing of first-pass liver metabolism.

The aim of this work was comparative evaluation of anti-influenza rimantadine efficacy after its oral and transdermal administrations.

Rimantadine was administered either orally (for prophylaxis once, a day before infection and after virus administration in doses 0.5, 1, 2 and 5 mg/mouse) or transdermally (application of matrix (1 sm^2), containing 0.5, 1, 2, 5 mg/sm^2,

on the shaved back of mice 24 h before infection and 1.2-propylenglycol as permeability enhancer). Experimental infection of mice was carried out with intranasal administrations of highly pathogenic influenza virus strain A/PR/8/64 (H1N1) in dilutions in range from 10^{-1} to 10^{-7}. The animal mortality was recorded for 14 days after infection and the LD_{50} were estimated.

It was shown that transdermal rimantadine administration was more effective for prophylaxis and therapy (LD_{50} difference was from 1.0 to 0.25 in compare to oral administration). Also it was found that transdermally rimantadine effective at doses 0.5 mg/sm^2, though doses higher 2.0 mg/sm^2 did not cause the higher protective action.

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Model of Severe Acute Respiratory Syndrome on Macaca Rhesus

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To create the model of severe acute respiratory syndrome (SARS) on macaca rhesus, Coronavirus (CoV) was detected in different samples coming from six macaca rhesuses in different days by virus isolation, immunoflurescence assay, pathological inspection and reverse transcription polymerase chain reaction (RT-PCR). The results showed that SARS CoV were isolated from the above samples, furthermore SARS CoV RNA could be detected in bloods of the second and fifth day, secreta of nose-throat of the seventh and ninth day, feces of the third day, and feces and urines of the fifth day after infection (Table 1). Under the microscope, it was found in the group infected by SARS CoV that pulmonary alveolus interval had been broadened and had many lymphocytes and monocytes infiltrated (Fig. 1). There was much exudation in the chamber pulmonary alveolus, which even formed the hyaline membrane finally. The organized pneumonia could be found in several pulmonary alveolus. It was also observed great necrosis foci in the liver, companied by some inflammatory cells infiltrated. It may be successful to create the model of SARS on macaca rhesus, and show that the animal model can be used to evaluate anti-SARS CoV drugs and vaccines.

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Summary of the Activity of Antiviral Agents in a Murine SARS-Associated Coronavirus (SARS-CoV) Replication Model

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Severe acute respiratory syndrome (SARS) is a respiratory illness caused by SARS-CoV. It is a life-threatening, highly contagious, febrile respiratory illness that was initially described in early 2003. Treatment for the disease is supportive as there are no approved or universally recommended therapies for SARS. Therefore, compounds found to be active in vitro and several immunomodulators were evaluated for efficacy in a murine SARS-CoV replication model. Partially anesthetized BALB/c mice (10–16 g) were inoculated intranasally (i.n.) with $10^{4.5}$ $CCID_{50}$ virus. Normally, 4 h prior to virus exposure, mice were pretreated i.n. or intraperitoneally (i.p.) with compound using one or more concentrations of drug. After infection, mice were then treated (i.n. or i.p.) with compound (1× per day or bid) for a total of 3 days. Mice were sacrificed on either day 3 (day of maximum lung titer in untreated mice) or on day 7 (day of complete viral clearance from lungs in untreated mice). Net body weight gains/losses were then calculated. Lungs were weighed and scored for lung consolidation and/or for surface inflammation. Lung virus titers were done by cytopathic effect (CPE) assay. For some experiments, cytokine arrays were done on serum and lung samples. The following compounds reduced viral titers by 0.5 $\log_{10}$–1.0 $\log_{10}$: calpain inhibitor VI (i.p. 1 mg/(kg day), 1× per day) and nelfinavir (i.p. 30 mg/(kg day), bid). In contrast, ribavirin, at 75 mg/(kg day) (bid by i.p. or i.n.), promoted virus infection; virus (3 $\log_{10}$) was still present at day 7. However, ribavirin at 20 mg/(kg day) (bid, i.p.) only enhanced virus titers at day 3, although virus was cleared at day 7 as in the placebo-treated animals. Mycophenolic acid and 5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide (EICAR) also seemed to increase virus titers at day 3. The data suggest that some compounds only modestly inhibit viral replication in lungs of BALB/c mice. In contrast, ribavirin appeared to prolong viral infection BALB/c mice and other compounds seemed to increase virus titers [supported by contract no. N01-AI-15435 from the Virology Branch, NIAID, NIH].

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Anti-Coronavirus Activities of Polyoxometalates

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Severe acute respiratory distress syndrome (SARS) emerged in late 2002 in Guandong, China and spread to world wide. By July 2003, almost 8000 patients thought to correspond to the probable cases and it costs the lives of 750 persons. Although there has been no successful antiviral chemotherapy for SARS established, several anti-SARS CoV agents were reported by in vitro examination during 1.5-year abatement of SARS outbreak in the world. By further researches on the SARS CoV replication, mechanisms of virus binding to cells,

fusion between virus infected and uninfected cell membrane, cleavageÅ @ and processing of essential viral proteins, etc. have been disclosed. Polyoxometalate (POM) is negatively charged inorganic compound with core of transitional metal (such as W, V, Ti, Mo) and surrounding oxygen atoms. Some V or Ti substituted polyoxotungstate have shown broad-spectrum anti-RNA virus activities in vitro. The RNA viruses include several human respiratory disease viruses such as influenza A and B, RSV, parainfluenza 2, etc. We examined these compounds for antiviral activity against several coronaviruses (CoV) which cause porcine, bovine, feline diseases and SARS. Some compounds showed common anti-CoV activities against all CoV examined. The inhibition of virus replication occurred at early stage of the virus infection and thought to be inhibition of adsorption or fusion of virus particles to cells. POMs are promising compounds to be developed as broad spectrum anti-respiratory infectious disease drugs.

West Nile and Other Viruses

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Structure Activity Relationship Studies on Biaryl Derivatives with Anti-Picornavirus Activity

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The genera rhino- and enteroviruses of the family picornaviruses consist of a large number of serotypes. Whereas rhinoviruses are estimated to cause approximately one-third of all upper respiratory tract viral infections, enteroviruses may cause severe acute and chronic diseases like aseptic meningitis, encephalitis, hand–foot–mouth disease, and myocarditis as well. Specific antivirals for the treatment of these infections are not available. Until now the most promising compounds discovered are inhibitors of viral adsorption and/or penetration.

During this study, we synthesized a series of biaryl derivatives of pleconaril in order to evaluate the structural features required for anti-rhinovirus and anti-enterovirus activity. These derivatives were tested for cytotoxicity and activity against the pleconaril-resistant coxsackievirus B3 (CVB3) Nancy, the pleconaril-sensitive CVB3 97–927, rhinovirus 2 (HRV-2) and 14 (HRV-14) in cytopathic effect (CPE)-inhibitory assays in HeLa cells. Pleconaril was included as control compound.

Some of the biaryl analogues demonstrated an moderate antiviral effect against CVB3 Nancy and CVB3 97–927. A series of compounds inhibited HRV-2 replication as good as or even better than pleconaril. The results from studies of structure–activity relationship revealed that only compounds containing small substituents like F, CF3, Me, CH=CH_2 in benzene ring showed anti-CVB3 and anti-HRV-2 activity. The antiviral activity was reduced or lost after substitution with 4-Ph, 4-OCF_3, 3,4,5-triMe, as well as 2,3-(1,4)butadiene. Most active compounds have a small electron acceptor group or atom in *para*-position of the phenyl ring. Derivatives with same substituents in *meta*-position or with two substituents in *para*- and *meta*-positions have comparable or some less activity. In contrast, all of the novel synthesised analogues were fully inactive against HRV-14.

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Discovery of Antiviral Agents against RNA Viruses: Correlation with Inhibition of IMPDH

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Our interest in the discovery of molecules with antiviral activity against RNA viruses led us to the design of ribonucleosides with surrogate bases with the intent of using inhibition of inosine monophosphate dehydrogenase (IMPDH) as a probe for antiviral drug discovery. IMPDH catalyzes the conversion of IMP to XMP, utilizing the coenzyme, NAD+, as the hydride acceptor. IMPDH is a key rate-determining enzyme of de novo guanine nucleotide biosynthesis. It has been considered a significant target enzyme for the discovery of therapeutic agents, including antiviral agents. Our molecular design of inhibitors of IMPDH was based on the mechanism of substrate action of IMPDH which involves covalent interaction of the sulfhydryl group of Cys-331 with the C-2 position of IMP. Thus, modification of the C-2 position of purine nucleosides involving Michael acceptors was an important consideration in the design of IMPDH inhibitors. The synthetic work required chemoenzymatic approaches to specific functionalization at the C-2 position of purine nucleosides. The final deprotection step utilized adenosine deaminase which served as an excellent reagent for the clean removal of the masking group. Details of the synthesis will be presented. IMPDH inhibition studies were carried out spectrophotometrically by monitoring the formation of NADH. Antiviral data, which were obtained through collaborative studies, will be presented. Correlation of antiviral activity with IMPDH inhibition will be discussed.

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Antiadenoviral Activity of Plants Compounds

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Activity of helepine, alpizarine and hyporamine against adenoviruses (Ad) type 2 has been studied in the cells culture Hep-

2. Hyporamine shows anti-adenovirus activity in case presence of compound adsorbtion of Ad on cells. Hyporamine in concentration 10 μg/ml decreased the number of infected cells with intra nuclear inclusions on 95% (in control was infected 90% of cells). ED50 of Hyporamine was 5 μg/ml, selectivity index (SI) was 50. In case infection of 50% of cells ED50 was 1 μg/ml, SI-250. Hyporamine antiviral effect was absent when cells were treated before or after adsorption of virus. Antivirus effect of Hyporamine is not connected with virucidality of compounds. It did not reduce virus titer at incubation during 4 h. Hyporamine has not caused any changes in morphology of Ad treated particles and in interaction of them with antibodies to hexone—major protein of capside.

Studying of Ad adsorbtion labeled by 3H-thymidine at presence of Hyporamine has shown that it did not effect virus attachment. Antiviral effect of Hyporamine, probably takes place in the stage of penetrating Ad into a cell, or in the stage of its uncoating.

The impotent peculiarity in antiadenovirus action of Hyporamine is blocking of adenovirus reproduction on the first stages of interaction of the virus with the cell up to the synthesis virus macromolecules.

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Discovery of West Nile Virus Inhibitors

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Multiple members of the Flavivirus genus of the family Flaviviridae cause lethal hemorrhagic fever or encephalitis. Among these Dengue Virus, Yellow Fever Virus, Omsk hemorrhagic fever virus, Kyasanur Forest disease virus infections associated with fulminant hemorrhagic disease; whereas, West Nile virus, Japanese Encephalitis Virus, and tick-borne encephalitis virus cause often-fatal encephalitis. The public health significance of the hemorrhagic fever and encephalitis causing flaviviruses is enormous and global. Each year more than 50 million people are infected with DV, alone. As part of our program to discover and develop new compounds for the treatment of flaviviruses of bioterror concerns, we are evaluating the use of imino sugars against West Nile virus (WNV) in both WNV replicon and live virus infection systems. We utilized an initial screen with another member of the flaviviridae, bovine viral diarrhea virus (BVDV) before analysis in the appropriate WNV model since the imino sugars affect a common host pathway. Using this system we have identified several inhibitors of WNV with distinct mechanisms of action against WNV. The activity of these compounds and mechanisms of action of these compounds will be discussed. In addition, we have started a high-throughput effort to discover novel small molecule compounds that inhibit West Nile virus RNA replication in a cell based replicon assay. Preliminary hits demonstrate great potential for this approach.

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Reactions of Guanidine with Vinylogous Ester–Aldehydes: Synthesis and Anti-West Nile Virus Activity of a Novel Imidazole Nucleoside Containing a Diaminodihydro-*s*-triazine as a Substituent

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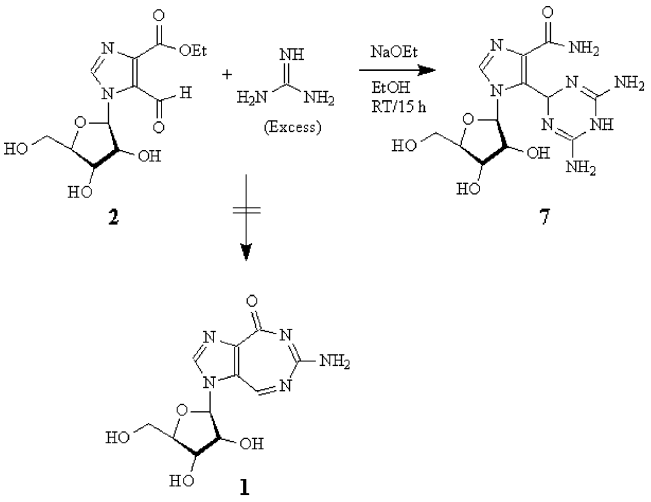
The attempted synthesis of a ring-expanded guanosine (**1**) containing the imidazo[4,5-e][1,3]diazepine ring system by condensation of 1-(2′-deoxy-D-erythropentofuranosyl)-4-ethoxy-carbonylimidazole-5-carbaldehyde (**2**) with guanidine, resulted in the formation of an unexpected product, 1-(2′-deoxy-D-erythropentofuranosyl)-5-(2,4-diamino-3,6-dihydro-1,3,5-triazin-6-yl)imidazole-4-carboxamide (**7**). The structure as well as the pathway of formation of **7** was corroborated by isolation of the intermediate, followed by its conversion to the product. Nucleoside **7** showed promising in vitro antihelicase activity against the West Nile virus with an IC_{50} of 3–10 μg/mL.



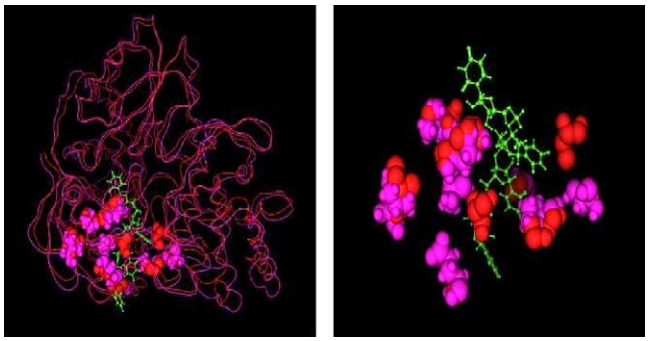


Fig. 1. Superimposition of the HCV and Dengue polymerases. The conserved residues of the active sites of either proteins are in space-fill rendering. Red is the HCV templste polymerase, magenta is the Dengue polymerase model and in green the oligonucleotide (ssRNA).

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Selective Functional Group Transformation: The Conversion of an Ester Group into an Amide or Acid in Vinylogous Ester–Aldehydes Attached to Aromatic or Heterocyclic Rings

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As a corollary to our efforts on the synthesis of analogues of a novel imidazole nucleoside that recently exhibited an excellent in vitro activity against the West Nile virus, an efficient general method has been developed for the selective conversion of an ester group into the corresponding carboxamide or carboxylic acid in a vinylogous ester–aldehyde attached to an aromatic or heterocyclic ring. The method uses excess guanidine, which mediates the observed conversion, while also protecting the aldehyde function as a diaminodihydro-*s*-triazine moiety. A tentative mechanism for the conversions will be presented. The carboxaldehyde group is regenerated by hydrolysis of the triazine moiety to provide either a vinylogous amide–aldehyde or acid–aldehyde as the final product.

Virological Methods

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The Three-Dimensional Structures of the Dengue Virus, West Nile Virus, Japanese Encephalitis and Yellow Fever Polymerase Proteins Predicted by Homology-Based Molecular Modeling

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Both Hepatitis C and Dengue are positive-sense RNA viruses. HCV is the major etiological agent of post-transfusion hepatitis worldwide. According to the World Health Organization, 3% of the world's population is suffering from HCV infection. HCV infection leads to chronic hepatitis that may cause liver cirrhosis and may lead to hepatocellular carcinoma and liver failure. Dengue virus can cause from mild fevers to a severe haemorrhagic disease. Dengue virus is currently considered to be the most important human affecting, mosquito-transmitted disease worldwide. There is no effective cure or means of efficient vaccination against either of the diseases, which makes the need for the development of new drugs and therapies much more urgent. The aim of this project is to design the 3D structure of the polymerase protein primarily of the Dengue virus and consequently those of the West Nile virus, the Japanese encephalitis virus and the Yellow fever virus. HCV polymerase (X-ray) was used as template with a homology identity of approximately 17%. The low homology identity percentage was overcome by performing ligand-supported homology modeling techniques and the conserved residue anchoring approach, which can be implemented into MODELLER.

The viability of the four model-polymerases was evaluated by an in silico scoring function and from the fact that the RNA binding motif and the ATP binding motifs were conserved between the models and the HCV template. Superimposition between HCV and the model-polymerases showed that seven residues of the active site (all involved in the RNA-binding motif) are conserved and yield an overall RMSd between 0.2 and 3.0 Å (Fig. 1).

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The Inadequate Knowledge about Sexually Transmitted Diseases [STDs] and Risky Sexual Behaviour: The Risk Factors for Wild Spread of STDs Among Youth in Developing Countries

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Issues: This abstract shows that the low level of knowledge and risky sexual behaviour of youth are the risk fac-

tors for the high rate of spread of STDs among Nigerian youth.

Description: A self developed validated and reliable questionnaire [$r = 0.77$] was used to collect the data needed for the study and percentage was used to analyze the data. The population of the study was made up of the resident undergraduate/graduate students in male hostels in the Obafemi Awolowo University, Ile-Ife, Osun state, Nigeria. The sample size is 636 selected through simple random sampling technique. The demographic data is as followings: out of 636 respondents, 11 were below 16 years old, 95 were between 16 and 20, 309 were between 21 and 25, and 223, between 26 years and above. Relative risk (RR) calculated is 1.7, i.e. RR > 1, indicating that the factors are risk factors, and the confidential interval (CI) for RR at 95% significant level is 1.61 < 1.7 < RR < CI upper limit. RR and CI are both used to validate the instruments.

Lessons learned: Table 1 revealed that 36.6% responses on STDs knowledge was below average knowledge about the diseases expected from the higher education students. While, Table 2 showed that Nigeria students do engage in one risky sexual behaviour or the other.

Conclusion: It is clearly seen that low level of knowledge and engagement in risky sexual behaviour are the obvious risk factors for the high rate of STDs, not only among Nigerian youth but also in most developing countries.

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Genetic Screen for Monitoring Viral Proteases

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The activity of specific proteases is essential in many fundamental cellular and viral processes. Viral polyprotein processing is indispensable in the replication and maturation of many viruses. Consequently, site-specific proteolysis has been an attractive target for the development of antiviral therapies based on potent and selective viral inhibitors. The generation of such therapies based on the inhibition of site-specific proteolysis has been clearly illustrated in the development of effective inhibitors of human immunodeficiency virus type 1 (HIV-1) and hepatitis C virus (HCV). It has been demonstrated that a bacteriophage lambda based genetic screen can be used to isolate and characterize site-specific proteases. We have previously adapted this system to study the HIV-1 and HCV proteases. This genetic screen system is based on the bacteriophage lambda cI-cro regulatory circuit, in which the encoded repressor cI is specifically cleaved to initiate the lysogenic-to-lytic switch. The inherent difficulties and safety requirements for the ex vivo propagation of severe acute respiratory syndrome (SARS) coronavirus (CoV) (SCoV) prompted us to explore this genetic system as a simple alternative approach for the characterization of SCoV 3C-like protease activity. A specific target for the SCoV 3C-like protease, P1/P2 (SAVLQ/SGFRK), was inserted into the lambda phage cI repressor. The target specificity of the SCoV P1/P2 repressor was evaluated by co-expression of this repressor with a chemically synthesized SCoV 3C-like protease gene construct. Upon infection of *Escherichia coli* cells containing the two plasmids encoding the cI. SCoV P1/P2-cro and the β-galactosidaseñSCoV 3C-like protease constructs, lambda phage replicated up to 2000-fold more efficiently than in cells that did not express the SCoV 3C-like protease. Therefore, this simple and highly specific assay can be used to monitor the activity of proteases with different mechanisms of action, and it has the potential to be used for screening specific inhibitors.

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The Hierarchical QSAR Technology for Effective Virtual Screening and Molecular Design of the Promising Antiviral Compounds

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Drug design and development of new antiviral agents are permanently actual tasks. The use of modern computer technologies could allow solving these problems more effectively.

The hierarchic system of QSAR models from 1D to 4D based on the simplex representation of molecular structure (SiRMS) has been developed. The essence of this system is that the QSAR problem is solved sequentially in a series of the improved models of the description of molecular structure. Thus, on each subsequent stage of a hierarchic system, the QSAR problem is not solved ab ovo, but the information obtained from the previous step will be used. Actually, we deal with a system of solutions defined more exactly.

In the SiRMS approach, a molecule is represented as the system of different simplex descriptors (tetratomic fragments with fixed composition, structure, chirality and symmetry). The level of simplex descriptors detailing increases consecutively from 1D to 4D representation of molecular structure. The taking into account of different atom characteristics (for example, charge, lipophilicity, etc.) is the principle feature of offered approach. It enables to determine easily the fragments of structure both promoting and interfering the given biological activity. Realization of molecular design of the compounds with the given level of activity is possible on the base of SiRMS.

The principle feature of the offered strategy is that not only a hierarchy of models but also hierarchy of purposes are taken into account. Evidently, there cannot be only one model that will solve all of the problems related to the influence of structure of the set of the studied molecules on the

examined property. Hereby, for solving every concrete task, it is necessary to develop the set of different QSAR models, some of which are more suitable for the prognosis of the studied property, the other for interpretation of the obtained relations, and the third for a molecular design. These models all together, in complex, work out problem of creation of new perspective compounds and matters with the given set of properties. The important feature of such approach is that the general results obtained by a few different independent models always are more relevant. Thus, the proposed strategy allows solving all the problems dealing with the virtual screening, modeling of functional (biological) targets, advancing of hypotheses about mechanisms of action, and at last, designing of new compounds with a complex of useful properties. The efficiency of method was demonstrated on the series of macrocyclic pyridinophanes, their analogs and some well-known antiviral agents.

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Screening Program Targeting Viral Enzymes: An Alternate Method To Discover Antiviral Drugs

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Our group is devoted to the understanding of fundamental mechanisms during viral replication. The emergence of new viruses during the last few years (for example SARS) has highlighted the urge to discover new bio-active molecules against viral targets.

The need to speed up the discovery of potential inhibitors as therapeutic agents prompted us to develop a medium-throughput screening program. However, such a technique cannot be easily applied to hazardous and contagious viruses. This problem can be overcome by designing assays directed toward isolated essential viral enzymes, such as polymerases. Our expertise in various fields ranging from enzymology to molecular modeling, and including molecular and structural biology and organic chemistry, enable us to envision each level of the overall project with good confidence.

The different steps of this project include for each target enzyme:

1. the design of a reliable assay on the targeted enzyme;
2. its miniaturization and automatizing to 96-well plate format;
3. the constitution of diversified chemical compound libraries;
4. the storage and analysis of the data gathered;
5. the identification of specific “hits”;
6. the optimization of the obtained “lead” compounds to potential active drugs.

The problems encountered and the proposed solutions, including the development of a new computer software to manage the data, will be presented together with some interesting results.

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Luminescent Microscopy and Fractal Microscopy in Virus–Cell Imaging: A Comparative Study

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Luminescent microscopy is approved and used widely as a standard mean of virus–cell interaction efficacy monitoring. It is necessary to take about 10 million frames to cover the regularly sized substrate (series imaging). Instead, the fractal microscopy proposes fully automated imaging process with full covering of the substrate plate with area of about 100 mm^2 (parallel imaging). We have used the data obtained during the laboratory trial of Acyclovir as anti-herpetic mean. Herpes simplex virus (HSV) of US-1 strain was applied to the Hep-2 substrate with and without the preliminary action of E-aminocaproic acid (E-ACA). It was shown that the best anti-herpetic result was achieved during the combined use of Acyclovir and E-ACA with the minimization of the curative dose of Acyclovir minimally by two times. Diffraction patterns were obtained by optical Fourier transform of the microscopic object used as the filter for stabilized diode laser. The fractograms were obtained both for the data of regular photography, digital photography and direct input of the image in electronic version. The time of HSV (US-1) multiplication was established with the use of regular luminescence microscopy as equal to 34–36 h.

Results: The fractal microscope has allowed us to monitor the influence of E-ACA presence on D values up to the cell toxicity limit. We have registered fractograms with statistically reliable different D values for various stages of virus–cell interaction, such as eclipse, DNA duplication and virus release. The fractal approach used hereby has made it possible to evaluate the minimal size of the fractal cluster structural elements with the size up to submicron values. The structure of the microscopic object as a system of circles with different diameters was restored through the use of the inverse Fourier transform of the diffraction pattern with following image processing. The comparison made during present study has shown that fractal microscope is a powerful laboratory tool for drug design and clinical practice with much better possibilities that the regular microscopy. Present work was supported in part by STCU Grant #3147 and authors are deeply indebted to this Foundation.

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Mathematical Analysis of Fractal Approach to General Cell Stability and Model Virus–Cell Interaction

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The fractal microscope proposed for dynamic system of virus–cell interaction's study, is based on the diffraction. The bright spots of the diffraction pattern (DP) are formed by laser rays diffraction on the transparent points of the system. The fractal dimension D of the DP is equal to that of the object.

We have studied the changes of the fractal dimension D for the system of Herpes simplex virus (HSV) of US-1 strain interacting with cell culture Hep-2. The anti-herpetic mean under investigation was Acyclovir taken in 10^{-4} M concentration. We have modified the cell rigidity with the use of E-aminocaproic acid (E-ACA), being an effective proteolysis inhibitor. We depict the cell stability with the use of exchange coefficient $K = S(\mathrm{c})/V(\mathrm{c})$, where $S(\mathrm{c})$ is the surface of the cell and $V(\mathrm{c})$ its volume.

The data of fractal and regular luminescent microscopy both indicate that HSV radius is about 1/10 of that of Hep-2 cell and that the nuclear inclusions' number is diminished by 75–80% as the result of Acyclovir treatment. The minimal element size was minimized for the times of 10 and 34 h beginning from the infectious start. These times correspond to the stages of virion engulfment and release. It was found that for the stable spherical cell structure $R(\mathrm{c}) = \text{constant}$ and $K = 3/R(\mathrm{c})$. At the stage of engulfment the surface area of the cell is diminished by $S(\mathrm{v}) = 4\pi R^2(\mathrm{v})$ and surface of the virion and the cell volume is increased by the volume of virion $V(\mathrm{v}) = (4\pi/3)R^3(\mathrm{v})$ taking into account the mass conservation law. Assuming the surface tension coefficient σ to be constant, the less possible surface for given volume corresponds to stable structure. The release stage is characterized also by corresponding cell surface and volume decrease.

Proposed mathematical analysis of fractal properties and cell stability parameters shows us that fractal microscope could detect in real time all the changes that occur in the cell during the virus–cell interaction. The authors are grateful to the partial support from STCU Grant #3147 and hope that it will serve for the fractal microscope's wider application to the problems of virus–cell interaction study.

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Structural Genomics on Viral Replicative Proteins: A Tool for Antiviral Drug Discovery

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Drug discovery will increasingly depend on the availability of the three-dimensional structure of the target protein. Indeed, this structure enables modelers to perform in silico drug screening as well as SAR; as it is easier to improve a chemical scaffold on a structural basis rather than on a "blind" approach, binary complexes are used to reveal the actual interactions between the compound of interest and the protein; finally, screening of chemicals can also be performed within protein crystals.

Our laboratory is coordinating the VIZIER (Comparative Structural Genomics of Viral Enzymes Involved in Replication) European project, whose goal is to lay down solid foundations for the rational screening of drugs against a wide range of RNA-based viruses which belongs to three genetically different classes, namely double-stranded and single stranded RNA viruses with positive and negative polarity, dsRNA, ssRNA+ and ssRNA−, respectively. These classes of viruses employ widely different replicative mechanisms driven by poorly characterized replication machineries. Although virus-specific, the latter are the most conserved and essential viral components and, thus, attractive targets for antiviral therapy.

The VIZIER project will perform large-scale RNA virus genome sequencing, predicting potential enzymatically active subdomains from genome sequences, robot-mediated HTP processing the selected targets for the X-ray analysis, and integrating the accumulated knowledge in an effort to screen for inhibitory (antiviral) compounds.

VIZIER will aim at the identification of lead molecules inhibiting the replicative enzymes, but will not enter into the broad field of clinical drug development. Offers of cooperation will be made to the pharmaceutical and biotechnology industry for further drug development, on a contractual basis.

Although at a preliminary stage, a progress report will be presented on clinically relevant emerging pathogens from Flavivirus and Coronavirus families.

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Simple and Rapid Method for the Simultaneous Quantification of Zidovudine and its Monophosphate in Cell Extract by High-Performance Liquid Chromatography

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In the past decade, an increasing number of research groups have focused their attention on mononucleotide prodrug study, namely pronucleotides. Such entities were designed to give rise to the intracellular delivery of 5′-mononucleotide, the later being further metabolized to the corresponding triphosphate analogue in order to exert antiviral activity. To prove that new series of 5′-mononucleotide analogues can act as effective pronucleotides, their decomposition pathway were studied in cell extracts. Thus, we developed and validated a simple and rapid method by high-performance liquid chromatography for the simultaneous quantification of zidovudine (AZT) and its monophosphate (AZTMP).

Without pretreatment, biological samples could directly be injected on an analytical system. Using an ion-pair agent, AZT, AZTMP and an internal standard (IS) were trapped on the cleaning precolumn and the proteins quickly eluted. Thereafter, the precolumn was connected to a reverse-phase analytical column where analytes were separated, and detected using UV detection at 266 nm. This method was validated over the range of 5–125 μg/ml for AZT and AZTMP. Extraction recoveries of the analytes and IS from cells extracts were higher than 95%. This method is currently used to study the decomposition pathway of AZT prodrugs in cell extracts.

Poster Session II: Herpesviruses, Poxviruses, Other Viruses, Prodrugs and New Antivirals

Herpesviruses

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A Recombinant Guinea Pig Cytomegalovirus (GPCMV) Expressing the Human Cytomegalovirus (HCMV) *UL97* Gene Demonstrates Significantly Improved Susceptibility to the Antiviral Agent, Ganciclovir

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Among the cytomegaloviruses (CMVs) of small animals, the guinea pig CMV (GPCMV) is of interest for antiviral research, due to a similar disease pathogenesis to human cytomegalovirus (HCMV), including transplacental transmission. Unfortunately, GPCMV is resistant to the most commonly used HCMV antiviral, ganciclovir (GCV), rendering in vivo study of this agent problematic in this animal model. Using the GPCMV genome cloned as an infectious bacterial artificial chromosome in *E. coli*, we used an inducible recombination system to engineer a series of mutant GPCMVs, including a virus deleted of the GPCMV *UL97* (*GP97*) homolog, and viruses which replaced the native *GP97* gene with the HCMV (Towne strain) *UL97* gene. These studies demonstrated that a *GP97* deletion virus retained replication competence, but was severely attenuated for in vitro replication. Two chimeric viruses which replaced the *GP97* gene with the HCMV *UL97*, in contrast, replicated efficiently in cell culture. Evaluation of the GCV susceptibility profile of wild-type and mutant GPCMVs revealed that replacement of the *GP97* with HCMV *UL97* resulted in substantially improved susceptibility of the chimeric virus to the inhibitory effect of GCV. Plaque reduction assays revealed that the IC_{50} for wild-type (BAC-derived) GPCMV for GCV was approximately 200 μM. In contrast, two independently derived chimeric GPCMVs which expressed HCMV *UL97* were GCV-susceptible, demonstrating an IC_{50} of 15 μM. Northern blot, using HCMV *UL97*-specific probes, was performed. These probes hybridized with RNA purified from reconstituted chimeric viruses, but not wild-type BAC virus. These studies demonstrate the usefulness of employing recombination systems in *E. coli* to generate BAC-derived chimeric GPCMVs expressing HCMV genes. The improved GCV susceptibility profile of the recombinant GPCMV should facilitate the testing of GCV, and, potentially, other antivirals which target *UL97*, in this uniquely useful small animal model.

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A New Family of Non-Nucleoside Inhibitors of Human Cytomegalovirus (HCMV) and Varicella-Zoster Virus (VZV) Based on the β-Keto-γ-Sultone Template

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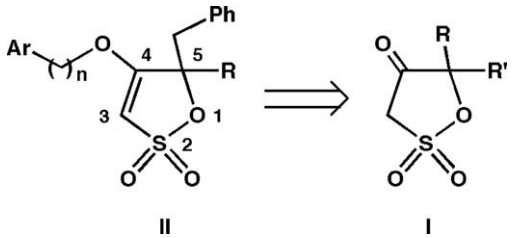
Human herpes viruses cause a variety of clinically significant diseases: HSV-1 (cold sores), HSV-2 (genital herpes), VZV (chicken pox, shingles), and HCMV (retinitis, pneumonitis). The current treatment of these diseases uses nucleoside (acyclovir, ganciclovir, cidofovir) and phosphate (PFA) substrate analogues. Because of the toxicity associated with PFA and ganciclovir, together with the emergence of mutants resistant to acyclovir, there is a need for a new class of antiherpes compounds with novel mechanism of action.

During the course of our search for novel small molecule heterocycles based on the β-keto-γ-sultone template **I**, an antiviral screening program against a panel of viruses in cell culture was initiated. From these tests, we discovered an entirely new class of non-nucleoside inhibitors of general formula **II** which showed a marked activity against HCMV and VZV infection, being inactive against a variety of other DNA and RNA viruses. Structure–activity relationship investigations (SAR) were initially focused on the substituents at the 4- and 5-position of the sultone moiety. These studies showed the importance of two aromatic rings for activity. Substitution on the phenyl ring at the 4-position and the length of the linker have a significant influence on the activity of these compounds. The synthesis, biological evaluation and preliminary SAR studies of this new family of compounds will be described.



II I

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The In Vitro Inhibition of Cytomegalovirus by Novel Ribonucleotide Reductase Inhibitors Didox and Trimidox

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Cytomegalovirus (CMV) infection still contributes significantly to the increase in morbidity and mortality of immunocompromised individuals. Ganciclovir (GCV) has been successful in reducing CMV viral titres; however, mutations in UL97, UL54 genes and point mutations in viral DNA polymerase, have resulted in an increase in the emergence of resistant strains, which has reinvigorated the search for alternative antiviral therapies. In this study, we investigated the efficacy of novel virostatic, which inhibit the enzyme ribonucleotide reductase (RR) that plays a critical role in the de novo synthesis of deoxyribonucleotides. Didox (DX; 3,4-dihydroxybenzohydroxamic acid) and Trimidox (TX; 3,4,5-trihydroxybenzamidoxime) are second-generation ribonucleotide reductase inhibitors. They are polyphenols with a hydroxamic acid or an amidoxic side chain, which inhibit the RR enzyme. We have previously shown the effectiveness of these compounds in inhibiting MAIDS induced perturbations in C57BL/6 mice. In the present study plaque reduction assays were used to determine the effect of antiviral drugs in varying concentrations (IC_{50}, μM) over a 5-day incubation period. The results obtained show: (a) both DX and TX inhibited viral plaque formation in a dose dependent manner; (b) there was no significant difference in plaque reduction between pre and post-treatment compared to post infection treatment alone; c) Antiviral activities GCV > TX > DX, correspond to an IC_{50} of 3.2, 7.1, and 20.7 μM, respectively. Further work is currently under way to examine the antiviral effects and toxicity profile of these drugs in GCV resistant strains of CMV.

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Mechanism of Action against Human Cytomegalovirus of First and Second Generation Methylenecyclopropane Purines

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We have previously described series of methylenecyclopropane purines consisting of first-generation hydroxymethyl compounds [Qiu et al., 1998. J. Med. Chem.] and second-generation bis-hydroxymethyl compounds [Zhou et al., 2004. J. Med. Chem.] that have potent and selective activity against HCMV. Towne strains of HCMV selected separately for resistance to first-generation adenine (synadenol) and guanine (synguanol) analogs were approximately 10–20-fold resistant in plaque and yield reduction assays to several first-generation purine analogs. Similar resistance was observed to the second-generation guanine analog cyclopropavir (IC_{50}'s in plaque reduction assays = 0.35 and 21 μM, respectively for wild-type (wt) and synguanol-resistant virus). Likewise HCMV with a large deletion in UL97 [Prichard et al., 1996. J. Virol.] was resistant to both first and second-generation compounds (IC_{50}'s = 2.1 and 0.25 μM in wt; 100 and 15 μM in $UL97^{del}$, respectively for synguanol and cyclopropavir). In contrast, HCMV with UL27 deleted (Komazin, U. Mich. Ph.D. dissertation) was sensitive to both compounds. UL97 from the HCMV strain selected for resistance to the other first generation compound (synadenol) was sequenced and two mutations were identified: M460I and C603Y. Because HCMV with either M460I or the related C607Y mutation alone was sensitive to synadenol and synguanol [Baldanti et al., 2002. Antiviral Res.], we hypothesize that two mutations are required for resistance to first- and second-generation analogs. This hypothesis is being tested by construction of three strains of HCMV from HCMV AD169 BAC with one, the other, or both mutations in UL97. We conclude that a functional UL97 is required for activity against HCMV and that is likely that two mutations in UL97 are required for resistance to the methylenecyclopropane purines. This study was supported by grants U19-AI31718, P01-AI46390 and R01-CA32779 from N.I.H. and by funds from the University of Michigan.

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Inhibition of Drug-Resistant Human Cytomegalovirus Replication by Kampo (Japanese Herbal) Medicine

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Human cytomegalovirus (HCMV) is a ubiquitous herpesvirus infecting 50–90% of normal adults, depending on geographic location. HCMV infects most individuals early in life and establishes thereafter a lifelong latent infection. However, latently infected HCMV is frequently activated in immunocompromised individuals such as patients with AIDS or organ and bone marrow transplants, thereby causing severe morbidity and eventually mortality. Symptomatic HCMV infection has been successfully treated with ganciclovir (GCV), but the appearance of GCV-resistant virus is a current problem in the treatment of immunocompromised patients with HCMV infection. New or alternative efficacious anti-HCMV agents need to be developed. Several Kampo (Japanese herbal) medicines (KM) are widely used in Japan and China as an effective medication against some disorders of the human body. In order to clarify the interaction between HCMV and KM, we examined the effects of KM on the HCMV replication in human embryonic fibroblast (HEL) cells.

Methods: Monolayered HEL cells were infected with GCV-resistant or -sensitive strains of HCMV and treated with KM. Produced infectious virions and DNA levels of HCMV

were measured by a plaque assay and dot blot hybridization, respectively.

Results and conclusions: Treatment by KM inhibited cytopathic effects of HCMV infected cells and replication, with concomitant decrease in DNA levels. In the meanwhile, KM had no virocidal effects on the cell-free HCMV. We also found that anti-IFN-b antibody recovered KM-induced decrease HCMV replication. These results suggest that KM have a potential value as a source of new powerful compounds against GCV-resistant HCMV.

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Intracellular Localization of Herpes Simplex Virus Type 1 Thymidine Kinase in Virus-Infected Cells

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Intensive studies on HSV-1 thymidine kinase (TK) have been performed in many aspects. The viral TK expressed in cells as fusion proteins with fluorescent proteins by gene-transfection found mainly in the nucleus, but the viral TK in virus-infected cells has been considered locating mainly in the cytoplasm. We have performed intracellular localization of the vTK in virus-infected cells using immunofluorescence confocal microscopy. Cell fixative was an important factor causing different localization profiles. Regardless of viral strains or cell types, the vTK were observed mainly in the nucleus. They were detected in the nucleus of Vero cells from 2 h p.i. to the end of viral growth in one-step growth experiments. Apart from virus amplification imaging the localization of the vTK could be a good tool to confirm cell susceptibility to virus and to check homogeneity of cell population.

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Intracellular Localization of Herpes Simplex Virus Type 1 Thymidine Kinase in Cells Infected with Virus and Treated with Various Antiviral Agents

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Intensive studies on herpes simplex virus type 1 (HSV-1) thymidine kinase (TK) have been performed in many aspects. The intracellular localization of the viral TK using immunofluorescence confocal microscopy was a powerful tool to confirm whether virus entered its host cells. The TK polypeptides were detected in the nucleus of Vero cells from 2 h p.i. to the end of viral growth in one-step growth experiments. We compared the efficacy of various antiviral agents with different mode of action, such as inhibitor of DNA synthesis and inhibitors of virus binding and/or fusion under one-step conditioned experiments. DNA replication inhibitors such as acyclovir, ganciclovir, penciclovir, phosphonoformic acid could not inhibit the expression of the TK gene, but entry inhibitors such as dextran sulfate 8000, pentosan polysulfate and aurintricarboxylic acid could inhibit it. This method might be useful to confirm whether a new antiherpetic agent inhibits early phase of the virus replication cycle and also the homogeneity of cell population susceptible to entry inhibitors.

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Human UMP-CMP Kinase Specificity for Natural and Antiviral Analogs Using Competition Fluorescence Experiments

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While human UMP-CMP kinase structure is recently known from X-ray crystalography of the free monomeric protein (Segura-Pena et al., 2004), the nature of the binding site is still discussed (Hsu et al., 2004). By competition experiments using fluorescent probes (mant-ATP, MABA-CDP, Rudolph et al., 1999), we studied the specificity of several triphosphate ("donor") and monophosphate ("acceptor") nucleosides as well as bisubstrate analogs (Ap5U, for example). Cidofovir, a cytidine phosphonate presenting anti-Herpes and anti-Variola properties, is shown to bind to the acceptor site as well as CMP and dCMP. The relative affinities for the acceptors are compared with the enzymatic reactivities (Deville-Bonne et al., 2003).

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Anti-Herpesvirus Activity of an Extract of Emodin

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Rheum officinale is a medicinal plant grown in the west and south of China. We have reported previously that Rheum palmatum, prepared from the rhizome of Chinese rhubarb (Rheum officinale) have demonstrated good activity against the herpesvirues. To determine the extent of anti-herpes simplex virus (HSV) activity present in a number of plant extracts, emodin extracts were extracted from Rheum officinale. And the quantity of emodin was achieved 83.79% in extracts and it was verified to be emodin monomer by high performance liquid chromatography (HPLC). The anti-HSV effects were investigated by observing cytopathic effect (CPE), adopting MTT colorimetric assay for viable cell rate, detecting HSV-DNA with polymerase chain reaction (PCR) and testing viral titers. We found that the emodin had no antiviral abilities of the directly killing and adsorption blocking. But in the group of anti-biological synthesis, the median inhibitory concentration (IC_{50}) of emodin anti-HSV-1/-2were 1.21 and 1.63 μg/mL, respectively, so the treatment index (TI) were relatively figured out as 2.07 and 1.54 regarding a median toxic concentration (TC50) of 2.51 μg/mL. With the enhancement of the dose, the degree of CPE, viral titers, titers of HSV-DNA in culture media decreased correspondingly, whereas viral inhibition rate increased. The results showed that emodin may inhibit HSV biological synthesis other than directly inactivate these viruses or block their adsorption to susceptible cells in vitro.

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Investigation of Antiherpetic Activity Using Hierarchic QSAR Technology on the Base of Simplex Representation of Molecular Structure

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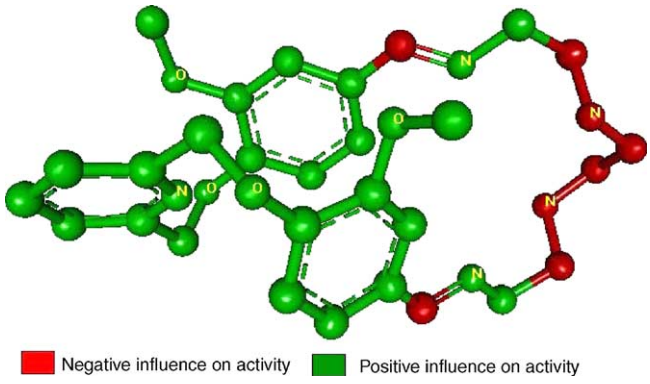
The diseases caused by Herpes simplex virus (HSV) are widely distributed. Prophylaxis and treatment of these infections are the most significant problems of health care. Drug design and development of new medicines directed against HSV are permanently actual tasks. The system of effective drugs' choice based on total screening is non-effective. The use of modern computer technologies could allow solving these problems more effectively.

The hierarchical technology of QSAR models from 1D to 4D based on the simplex representation of molecular structure (SiRMS) has been used for the series of macrocyclic pyridinophanes, their analogs and some well-known antiherpetic agents.

The compounds ability to inhibit herpetical reproduction was estimated on the reduction of the percentage of infected cells in treated cell cultures in comparison.

Statistic characteristics for partial least squares model are satisfactory ($R^2 = 0.867$, $CVR^2 = 0.653$). The molecular fragments that promoting and interfering the antiviral activity were defined.



Using this information structure $C_{24}H_{54}N_{10}$ with high antiherpetic activity was designed, synthesized by us and tested in vitro. Thus, developed hierarchical approach is the effective instrument for design and directed synthesis of novel effective antiherpetic agents.

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Anti-Herpetic Activity of Synthetic Proteolysis Inhibitors and Their Analogues

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Elaboration of the effective anti-herpetic agents and of computer-assisted hierarchical system are permanently of researchers' interest. The basic compounds for antiherpetic are as follows: E-aminocaproic acid (E-ACA), paraaminomethylbenzoic acid (Ambenum) and E-ACA analogs—sodium salt acetylaminocaproic acid (Aceminum) as well as newly synthesized compound 429, containing E-ACA and its sodium as terminal fragments and pyridine

as the "bridge" (-6-[6-carboxy-penthyl-carbomyl-pyridine-2-carbonil]-amino) gexane acid. The compound 429 was designed with the use of 4D QSAR-method. We have studied the anti-herpetic activity against Herpes simple virus (HSV-1) (strain-US) and HSV-2 (strain and BH) titration in the presence of the preparations presented above cultivated on the culture chicken embryons' fibroblasts. The anti-herpetic action of the preparations towards HSV-1 was studied with the use of cytomorphologic method on the cell culture Hep-2. The rate of HSV-like viral reproduction's inhibition was evaluated by the decrease percentage of the cells with specific viral intra-nuclear inclusions. We have registered that E-ACA, taken in concentrations 1000 and 3000 mkg/ml and Ambenum, taken in concentrations 50 and 200 mkg/ml do hinder the HSV reproduction in a statistically reliable in the culture of chicken fibroblasts. Ambenum also hindered the HSV on MDCK cell culture, but its effective dose exceeds the toxic one by 12 times. Aceminum, taken in 1.5% concentration hindered the HSV-1 and HSV-2 reproduction on chicken fibroblasts' cell culture by 2.5 and 2.25 log, correspondingly. Aceminum, taken in concentration of 0.02% has decreased the number on intra-nuclear virus specific inclusions of HSV-1 on cell culture of Hep-2 by 53%. The preparation 429, taken in concentration of 10E-4 M, has decreased the number of intra-nuclear inclusions on cell culture Hep-2 by 60.5%. The same concentration of E-ACA was effective by 45% only. The authors are deeply indebted to the STCU Foundation for the financial support of the present research under the Grant #3147.

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Complex Use of Myramistin and Interferon in Herpetic Stomatitis Treatment

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Acute herpetic stomatitis (AHS) is registered most frequently in the early childhood beginning from the age of 6 months to 3 years and older. It was shown that in every tenth child this disease transforms into the chronic form of recurring herpetic stomatitis (RHS). We have studied both in laboratory and clinic the efficacy of the use of leukocytic interferon (LI) and the protein with low molecular mass with antiviral properties and that of Myramistin and benzyldimethyl [3-myristoilaminopropyl] ammoniumchloridemonohydrate and cationic surface active compound with antiseptic action. The specific herpetic antibodies, which were not detected in the healthy children, were registered in the patients' saliva using the method of fluorescent antibodies. We have made the cytological analysis of material taken from the patients' mouth cavity and the presence of degenerative epithelial cells, simplasts and multinuclear cells in the preparations was shown experimentally.

The first group of AHS patients, contained 28 children, was treated with LI (300 units/ml) applications and subsequent gargling of the mouth cavity four and five times daily. The second group of children (32 persons) was treated with the local applications of Myramistin 2% grease on the hyperemied surface, accompanied with the washing of the mouth cavity with the water solution of Myramistin (100 mkg/ml) as well as the application of the preparation all over the inner surface of the patient's mouth cavity. The third group of AHS patients (30 persons) was treated with the combined therapy of LI and Myramistin.

The most effective has proved to be the combined therapy with the use of LI and Myramistin. The patients of this group has not demonstrated the repetitive eruptions and the tests, taken from the surface of the mouth cavity have shown the active regeneration of the epithelial cells with the full form structure as well as the fagocytic activity. The average longitude of AHS patients' treatment was shortened as a result of the combined LI and Myramistin therapy use from 8.5 to 3.5 days. The analysis of the results obtained shows that the maximal therapeutic effect is achieved with the use of medicines with various mechanisms of antiviral action.

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Influence of Doxorubicin and Etoposide on the Process of Cd95-Mediated Apoptosis in EBV-Infected Lymphoma Bl-41 and Dg-75 Cells

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Viruses affect infected cells in different ways. Along with deep alternations of the metabolic processes and their redirection on the synthesis of the virion components, infection can lead to the changes of functional state and regulatory processes in a cell. The purpose of our activity was the analysis of influence of antitumoral drugs, namely, commercial Doxorubicin (EBEWE, Austria) and "Vepesid (Etoposide)" (Titolare, Italiana), on processes CD95-mediated apoptosis in lymphoma BL-41 and DG-75 cells infected by Epstein-Barr virus.

To study the CD95-mediated apoptosis in the cells BL-41, DG-75 (EBV-negative lymphoma cells) we used monoclonal antibodies to CD95 antigen, received from Prof. S. Sidorenko. Apoptic cells were stained with Hoechst 33482 and determined by fluorescent microscopy. The scheme of investigation of each cell line included following variants: (1) study of the level of expression of the CD95-mediated apoptosis in a non-infected cell culture after addition of apoptosis-stimulating Monoclonal antibody IPO-4; (2) study of the level of apoptosis expression in the cells infected with EBV; (3) study of the CD95-mediated apoptosis in the EBV-infected cells after addition of the Mab IPO-4.

The affect of drugs was estimated by calculation of survival index (ID_{50}) by using the MTT method. It was shown that concentration of 20 mg/ml of Etoposide as well as Doxorubicin for cell line DG-75 causes the decreasing of cell proliferation level on 50% after 24 h after infection. BL-41 cells were more sensitive to investigated drugs: ID_{50} amounted to 5 mg/ml of drugs. In the system DG 75 + EBV the addition of Doxorubicin in concentration of 20 mg/ml was resulted in apoptosis of 89% cells after 24 h, though Etoposide-induced apoptosis in this virus–cell system took place only in 35% cells. Appearance of more than 50% apoptotic cells took place within 1 mg/ml dose of added drugs, but in the system of super infected cells + Doxorubicin revealed only 10% apoptic cells.

Using of apoptotic test set, which consist of Annexin-Cy3.18, allowed to determine that already in 3 h 10% of cells had the characterized red fluorescence, that was evidence of start of the apoptotic process.

Thus, it was investigated the influence of Doxorubicin and Etoposide on the process CD95-mediated apoptosis in lymphoma BL-41 and DG-75 cells infected with EBV.

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Studying of Anti Epstein-Barr Virus Activity of New Nitrogen-Containing Heterocyclic Compounds

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Search of new effective preparations capable to inhibit herpesviruses reproduction is stipulated by their certain resistance to different groups of chemical preparations. Bi- and tricyclic nitrogencontainig structures (non nucleoside protease inhibitors) are widely used as potential antiviral agents. They are used against retroviruses and some herpesviruses. 2′,3′-Dideoxy-2′,3′-didehydro-6-asacytidine and 2′-deoxy-6-asacytidine are original cytidine analogues.

The objective of the present investigation was to study the activity of 2′,3′-dideoxy-2′,3′-didehydro-6-asacytidine (N1) and 2′-deoxy-6-asacytidine (N2) against Epstein-Barr virus—lymphotropic and oncogenic virus from Herpesviridae family. As a model of EBV-infection in vitro we used the line of lymphoblastoid B-cells Raji, which infected by EBV. An inhibition of reproduction of EBV in a cell culture by N1 and N2 was determined by reduction of a number of genome-equivalents of EBV DNA on a cell, which were revealed by quantitative PCR with use of primers and reagents (Amply-Senc-100 R) (Russia). The first stage of investigation of N1 and N2 was the analysis of their cytotoxicity for cell line Raji. We have studied N1 and N2 in concentrations of 1000, 500, 250, 125, 64, 32, 16, 4, 1, 0.5 and 0.1 μg/ml. The concentrations that inhibited the quantity of alive cells on 50% (ID_{50}) were equal to substances N1 500 μg/ml and N2 250 μg/ml. The minimal inhibiting concentration (MIC) of N1 was equal to 4 μg/ml, because the amount of genome-equivalents of DNA EBV on a cell were reduced with 22.0 up to 10.4. MIC for N 2 was equal to 16 μg/ml (the amount of genome-equivalents were reduced with 22 up to 7). Hence, the index of selectivity (IS) was equal to 125 and 16 for 2′,3′-dideoxy-2′,3′-didehydro-6-azacytidine and 2′,3′-dideoxy-6-azacytidine accordingly. It was investigated the influence of N1 and N2 on CD95-mediated apoptosis in Raji cells infected by EBV.

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The EBV Transcription Profile Upon the Treatment with Acyclovir and Maribavir

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Expression of herpesvirus genes in the lytic cycle falls into kinetic classes (α, β, and γ) that proceed in an orderly manner. This order has been defined in part by responses to various inhibitors, and the analyses were mainly performed on single genes; however precise kinetic assignment for a number of genes remained unclear. Here we present a comprehensive view of EBV lytic gene expression as affected by two antiviral compounds with quite different mechanisms of action. Akata cells latently infected with Epstein-Barr virus (EBV) were treated with anti-human immunoglobulin which results in initiation of a lytic viral replication cycle. Acyclovir (ACV), a nucleoside-analog EBV DNA polymerase inhibitor, and maribavir, an antiviral compound that does not inhibit EBV DNA polymerase directly, were used to study side-by-side the impact of drug treatment on EBV gene expression and identify viral genes that require DNA replication for optimal expression. EBV gene expression was analyzed by whole genome real-time quantitative PCR array [Papin, J., Vahrson, W., Hines-Boykin, R., Dittmer, D.P., 2004. Methods Mol. Biol. 292, 449–480]. The analyses show that the majority of genes fit the expected profile: proteins involved in DNA replication fall in the early gene group and are not inhibited by the drugs; and structural proteins fall in the late gene group, and are inhibited by the drug treatment. However, some genes showed discordant patterns, which may imply additional functions for the corresponding proteins. The results

independently confirm previous observations on kinetics of EBV gene expression while providing additional insights into EBV replication strategies and action of these drugs.

Poxviruses

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Smallpox Antivirals: In vitro Assay for Vaccinia Virus I7L Enzymatic Cleavage of Core protein Precursors

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Development of an effective antiviral drug requires the identification of a specific interaction or activity whose disruption will be lethal to the virus and relatively benign to the host. Since viruses, such as orthopoxviruses, are obligate intracellular parasites which utilize many of the host cell enzymes and metabolic pathways during their replication, this task has proved quite difficult and this fact is chiefly responsible for the absence of successful antiviral drugs for use against smallpox. Fortunately, the realization that viruses use proteolysis catalyzed by viral-encoded proteinases as a key step in their developmental cycle, has opened up a new class of targets for antiviral drug development. In recent work, we have identified the poxvirus gene that encodes the viral core protein proteinase (vCPP). This gene (I7L) is highly conserved in all pathogenic poxviruses and is predicted to encode a cysteine proteinase that cleaves at AGX sites to produce several major core components of the viral particle. We have taken advantage of this attractive target to develop an effective drug that blocks orthopoxvirus replication based on specific mechanistic inhibition of vCPP. In order to screen compounds for their ability to inhibit the enzymatic activity of I7L an in vitro assay was developed. We are currently working on the development of a biochemical assay for use in high-throughput screening. The results from development of a fluorescent-quench peptide specific assay will be presented. Here we also show that I7L is capable of cleaving the gene product of the A3L ORF, p4b, when p4b is expressed in a cell-free transcription and translation assay. Mutation of I7L inhibits this processing as does the addition of specific I7L inhibitors.

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Efficacy of Smallpox Vaccination in the Presence of Antiviral Drugs, Cidofovir and Hexadecyoxypropyl-cidofovir

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The current smallpox vaccine is very reactogenic in humans. Standard vaccination is a percutaneous scarification of $\sim 2 \times 10^5$ plaque forming units (PFU) of live vaccinia virus (Dryvax). The resulting lesion can take up to 42 days to resolve. Any treatments that would hasten the resolution of lesion without affecting vaccine efficacy would be welcomed in the clinic. Vaccination in the presence of an antiviral would limit the replication of the virus, leading to a smaller and more rapidly resolving lesion. One drawback to this approach would be a reduction in the strength of the immune response due to a lower mass of viral antigen. We tested this hypothesis using cidofovir (CDV) and alkoxyalkyl cidofovir analogs.

To determine if antiviral treatments affected the efficacy of vaccination, groups of A/NCR mice were vaccinated in the presence or absence of antivirals with decreasing doses of Dryvax. We observed similar levels of protection in the presence or absence of CDV with each dose of vaccine as well as a reduction in lesion size and resolution time.

Next, we evaluated whether CDV had an effect on protection when sub-optimal amounts of vaccine were employed. Groups of mice were vaccinated in the presence or absence of CDV with 1.0×10^2 PFU of Dryvax. If the Dryvax vaccine dose was limiting, we would expect to see a greater effect of CDV on vaccine efficacy as CDV would restrict viral replication and the generation of a threshold antigenic mass needed to trigger a protective immune response. This sub-optimal vaccination was tested against increasing doses of aerosolized ectromelia virus with no statistically significant differences in mortality and mean time to death between mice vaccinated in the presence or absence of CDV. To further extend these studies, we are examining key components of the primary and memory immune response following vaccination in the presence and absence of antiviral.

Taken together these experiments support the hypothesis that the presence of CDV during vaccination will reduce the size of the primary lesion without affecting vaccine efficacy.

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Synergistic Combination Effect of Cidofovir and Idoxuridine on Vaccinia Virus Replication

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An intensive search of chemotherapeutic agents active against orthopoxviruses is actually in course in view of a potential menace of terrorism attacks with smallpox virus. Development of effective combinations of antivirals is considered as a prospective approach in this respect. We studied the combination effect of cidofovir and idoxuridine on vaccinia virus (VV) replication in cell cultures of chick embryo fibrob-

lasts (CEF). Cidofovir (CDV) is an acyclic nucleoside phosphonate manifesting therapeutic potential in the treatment of a large scope infections caused by DNA viruses, poxvirus included. Idoxuridine (IUdR) is the pioneer antiviral substance used in the treatment of lethal ortopoxvirus infections on systemic administration till the time of smallpox eradication. Two VV strains were used, from the collections of Institute of Virology, Bratislava, and of Institute of Microbiology BAS, Sofia. The experimental design of both combination antiviral effect and cytotoxicity testing followed the Prichard and Shipman three-dimensional model (1990). Firstly, the individual IC_{50} values of the compounds in the CPE inhibition test in monolayer CEF cultures in 96-well plates were determined. It was found the antiviral effect is strongly dependent on viral inoculum size. No marked susceptibility to CDV and IUdR between two VV strains was established. Cytotoxicity of the compounds for CEF cells was assayed in both monolayer (maximal tolerated concentration, MTC) and in growing cell cultures (50% cell growth inhibitory concentration, $CGIC_{50}$). Selectivity index values of individual antiviral effects of CDV and IUdR versus VV were found to be close when evaluated MTC/IC_{50}, and markedly higher for CDV at $CGIC_{50}/IC_{50}$. The combination effect of CDV + IUdR on VV replication in CEF cultures was characterized as a markedly synergistic one.

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Effect of Oral Treatment with HDP-(*S*)-HPMPA or ODE-(*S*)-HPMPA on Cowpox or Vaccinia Virus Infections in Mice

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We have previously reported that (*S*)-9-[3-hydroxy-2-(phosphonyl-methoxy)propyl]adenine, or (*S*)-HPMPA, is active in vitro against cowpox virus (CV) and vaccinia virus (VV), but is not orally active. However, the ether lipid esters of (*S*)-HPMPA, hexadecyloxypropyl-(*S*)-HPMPA (HDP-(*S*)-HPMPA) and octadecyloxyethyl-(*S*)-HPMPA (ODE-(*S*)-HPMPA), had significantly enhanced activity in vitro and are 74% orally bioavailable in mice. In this study, HDP-(*S*)-HPMPA and ODE-(*S*)-HPMPA were prepared in water and administered once daily by oral gavage to mice using 30, 10 and 3 mg/kg for 5 days beginning 24, 48 or 72 h after inoculation with CV or VV. Both HDP-(*S*)-HPMPA and ODE-(*S*)-HPMPA were highly effective ($p < 0.001$) at preventing mortality due to CV at 30 mg/kg even when treatments were delayed up to 72 h post infection. Also, ODE-(*S*)-HPMPA or HDP-(*S*)-HPMPA were highly effective ($p < 0.001$) at preventing mortality in mice infected with VV at 30 mg/kg when treatments were delayed up to 48 or 72 h, respectively. When ODE-(*S*)-HPMPA or HDP-(*S*)-HPMPA were given at 30 mg/kg beginning 24 h after inoculation with CV, replication in target organs of liver, spleen and kidney were reduced to below detectable levels, however, virus titers in lung were not significantly altered. In a similar study using VV, HDP-(*S*)-HPMPA also reduced viral replication in liver, spleen and kidney to below detectable levels. Oral treatment with ODE-(*S*)-HPMPA not only reduced replication in liver, spleen, and kidney but also in lung on day 12. These data indicate that HDP-(*S*)-HPMPA and ODE-(*S*)-HPMPA given orally are very active against CV and VV infections in mice and are as effective as cidofovir. The results further suggest that these compounds should be pursued to determine their potential for treatment of human orthopoxvirus infection.

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Pharmacodynamics of Cidofovir, an Inhibitor of Poxvirus Replication, in an In vitro Hollow Fiber Model System

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Background: Cidofovir has in vitro and in vivo activity against poxviruses. To use the drug effectively for the prevention and treatment of people exposed to Variola major virus or monkeypox virus, the correct dose and schedule of administration must be known. To this end, Cidofovir was evaluated in our hollow fiber pharmacodynamic model of vaccinia virus infection to ascertain the dose–response effect and the pharmacodynamic variable most closely linked to the inhibition of vaccinia virus replication.

Methods: The EC_{50} value of Cidofovir for vaccinia virus grown in HELA-S3 cells was determined by growing virus in the presence of various concentrations of drug in T flasks. The dose response effect of Cidofovir on virus replication in the hollow fiber system was determined by growing vaccinia virus in HELA-S3 cells in the presence of continuous infusions of 2×, 4× and 8× EC_{50} concentrations of Cidofovir. To determine the pharmacodynamically-linked variable, hollow fiber units containing vaccinia virus infected HELA-S3 cells were either continuously infused with 3 or 250 μM drug or given a bolus of drug equivalent to the 72 h AUC of 3, 100 or 250 μM followed by a washout to simulate the concentration time profile of Cidofovir reported in man. In each case, efficacy was determined by FACS analysis of virus-infected cells treated with a Mab specific for vaccinia virus and drug concentrations were confirmed by LC/MS analysis.

Results: The EC_{50} value of Cidofovir for vaccinia virus grown in HELA-S3 cells was 56.93 ± 1.13 μM. In the hollow fiber model, continuous infusion of Cidofovir at 100 or 200 μM reduced the number of virus-infected cells by 62 and 98%, respectively. In a dose fractionation experiment, 3 μM Cidofovir given either as a continuous infu-

sion or as a bolus followed by a washout had no effect on virus replication. In contrast, continuous infusion of 250 μM or a bolus equivalent to AUC_{72h} of 100 or 250 μM drug followed by washout inhibited virus replication by greater than 90%.

Conclusion: The data suggest that the AUC_{72h}/EC_{50} ratio was the pharmacodynamically linked variable that best correlates with antiviral activity of Cidofovir for vaccinia virus-infected cells in the hollow fiber model.

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Antiviral Activity of Nucleoside Analogs Against Orthopoxvirus Replication is Limited Predominantly by Their Phosphorylation

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Orthopoxviruses and herpesviruses are both large DNA viruses with DNA polymerases containing rather conserved active sites, yet these virus families exhibit very different susceptibilities to nucleoside analogs. We speculated that the observed differences were due largely to the capacity of the viruses to phosphorylate these compounds, rather than the substrate specificity of the DNA polymerases. The activity of selected antiviral drugs were tested against both thymidine kinase positive (TK+) and TK negative (TK−) strains of cowpox virus (CV) and herpes simplex virus type 1 (HSV-1), using cidofovir serving as a control since it does not require phosphorylation to the level of the monophosphate. The degree to which the kinases confer activity on the drugs was determined by calculating the ratio of the EC50 values against TK− and TK+ viruses. In HSV-1, each of the 11 compounds tested was markedly less effective against the TK− strain, suggesting that this viral kinase was required to activate the drugs. This was not observed when the drugs were tested against CV. Only halogenated uridine derivatives were shown to be less effective against the TK− strain of this virus, and the relative differences in EC_{50} values for these compounds were small compared to the differences observed in HSV-1. We infer from these data, that the TK encoded by CV has a narrow substrate specificity compared to the HSV-1 TK, and that the compounds that are substrates for this enzyme are phosphorylated to a limited extent. These data suggest that the limited activity of herpesvirus drugs against orthopoxviruses is a result of their inefficient activation by the kinases encoded by this family of viruses. Additional screening of nucleoside analogs against orthopoxviruses is required to identify analogs that are efficiently activated by viral kinase, and should identify compounds with a high degree of selectivity against this group of viruses.

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Reduced Pathogenicity of Phenotypically and Genotypically Characterized Cidofovir (CDV)-resistant Vaccinia Virus (VV)

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We have recently described the phenotypic and genotypic characterization of several plaque-purified VV (Lederle strain) clones isolated following selection with CDV. Different amino acid substitutions at positions 246, 314, 420 and 684 were observed in the viral DNA polymerase of several plaque-purified CDVr clones. Changes at positions 246 and 420 were associated with gene polymorphism as suggested by comparison with the DNA polymerase sequences of several orthopoxviruses. The A314T and the A684V mutations were considered associated with resistance to CDV and other HPMP (3-hydroxy-2-phosphonylmethoxy) derivatives, since the A314 and A684 are conserved residues among orthopoxviruses and have partial or total homology with the corresponding amino acid in herpesviruses DNA polymerases. We have now found that clones isolated under cyclicCDV (cCDV) pressure present only the 314 mutation, suggesting that this mutation alone can confer resistance to the HPMP derivatives. Interestingly, the DNA polymerase of several clones isolated following plaque purification of the Lederle strain revealed that the vaccine strain is a heterogeneous population: some clones, named ΔLederle, have an amino acid deletion in the viral DNA polymerase (sequence Asn-deletion-Gly at positions 936–938), while the corresponding sequence for the other clones, named non-ΔLederle, is Ala-Asn-Val. Both types of clones proved equally sensitive to all drugs tested. The CDVr clones were selected from the non-ΔLederle population, while the cCDVr clones originated from the ΔLederle population. To evaluate the pathogenicity of the different clones, mice of 13–14 g were inoculated intranasally with different viral doses and body weight was recorded daily. Significant differences in the percentage of increase of body weight were noticed between the strains tested. Thus, at 7 days post-inoculation, the percentage increase in body weight was 19% for the Lederle vaccine strain, 7% for a non-ΔLederle clone, 33% for a ΔLederle clone, and 41% for a CDVr non-Δclone as compared to 45–52% for uninfected control mice, when animals were inoculated with 4×10^4 PFU. Our results indicate that the CDVr non-Δclone was much less pathogenic than the original vaccine strain.

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Group-specific and Neutralizing Human SCFV to Orthopoxviruses from a Combinatorial Phage Library

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The genus Orthopoxvirus includes several species of well-known pathogens, e.g. variola, vaccinia, cowpox and monkeypox viruses. Vaccinia virus (VACV) was used in the past as an effective vaccine against smallpox. Although VACV is generally safe vaccine, disseminated, life-threatening infections occur infrequently, especially in individuals with impaired immunity. Such infections can be treated by therapeutic administrations of human VACV immune globulin (VIG). Human monoclonal antibodies offer an obvious alternative to VIG. Fully human Mabs can be constructed using the specific variable Ig domains selected from a combinatorial phage library.

A collection of 64 human scFvs against orthopoxviruses have been obtained from a combinatorial phage scFv library constructed from the variable domains of light and heavy immunoglobulin chains derived from the populations of lymphocytes, which were obtained from the donors, vaccinated by vaccinia virus. All selected scFvs were tested in simultaneous ELISA experiments for binding with vaccinia (strain Elstree), cowpox (strain Grishak) and ectromelia (strain K-1) viruses. Most of the selected scFvs reacted with the viruses at the same manner. Six scFvs were found to be group-specific: 2VA8 bound vaccinia and cowpox viruses; 2VC3, 2VD4, 2VD10 bound vaccinia and ectromelia viruses; 4VF2, 4VE7 reacted with vaccinia virus. A standard assay of virus neutralization as the ability of antibodies to inhibit virus plaque formation in eukaryotic cells monolayer (PRNT) was performed with the viruses. Two scFv showed neutralizing activity against all tested viruses, 2VA8 neutralized vaccinia and cowpox virus but did not neutralize ectromelia virus.

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Phage Display Immune Library of Human scFv Against Orthopoxviruses

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A combinatorial phage display library of human scFv antibodies was generated from IgG heavy and light chain variable domain genes from the lymphocytes of four vaccinia virus (VACV)-immune donors. The titers of anti-VACV antibodies in the sera were examined by ELISA for each donor after vaccination. Populations of peripheral lymphocytes were educed when the titers peaked up to the plateau. The genes encoding the variable heavy and light chain domains were amplified by RT-PCR using the primers specific to the conservative regions of these genes. Vh and Vl genes were randomly combined via the DNA linker whose structure corresponded to Ser(Gly4Ser)2AlaArgGlySerGly4Ser sequence. The scFv genes were cloned into pHEN2 phagemid vector and the library was constructed using the *Escherichia coli* TG1 strain. The constructed library contained approximately 3×10^7 independent clones.

The library was enriched using biopanning procedures. Two antigens were used for panning: vaccinia virus Elstree and A30L variola virus India-1967 recombinant protein (corresponding to A27L for VACV Copengagen). Individual clones from the enriched populations were screened against both VACV and A30L antigens simultaneously and 18 positive clones were selected. All the selected scFvs were assayed for their bindings with vaccinia, variola, cowpox and ectromelia viruses. A standard assay of virus neutralization as the ability to inhibit plaque formation for the orthopoxviruses (PRNT) was performed to identify neutralizing scFvs.

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Full-size Human Antibodies Against Orthopoxviruses

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Fully human Mabs against Orthopoxviruses were constructed from human Vh- and Vl-fragments and constant domains of human IgG1. Vh- and Vl-fragments were generated from the scFvs selected from the synthetic combinatorial phage library of human scFv antibodies through biopanning against live variola virus Ind-3a (major strain), variola virus Butler (alastrim strain) and vaccinia virus Elstree. Constructed human Mabs were produced in 293T human cells, from which the antibodies were purified by affine chromatography. Binding activities of the antibodies were tested by ELISA using vaccinia and variola viruses. Western-blot analysis was used to identify the target proteins for the selected Mabs. A standard assay of virus neutralization (PRINT) was performed to estimate neutralizing activities of the Mabs.

Other Viruses

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Evaluation of New Cell Culture Inhibitors of Protease-resistant Prion Protein Against Scrapie Infection in Mice

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Inhibitors of the accumulation of abnormal (protease-resistant) prion protein (PrP-res) in cell culture can sometimes prolong the survival of scrapie-infected rodents. Here, transgenic mice were used to test the in vivo anti-scrapie activities of new cell culture-active PrP-res inhibitors, which, because they are approved drugs or edible natural products, could be considered for off-label usage in human patients with transmissible spongiform encephalopathies (TSEs). These inhibitors were amodiaquine, thioridazine, thiothixene, trifluoperazine, tetrandrine, tannic acid and polyphenolic extracts of tea, grape seed and pine bark. Test compounds were administered for several weeks beginning 1–2 weeks prior to, or 2 weeks after, intracerebral or intraperitoneal 263 K scrapie challenge. Tannic acid was also tested by direct preincubation with inoculum. None of the compounds significantly prolonged the scrapie incubation periods. These results highlight the need to assess TSE inhibitors active in cell culture against TSE infections in vivo prior to testing these compounds in humans or livestock.

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Mouse Adenovirus Type 1-infected SCID Mice: A Unique Model for the Evaluation of Antiviral Compounds Against Systemic Adenovirus Infections

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The increasing importance of human adenoviruses in immunocompromised patients urges the search for new and effective anti-adenovirus compounds. Since human adenoviruses are species-specific, animal models for systemic adenovirus infections rely on non-human adenoviruses. We established mouse adenovirus type 1 (MAV-1) infection of SCID mice as a model for the evaluation of anti-adenovirus agents. In vitro studies in mouse embryonic fibroblasts pointed to the acyclic nucleoside phosphonate analogues cidofovir and 2,4-diamino-6-[3-hydroxy-2-(phosphonomethoxy)propoxy]pyrimidine (HPMPO-DAPy), and 3′-fluoro-2′,3′-dideoxythymidine (alovudine) as the most active compounds against MAV-1. SCID mice, infected intranasally with MAV-1, developed a fatal disseminated infection after 16–18 days, characterized by inflammation of the liver and duodenal hemorrhages. Several techniques were optimized to monitor viral, immunological and pathological aspects of the MAV-1 infection. Real-time PCR quantification of viral DNA revealed that MAV-1 disseminated to several organs, including the brain, lungs, liver, spleen, gut, kidneys and heart. Immunohistochemistry with a polyclonal antibody raised against an MAV-1 early protein showed that the virus was localized in the endothelial cells of the affected organs. Using multiplex RT-PCR assays, serum levels of inflammatory cytokines (i.e. IL-1β and TNF-α) were shown to be markedly increased. Studies are ongoing to evaluate the efficacy of antivirals (such as cidofovir) in this MAV-1 model.

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Compounds Reactive Against the Arenavirus RING Finger Z Protein Induce Z Oligomerization and Block its Interaction with the PRH Cellular Protein

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In previous studies, several electrophilic agents have shown potent antiviral and virucidal properties against arenaviruses. This report describes the mode of action of NSC20625, a disulfide provided by the National Cancer Institute (USA), against lymphocytic choriomeningitis virus (LCMV), the prototype species of Arenaviridae, demonstrating that Z protein is the target of this compound. Z is a 11 kDa protein with a conserved RING finger domain, which is a very attractive antiviral target. The treatment of LCMV particles with NSC20625 induced three concomitant effects: (i) viral infectivity was destroyed; (ii) virions were rendered unable to synthesize viral RNA upon infection of new host cells; (iii) the electrophoretic profile of Z protein was altered when analyzed under non-reducing conditions, whereas the pattern of the other main virion proteins remained unaffected. The interaction of NSC20625 with Z was confirmed by incubation of a purified recombinant LCMV–Z protein with the compound. Under non-reducing conditions, this agent induced the oligomerization of Z in high molecular weight aggregates, due to intermolecular disulfide bonds between the cysteine residues of the RING fingers. Furthermore, it has been reported that LCMV replication affect the subcellular localization of the proline-rich home-

odomain (PRH) cellular protein in human hepatocytes and that PRH can be physically associated with the Z protein. To determine whether the treatment with NSC20625 affects the Z–PRH interaction, LCMV-infected cells were treated with the agent and then stained with a purified antibody to monitor PRH bodies. No major differences between the PRH pattern in uninfected cells or infected-treated cells were observed, suggesting that the association of PRH bodies and Z protein through the RING finger is blocked by the compound. These results open the possibility of targeting Z protein as a new antiviral strategy against hemorrhagic fever arenaviruses.

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Antiviral Activity of Cyclooxygenase Inhibitors Against Bovine Viral Diarrhea Virus (BVDV) Replication

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A rapid and sensitive screening assay has been established for in vitro evaluation of antiviral compounds against bovine viral diarrhea virus (BVDU), which is widely used as a surrogate of hepatitis C virus (HCV). The procedure is based on colorimetric assessment for the viability of virus-infected cells via extracellular leakage of lactate dehydrogenase (LDH). Under optimized conditions, the LDH level was correlate well with the degree of viral replication. Using this system, we have evaluated several compounds for their anti-BVDV activities and found that some cyclooxygenase (COX) inhibitors are capable of inhibiting BVDV replication at their nontoxic concentrations. Among them, SC560 was the most active, and its 50% effective concentration (EC_{50}) and 50% cytotoxic concentration (CC_{50}) were 10 and 160 μM, respectively. The reference compound ribavirin was threefold more active but 11-fold more cytotoxic than SC560. Thus, the selectivity index of SC560 was higher than that of ribavirin. In addition, indomethacin and diclofenac, COX inhibitors widely used as anti-inflammatory drugs in clinics, also displayed selective inhibition of BVDV replication. These results suggest that this class of compounds should be further pursued for their mechanism of action, in vivo efficacy, and antiviral activities against the Flaviviridae family, including HCV.

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Substituted 5-Benzyl-2-phenyl-5H-imidazo[4,5-c]pyridines: Synthesis and Anti-BVDV Evaluation

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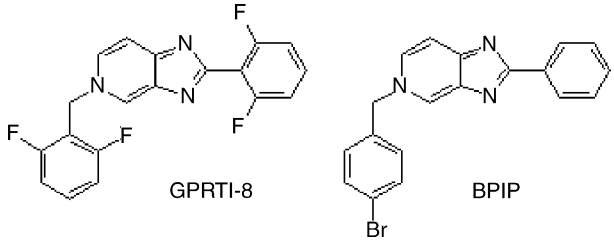
Bovine viral diarrhea virus (BVDV), a pestivirus, has often been used as a surrogate for hepatitis C. In a broad screening effort, GPRTI-8 was found to exhibit anti-BVDV activity (EC_{50}: 8 μg/ml, CC_{50}: >100 μg/ml) against the reference strain NADL (BVDV-type 1) and was chosen as a lead compound. Formal removal of all four fluorines resulted in an analogue with improved activity/selectivity (EC_{50}: 0.04 ± 0.03 μg/ml, CC_{50}: 46 ± 5.9 μg/ml). In a second step, substituents were introduced onto the benzyl ring (2-, 3- or 4-fluoro, chloro, methyl, methoxy, etc.), resulting in the discovery of BPIP (the 4-bromo analogue) as a highly active and selective inhibitor of BVDV (EC_{50}: 0.006 ± 0.001 μg/ml, CC_{50}: 29 ± 2 μg/ml, SI = 4830). BPIP proved also active against other pestiviruses including several CPE and non-CPE strains of BVDV-type 1 and BVDV-type 2 as well as against the classical swine fever virus (EC_{50}: 0.6 ± 0.4 μg/ml) and border disease virus (EC_{50}: 0.6 ± 0.2 μg/ml). BPIP was however inactive against hepatitis C in the subgenomic replicon system.



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Antiviral Strategies Against Bunyaviruses using Antisense Morpholino Oligonucleotides

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We have used our recently developed plasmid-based minigenome rescue systems for Crimean–Congo hemorrhagic fever virus, Rift-Valley fever virus, and Uukuniemi virus to screen antiviral compounds based on morpholino antisense oligonucleotides targeting different regions of the

minigenome vRNA and/or cRNA/mRNA. The antiviral effect of the compounds was appraised on the basis of reporter gene activity. The inhibitory activity of the same compounds was also tested by quantitating reduction in virus titer (plaque assay, $TCID_{50}$), and by monitoring reduction in virus antigen using indirect immunofluorescence/FACS procedures. Based on these results, we plan to confirm antiviral activity of the most promising compounds in suitable animal models.

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Inhibition of Coxsackievirus B3 PD by Specifically Sulfated Heparin and Lysosomotropic Agents

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Recently, we have shown that the coxsackievirus B3 variant PD (CVB3 PD) is able to enter coxsackievirus–adenovirus receptor (CAR)-lacking cells by using heparan sulfates (HS) as additional receptor (Zautner et al., 2003). Now, the possibility to inhibit CVB3 PD-induced cytopathic effect with growth factors binding to known HS sequences as well as specifically desulfated heparins was examined in Chinese hamster ovary cells (CHO-K1).

Hepatocyte growth factor (HGF) binding to HS sequences containing [–IdUA-GlcNSO3(6OSO3)–]*n* competes effectively with CVB3 PD virions for cell surface HS. In contrast, basic fibroblast growth factor (bFGF) binding to [–HexUA-GlcNSO3-HexUA-GlcNSO3-IdUA(2OSO3)]*n* was not able to hinder CVB3 PD attachment. Furthermore, natural heparin and 2-*O*-desulfated heparin inhibited the CVB3 PD-induced cytopathic effect, but there was a strong decrease in antiviral activity after chemical *N*-, *O*- and 6-*O*-desulfatation of heparin.

DSTP-27 a polykationic compound, blocking herpesvirus adsorption by binding to heparan sulfates showed only moderate antiviral activity by not even 50% of CPE inhibition using 800 μg/ml.

This confirms that 6-*O*- and *N*-sulfation of GlcNAc of HS are crucial for interaction of CVB3 PD with HS and points out that heparin of specific sulfatation pattern can be used to block virus adsorption.

In addition, lysosomotropic agents like ammonium chloride and monensine are able to reduce the CVB3 PD-induced cytopathic effect in HS-positive, CAR-negative CHO-K1cells but not in HS-lacking, CAR-expressing pgsD-677-hCAR cells.

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Combined Effect of Oxoglaucin and Other Inhibitors of the Enteroviral Replication in Experimental Coxsackievirus B Infection in Newborn Mice

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Oxoglaucin is an aporfinoid alkaloid, isolated from *Glaucium flavum* L., or obtained in a synthetic way (S. Philipov, Institute of Organic Chemistry, BulgAcadSci). As a result of a wide-spectrum in vitro antiviral screening, the compound was selected for further investigation due to its pronounced inhibitory effect against poliovirus 1 replication in FL cells. Its antiviral spectrum includes some Coxsackie and ECHO viruses as well. The individual effect of the substance in experimental infections in newborn mice with Cox B1 and Cox B3 was determined. Applied in a daily dose of 25 mg/kg s.c. against Cox B1, the compound showed a marked protective effect, expressed in lengthening the mean survival time and decreasing mortality rate. When used against Cox B3, oxoglaucin showed only lengthening the mean survival time. The combined effects of the substance with other enteroviral replication inhibitors (disoxaril, guanidine hydrochloride, diphenylthiourea derivative PTU-23) were tested on this experimental model in order to increase its effectivity. The compounds were applied s.c. in a double, triple and quadriple combinations. The triple combination of oxoglaucin, disoxaril and PTU-23/guanidine manifested the highest activity.

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Anti-Coxsackievirus B Activity of 2-(3,4-Dichlorophenoxy)-5-nitrobenzonitrile Analogues

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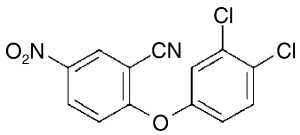
[2-(3,4-Dichlorophenoxy)-5-nitrobenzonitrile] (DNB) was previously shown to exhibit broad-spectrum antiviral activity against picornaviruses [Antimicrob. Agents Chemother. 22 (1982) 639–642]. To further study the potential of this scaffold as an antiviral against picornaviruses, more than 100 analogues of this compound were prepared, and their effect on the replication of Coxsackievirus B (CVB) was determined. Analysis of the structure–activity relationship of these molecules revealed that (i) modification of the substitution pattern on the left phenyl ring did not lead to an increase in antiviral activity and (ii) introduction of bulky substituents on the right side resulted in a dramatic decrease in activity. Several analogues with small, hydrophobic substituents in position 3 and/or 4 showed similar or slightly superior ac-

tivity compared to the lead compound DNB. The antiviral activity of DNB and several of its analogues was assessed by (i) cytopathic effect and virus yield reduction assays, (ii) Q-RT-PCR and (iii) viral antigen expression. Detailed single cycle time-of-drug-addition studies (in which viral replication is monitored by means of Q-RT-PCR) revealed that the compound did not inhibit binding to, or penetration into, the host cells. Following 35 passages of virus culturing in the presence of increasing drug concentrations, DNB-resistant virus was obtained. Drug-resistant virus will be genotyped, which will enable to identify the antiviral target at the molecular level.



DNB

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Inhibitory Action of Sulfated Polysaccharides on Dengue Virus Infection of Human Cells

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Sulfated polysaccharides are known to present a broad range of biological activities; in particular they represent an alternative in the search of antiviral substances against enveloped viruses, such as flaviviruses. The objective of the present work was to study the antiviral activity of diverse polysaccharides against dengue virus type 2 and 3 (DENV-2 and DENV-3) in human cells compared to monkey Vero cells, the reference host system. For this purpose, HepG2 cells (human hepatoma cells) and PH cells (human foreskin fibroblasts) were used. Sulfated polysaccharides of diverse structural types were assayed: λ- and ι-carrageenan, dextran sulfate, heparin and a DL-galactan hybrid. The cytotoxicity was measured by the MTT method to determine the cytotoxic concentration 50% (CC_{50}) and the antiviral activity was evaluated by a virus yield inhibition assay to determine the inhibitory concentration 50% (IC_{50}). No cytotoxicity was observed with any of the assayed polysulfates at concentrations up to 1000 μg/ml. The compounds were effective inhibitors of DENV-2 and DENV-3 in human HepG2 and PH cells with an antiviral effect similar to that observed in Vero cells, showing IC_{50} values in the range 0.09–13.9 μg/ml and selectivity indexes (CC_{50}/IC_{50}) in the range 1000–10,000. The mode of action of the polysaccharides was studied in HepG2 cells, analyzing the influence of time of addition of compounds on anti-DENV-2 activity by an infectious center assay. The highest inhibitory effect was observed when the compounds were added to cells together with the virus (99.8–99.9% inhibition) or when added immediately after adsorption at 1 h p.i. (91.1–99.4% inhibition), and no significant reduction in virus plaque formation was produced at later times. Also the inhibitory action of polysaccharides on DENV-2 adsorption and internalization was studied in PH, HepG2 and Vero cells. Significant antiviral efficacy was attained if compounds were present either only during DENV-2 adsorption or internalization. These results indicate that both initial events of dengue virus entry seem to be the main target for these compounds during in vitro infection of human cells.

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Inhibition of Dengue Virus Serotypes 1–4 in Cell Culture with Morpholino Oligomers

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Five antisense phosphorodiamidate morpholino oligomers (PMO), designed to hybridize to various regions of dengue-2 virus (DEN-2), were evaluated for their ability to inhibit dengue viral titer in mammalian cell culture. The PMOs were conjugated to short arginine-rich peptides in order to facilitate their entry into cells in culture. Vero cells were incubated with PMO agents, inoculated with Dengue virus, and viral titer determined by plaque-assay 1–10 days later. Three of the compounds, targeting the AUG translation start-site, the 5′-'cyclization sequence', and the 3′-terminus showed little inhibitory effect on viral titer. Two of the compounds, targeting the 5′-terminus (5′-SL PMO) and the 3′-'cyclization sequence' (3′-CS PMO), reduced viral titer of DEN-2 by over 3 orders of magnitude, compared to controls, in a dose-dependent and sequence-specific manner over a 4–6 days period. Ten micromolar solutions of the 5′-SL PMO and 3′-CS PMO each reduced viral titer of all four Dengue serotypes by over 2 orders of magnitude, in some cases to below detectable limits. The two highly positive compounds showed little non-specific cytopathology as evidenced by their lack of titer reduction of the related but non-homologous West Nile Virus grown under identical culture conditions, as well as by MTT assay. These data indicate that further evaluation of the 5′-SL PMO and 3′-CS PMO compounds as potential Dengue therapeutics is warranted.

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Single Chain Antibodies Against Ebola Virus from Naive Phage Display Library

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Human naive library of single chain Fv (scFv) was established from the variable domains of light and heavy immunoglobulin chains derived from the populations of

lymphocytes of six healthy human donors using the RT-PCR. To construct the library, the repertoires of light and heavy immunoglobulin domains were fused via flexible linker sequence and inserted into pHEN2 phagemid vector. ScFv library was expressed using *Escherichia coli* strain TG1, the repertoire of the scFv library exceeds 4×10^7 independent recombinant clones. To confirm that antibodies with a range of binding specificities can be isolated from the library, it was subjected to biopanning against different antigens, including viruses and recombinant proteins. Thus, the scFvs against TNF-α and the surface epitope of hepatitis B virus were selected after two rounds of independent panning procedures and assayed by ELISA and Western blot analysis. In addition, the scFv library was panned against inactivated Ebola Zaire virus, and 10 positive clones were selected. All the selected scFvs bound both inactivated and live Ebola virus. Western blot analysis demonstrated that seven selected scFvs reacted with VP40 and three scFvs bound both NP and VP40 of Ebola virus. Binding characteristics of the antibodies were tested in ELISA with subsequent dilutions with Ebola virus, and it was shown that the scFv selected from the naive library revealed picogramms of live Ebola virus while scFv selected from the synthetic scFv library earlier revealed nanogramms of the virus. Selected anti-Ebola scFvs, constructed from "natural" V-domains could be, useful for the development of fully human Mabs. The fact that scFvs bound live Ebola virus were obtained from the naive library suggested that the diversity of variable domains in the organisms could be a resource for antibodies against high pathogenic organisms without any immunized libraries.

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Generation of Human scFv Against Guinea Pig-adapted Variant of Ebola Virus

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Ebola virus (EBO) is a highly pathogenic agent for humans and primates causes hemorrhagic fever with high level of mortality. Guinea pigs inoculated with EBO normally develop a non-lethal febrile illness. Serial passage of initially non-lethal Ebola virus in outbreed guinea pigs resulted in the selection of variant 8MC with high pathogenicity. The guinea pig-adapted 8MC variant of EBO differed from wild-type viruses by mutations in the coding regions of nucleoprotein (NP), membrane-associated virion protein VP24 and RNA-dependent RNA-polymerase (L) genes, and by one mutation in the non-coding region.

A collection of human scFv antibodies against guinea pig-adapted 8MC variant of Ebola Zaire virus have been obtained from a combinatorial phage library of human single-chain antibody fragments, scFv (Medical Research Council Center, Cambridge, England) using biopanning procedure. In addition, several tens of human scFvs against wild-type Ebola Zaire virus have been selected. Cross-reactivity of the selected antibodies have been assayed by indirect ELISA in binding reactions with wild-type Ebola Zaire and Ebola 8MC viruses. Specificity of these antibodies was assayed using Western-blot analysis. Nine antibodies bound VP24 and 11 antibodies bound both matrix proteins VP40 and VP24 fractionated by electrophoresis. PCR products of scFv phage antibodies were gel-purified and sequenced using a cycle-sequencing protocol based on the chain termination method on automated DNA.

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Oxoglaucine: A Selective Inhibitor of Enterovirus Replication

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Enteroviruses are responsible for a variety of clinical syndromes and diseases, ranging in severity from mild disorders to life-threatening conditions. Enteroviruses are etiological agents of nearly 90% of the cases of viral meningitis and 20% of the cases of encephalitis. They can cause myocarditis and pericarditis, which may progress to dilated cardiomyopathy. Enteroviruses are suspected to play a role in the development of juvenile-onset diabetes mellitus. They are also causative agents of summer cold, herpangina, pleurodynia, hemorrhagic conjunctivitis. Until now, there are no enterovirus specific drugs available for clinical use. A great number of picornavirus inhibitors have been described so far, but just few of them have shown effectiveness in vivo and none has been approved for clinical use yet. Etiological therapy remains elusive. There is a clear need for continued development of new inhibitors of enterovirus replication is an originally synthesized aporphinoid alkaloid that proved its selective inhibitory effect on type 1 replication in preliminary screening tests by the agar-diffusion procedure. The objective of the present study was to test the antiviral effect of oxoglaucine on the replication of other enteroviruses, i.e. the coxsackieviruses. The end-point dilution method in the multicycle CPE inhibition setup in FL cells was used for determining the antiviral effect. Oxoglaucine revealed a marked inhibitory effect on all tested viruses. CV-B4 was the most sensitive, followed by CV-B5, CV-B3, CV-A9 and CV-B1. The concentrations that reduced the virus titer by $1 \log 10$ ranged from $0.01\ \mu$g/ml to $1.17\ \mu$g/ml for CV-B4 and CV-B1, respectively. The maximal tolerated concentra-

tion for monolayer FL cells was 6.4 μg/ml and the concentration that reduced the growth of the same cells by 50% was 4 μg/ml. The results reveal a promising antienteroviral spectrum and high selectivity of the antiviral effect of oxoglaucine.

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Degradation of Japanese Encephalitis Virus By Neutrophils

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Japanese encephalitis virus (JEV), an arthropod borne flavivirus is one of the major causes of acute encephalitis in South East Asia and Australia. Peripheral neutrophil leucocytosis or infiltration of neutrophils in extraneural tissue has been reported in human and experimental animals. Ability of neutrophils to degrade the phagocytosed Japanese encephalitis (JE) virion, via triggering respiratory burst and generation of toxic radicals was studied. JEV or JEV-induced macrophage derived factor (MDF) induces increase in intracellular oxidative signals with generation of superoxide anion (O^{2-}), via activation of cytosolic NADPH and subsequently form hydrogen peroxide, with maximum activity on day 7 post-infection. The response was sensitive to anti-MDF antibody treatment. Further, the study revealed rapid degradation of phagocytosed JE viral protein and nucleic acid. The viral protein degradation was partially dependent on the generation of toxic oxygen species as it could be partially abrogated by pretreatment of the cells with staurosporine. The data indicate that neutrophils on stimulation with JEV or MDF generate reactive oxygen metabolites and help in degradation of the phagocytosed virus that may be one of the early defense mechanisms for killing of the virus.

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Antiviral Strategies Against Nipah Virus: Exploring Gene Silencing Mechanisms to Identify Potential Antiviral Targets

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Nipah virus (NiV) is one of the several newly emerging viruses. Classified as a BSL-4 agent, it is a Category C emerging infectious disease threat pathogen on the NIAID and CDC Priority Pathogens list. Current evidence suggests that virus crossed species barrier from fruit bats to infect pigs and then humans, and was responsible for severe illness in hundreds of animals and humans in 1998–1999. This outbreak involved 265 humans, with a mortality rate of approximately 40% and one of the measures needed to control it was the culling of ~1 million pigs. The two recent (2004) outbreaks in Bangladesh involving humans only, were smaller but deadlier; 17 of 23 and 18 of 30 cases were fatal, a mortality rate of 75 and 60%, respectively. Moreover, the mode of spread in this out break was suggestive of human-to-human transmission. No vaccine or effective antiviral agents are currently available for the prevention or treatment of NiV disease.

We are in the process of appraising gene silencing as one of several approaches to identify potential targets to develop rational antiviral strategies for Nipah virus. To facilitate these studies, we have used an Reston Ebola virus minigenome rescue system as well as an infectious clone system to compare three small hairpin RNA (shRNA) delivery systems, namely plasmid-mediated pol I and pol III-driven shRNAs, and exogenously produced shRNA, for their ability to induce gene silencing. We will present the results of this comparison, and also preliminary results of our evaluation of shRNAs targeted to NiV N, P, and L genes to mediate gene silencing.

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Effect of Various 2′,5′-Oligoadenylates with Antipapillomavirus Activities on DNA-Polymerases and DNA-Topoisomerases

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2′,5′-Oligoadenylates are intermediates of interferon activity and are induced in the interferon treated cells. Although 2′,5′-oligoadenylates were discovered long ago and their role in cell cytoplasm is established, we know very little of their action in cell nuclei. Because they can be found there in amounts exceeding those in cell cytoplasm, some functional role in cell nuclei is indicated.

2′,5′-Oligoadenylates were tested against several nuclear enzymes. These enzymes were DNA-polymerase α, DNA-polymerase β and DNA-polymerase γ. Inhibitory activity of 2′,5′-oligoadenylates against DNA-polymerase α was discovered. The most active compounds were non-phosphorylated 2′,5′-oligoadenylates, however phosphorylated compounds were also inhibitory, although to a lesser degree. The most effective inhibition of DNA-polymerase α by 2′,5′-oligoadenylate was observed with non-phosphorylated trimers and tetramers.

2′,5′-Oligoadenylates with the most activity against DNA-polymerase α also had strong antipapillomavirus activity. Relation between 2′,5′-oligoadenylate structure, activity against DNA-polymerase α and antipapillomavirus activity will be presented.

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Inhibition of Sendai Virus by a Natural Cinnamon Extract

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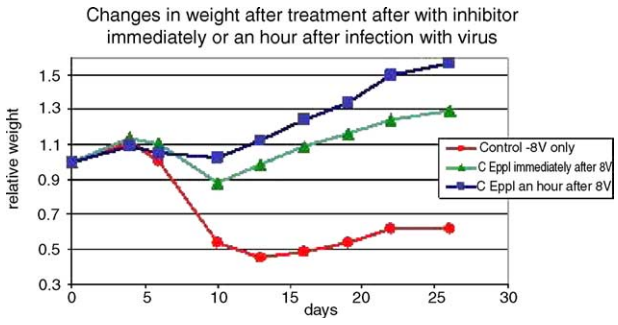
Finding treatment for viral diseases presents an important challenge in modern research and much effort is being invested in seeking substances to suppress viral activity. In this study, Sendai virus, a member of the Paramyxoviridae family, was used as a model. The aim of this research was to investigate fractions of Cinnamon Extract (CE) as a novel source for an antiviral inhibitor and to explore its commercial potential. The ability of CE to neutralize the virus was tested both in vitro on human erythrocytes and in vivo in mice. A dose of 50 μg/ml was sufficient to achieve total neutralization of 128 HAU of the virus within 1 min. CE has a long shelf-life of at least 2 years in the refrigerator or at room temperature. It still retained its antiviral activity after dialysis in bags with a cut-off of 10 kDa or after heating at various temperatures up to 121 °C. The antiviral activity was also stable at a wide range of pH between 1 and 12.5. CE has also proved its ability to inhibit the virus in vivo. When the virus was mixed with CE prior to infection of 25-day-old mice, the mice did not develop the disease nor did they lose weight or die. Moreover, same results were obtained when mice were treated with CE 1 h after infection with the virus (see Figure). Furthermore, immunization by a mixture of CE and the virus resulted in the immunized mice continuing to gain weight, whereas the non-immunized control group lost weight significantly after infection with the naïve virus. In conclusion, CE has exhibited an effective antiviral activity against Sendai virus both in vitro and in vivo. Since cinnamon is already included in the human diet, the investigated antiviral fraction could also be used as a prophylactic treatment.



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Affinity of (α-*P*-Borano)-NDPs to a Transition-state Analogue Complex of Rabbit Muscle Pyruvate Kinase

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Most antiviral nucleosides require stepwise phosphorylation to their respective triphosphates (ddNTPs) in order to exert their activity. The ddNTPs with α-*P*-borano-substitution were shown to be better chain terminators for drug-resistant reverse transcriptases. It was recently found that pyruvate kinase (PK) may be responsible for the last step of phosphorylation of 2′,3′-dideoxy- and acyclo-nucleoside diphosphates (ddNDPs). We synthesized several (α-*P*-borano)-NDPs and investigated their binding affinity with rabbit muscle PK and its transition-state analogue complex. We also investigated binding and inhibitory properties of (α-*P*-borano)-NTPs to PK, to determine possible toxicity of those derivatives.

Quenching of intrinsic PK tryptophan fluorescence upon addition of modified NDP was used to determine dissociation constant (K_d) values of the enzyme–substrate complex and the PK-Mg^{2+}-NDP-NO_3^--oxalate transition-state analogue complex. The K_d values for the PK transition-state analogue complex allow better differentiation of the affinity of NDP derivatives than do the K_d values for direct binding of NDPs with the enzyme. The α-*P*-boranophosphate modification decreases the binding affinities of ribo- and 2′-deoxyribo-NTPαBs and NDPαBs by 0.2–0.6 kcal/mol, but increases the binding affinity of 2′,3′-ddCTPαB analogues. No significant stereospecificity for binding of rabbit muscle PK with the Rp- and Sp-stereoisomers of NDPαB, NTPαB, dNTPαB, and ddNTPαB was observed.

From published crystal structure data, a comprehensive picture of the interactions of PK with ADP in the transition-state analogue complex has been derived. The fluorescence quenching approach allows us to characterize quantitatively each interaction and to determine its importance for binding affinity.

Prodrugs

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In Vitro Analysis of Iododeoxyuridine Ester Prodrugs for Activity Against Orthopoxviruses

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The potential re-emergence of smallpox through its use as a bioterrorist weapon has underscored the need for new agents for treatment of orthopoxvirus infections. Although cidofovir has been approved for emergency treatment of smallpox and complications from vaccination, there is general agreement that additional drugs are needed that are active when given orally and have less toxicity. Neyts, Verbeken and De Clercq (2002) reported that 5-iodo-2′-deoxyuridine (IDU) could reduce morbidity and mortality in a lethal model of vaccinia virus infection in mice. To increase the in vivo anti-poxvirus efficacy of IDU and to provide useful data for potential application for optimization of other anti-orthopoxvirus nucle-

osides, we synthesized several IDU carboxylate-based diesters. These IDU-derivative esters are expected to display a substantial variance in biological half-lives, which were determined experimentally by HPLC. In cell culture, as ascertained by both cytopathogenic effect and by plaque reduction assays, several of these prodrugs show significant activity against both vaccinia and cowpox viruses. Most notably, the 3′-5′-dipropanoate ester of IDU had EC_{50} values of 16 and 8.4 μm in plaque reduction assays against vaccinia and cowpox virus, respectively.

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Synthesis and Antiviral Activity of Alkoxyalkylesters of Cidofovir Monophosphate

Jacqueline C. Ruiz[1], James R. Beadle[1,2], Julissa Trahan[1], Kathy A. Aldern[2], Kathy A. Keith[3], Caroll B. Hartline[3], Earl R. Kern[3], Karl Y. Hostetler[1,2]

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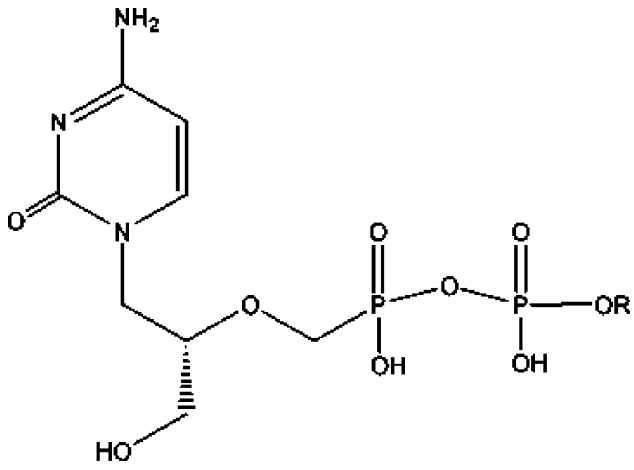
Hexadecyloxypropyl-cidofovir (HDP-CDV), an alkoxyalkylester of cidfovir (CDV), increased the antiviral activity of CDV by several logs. Studies on the cellular metabolism of HDP-^{14}C-CDV in MRC-5 cells indicate that the intracellular levels of the metabolite, cidofovir monophosphate (CDVp), are substantially lower than the levels of CDV or the active metabolite, cidofovir diphosphate (CDVpp). This suggests that the conversion of CDV to CDVp may be rate-limiting. We believe that bypassing the first phosphorylation step of CDV might increase cellular levels of the active metabolite CDVpp. Therefore, we synthesized hexadecyloxypropyl-phospho-cidofovir (HDP-P-CDV) and octadecyloxyethyl-phospho-cidofovir (ODE-P-CDV) by condensing protected dimethoxytritylcidofovir (DMTr-CDV) with HDP-phosphomorpholidate or ODE-phosphomorpholidate followed by deprotection with trifluoroacetic acid.

The alkoxyalkyl-phosphate esters of cidofovir were found to be more active than CDV. Compounds **1** and **2** had submicromolar EC_{50}s against HSV-1, MCMV and HCMV and were at least sevenfold more active than CDV. Compound **2** was particularly active against MCMV, vaccinia and cowpox. Although the newly synthesized alkoxyalkyl-phosphate adducts of CDV are somewhat less active than HDP-CDV, we believe that compounds of this type can bypass the slow first phosphorylation step. This approach may be especially useful for increasing the activity of poorly phosphorylated nucleoside phosphonates.



R= $CH_3(CH_2)_{15}O(CH_2)_3$- HDP-P-CDV **1**
R= $CH_3(CH_2)_{17}O(CH_2)_2$- ODE-P-CDV **2**

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Novel 5-Phosphono-pent-2-en-1-yl Nucleosides (PPen-Ns) and Their Alkoxyalkyl Phosphonoesters: Synthesis and Antiviral Evaluation

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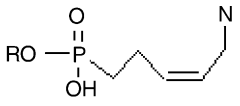
The acyclic nucleoside phosphonates (ANPs, e.g. cidofovir, adefovir, tenofovir), feature acyclic side chains within a phosphonate group or a phosphonomethoxy group. In an effort to evaluate ANPs with novel side chains, we designed, synthesized and tested a new series of unsaturated acyclic nucleoside phosphonates (**1**) against various viruses. The 5-phosphono-pent-2-en-1-yl nucleosides (**1**, PPen-Ns), were synthesized starting from 3-butyn-1-ol. Protected 3-butyn-1-ol underwent one-carbon homologation by hydroxymethylation. One of the two hydroxyl groups was converted to bromide, then phosphonic acid, while the other was replaced with various nucleobases using the Mitsunobu reaction. The PPen-Ns were also esterified with long-chain alkoxyalkanols, a modification that increases the intracellular delivery of ANPs. The hexadecyloxypropyl (HDP-PPen-Ns), octadecyloxyethyl (ODE-PPen-Ns) and oleyloxyethyl (OLE-PPen-Ns) phosphonoesters of each phosphonic acid were prepared. The unsaturated ANPs were evaluated against various viruses including HCMV, HSV-1, vaccinia, cowpox, HIV-1, VZV, HBV and EBV. Most PPen-Ns did not exhibit significant antiviral activity, but some of the alkoxyalkyl esters showed moderate to potent broad-spectrum antiviral activity. HDP-PPen-A was effective against EBV (EC_{50} = 0.1 μM), HBV

(EC_{50} = 2.0 μM) and HSV-1 (EC_{50} = 18 μM). HDP-PPen-G inhibited VZV (EC_{50} = 0.15 μM), HCMV (EC_{50} = 0.7 μM), HSV-1 (EC_{50} = 2.5 μM) and HBV (EC_{50} = 2.5 μM). These results indicate that several PPen-N alkoxyalkyl esters possess significant antiviral activity and should be investigated further for use against viral infections in humans.



1

R = H, HDP, ODE or OLE
N = adernine, guanine , uracil, cytosine and thymine

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Lung Targeted Antivirals: Studies with 1-*O*-Octadecyl-2-O-benzyl-sn-glycero-3-cidofovir

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Our previous studies showed that alkoxyalkyl analogs of acyclic nucleoside phosphonates are orally bioavailable but generate low levels of drug in the lung, a key early site of poxvirus replication. In this study, we examined the oral bioavailability and tissue levels of 1-*O*-octadecyl-2-*O*-benzyl-sn-glyceryl ester of ^{14}C-cidofovir (ODBG-CDV) in mice. We reported previously that ODBG-CDV was active against cowpox and vaccinia strains in the EC_{50} range of 0.09 to 0.4 μM. In pharmacokinetic studies, a single dose of 10 mg/kg of ODBG-[^{14}C]-CDV was administered orally or intraperitoneally (i.p.) to mice. Animals were sacrificed and plasma and various tissues were obtained at 1, 3, 6, 12, 24, 48, and 72 h. The organs were weighed, treated with TS-2 tissue solublizer, and aliquots of plasma and tissue were counted and drug content determined. After oral administration, peak plasma concentration of ^{14}C-labeled ODBG-CDV and metabolites was 1.1 μM, declining to 0.03 μM at 72 h; following i.p. administration of ODBG-CDV, peak plasma concentration was 10 μM declining to 0.07 μM at 72 h. Relative oral bioavailability, based on the area under curve (AUC) for plasma drug and metabolites, was calculated to be 32%. Surprisingly, oral ODBG-CDV gave peak lung levels of 111 nmol/g versus only 10 nmol/g with i.p. ODBG-CDV. Lung AUC was roughly 10-fold greater following oral administration of ODBG-CDV versus HDP-CDV in spite of the lower oral bioavailability of the former. When compared with oral hexadecyloxypropyl-CDV, ODBG-CDV provides a much higher AUC of drug and metabolites in the lung and a lower AUC in liver. In conclusion, esterification of CDV with 1-*O*-octadecyl-2-*O*-benzyl-sn-glycerol provides for a compound which, when given orally, appears to target the lung and could be useful for achieving greater therapeutic effects against viral infections in the lung.

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Activity of Alkoxyalkyl and Alkyl Esters of (*S*)-3-Hydroxy-2-phosphonylmethoxypropyl Derivatives of Cytosine (HPMPC, Cidofovir) and Adenine (HPMPA) and Cyclic Cidofovir Against Orthopoxviruses

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Cidofovir (CDV) and cyclic cidofovir (cCDV) have been shown to be potent inhibitors of poxvirus replication in vitro and in several animal models. However, these compounds are not active when administered orally. We have now evaluated the activity of several alkoxyalkyl and alkyl esters of CDV, cCDV and HPMPA, including hexadecyloxypropyl (HDP), octadecyloxyethyl (ODE), oleyloxyethyl (OLE) oleyloxypropyl (OLP) and 1-*O*-Octadecyl-2-*O*-benzyl-glyceryl (ODGB) derivatives against vaccinia virus (VV, Lederle strain) and cowpox virus (CPV, Brighton strain) in monolayer cultures of human embryonic lung (HEL) fibroblasts and in primary human keratinocytes (PHKs). Some derivatives were also tested in organotypic epithelial "raff" cultures, an ex vivo model representative of fully differentiated skin. All the analogues tested were more active than the parent compounds, the order of increasing activity against both VV and CPV in HEL cells being ODGB-CDV < OLP-CDV ~ HDP-CDV < OLE-CDV < ODE-CDV (a 50–800-fold increase in EC_{50} values compared to the parent compound) and in PHKs HDP-CDV ~ OLP-CDV < ODGB-CDV < ODE-CDV < OLE-CDV (a 10–200-fold increase in EC_{50} values compared to the parent compound). In both cell types, OLE-cCDV, HDP-HPMPA and ODE-HPMPA proved more active than the parent compounds, ODE-HPMPA being more active than HDP-HPMPA. To evaluate the effects of the compounds in the raft cultures, two series of cultures were run in parallel, one was used for histology and the other one for quantification of infectious virus. OLE-CDV, ODE-CDV and HDP-CDV proved more active than CDV; thus, at a concentration of 1 μg/ml both OLE-CDV and ODE-CDV were able to inhibit viral production by more than 5 logs, while at the same concentration a 3 log and a 1.5 log reduction was observed with HDP-CDV and CDV, respectively. Histological examination of the raft cultures correlated with quantification of virus yield.

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Amino Acid Ester Prodrugs of 2-Bromo-5,6-dichloro-1-(β-D-ribofuranosyl)benzimidazole Enhance Metabolic Stability In Vitro and In Vivo

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BDCRB is a potent and selective inhibitor of HCMV, but it lacks clinical utility due to rapid metabolism. We hypothesized that BDCRB prodrugs could evade BDCRB-metabolizing enzymes and that this bioevasion would enhance the in vitro efficacy as well as the in vivo half-life and extent of BDCRB systemic exposure. To this end, thirteen different amino acid prodrugs of BDCRB were synthesized and tested for N-glycosidic bond stability, ester bond stability, Caco-2 cell uptake, antiviral activity and cytotoxicity. Prodrugs exhibited significantly enhanced N-glycosidic bond stability and a wide range of ester bond stability. Most compounds were rapidly absorbed by Caco-2 cells, with the exception of the charged Asp- and Lys-prodrugs. Phe-BDCRB exhibited the most selective inhibition of HCMV replication with IC_{50} = 0.35 μM and CC_{50} = 100 μM, but it was not chosen for in vivo testing due to its rapidly cleaved ester bond. Asp-BDCRB was chosen for assessment of in vivo pharmacokinetics based on its favorable stability profile and antiviral activity that was well separated from cytotoxicity. In addition, D-Ile-BDCRB, which exhibited rapid Caco-2 cell absorption as well as the most stable ester bond and N-glycosidic bond, was chosen for in vivo testing. D-Ile-BDCRB was too stable to release sufficient BDCRB into the blood. Asp-BDCRB, however, exhibited a fivefold increase in the volume of distribution and roughly equivalent systemic exposure to the parent drug. Taken together with the fivefold improvement in half-life, Asp-BDCRB clearly exhibited bioevasion and further suggested that bioevasion can enhance drug delivery.

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A Possible Synthetic Strategy to Diastereomerically Pure *cyclo*Sal Prodrugs

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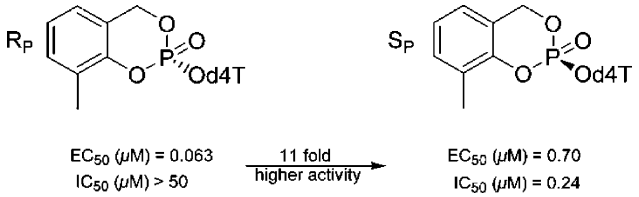
The *cyclo*Sal-prodrugs have been introduced as an intracellular delivery system for therapeutically active nucleotides.

Due to the synthesis of the *cyclo*Sal-phosphate triesters, the prodrugs are isolated as a 1:1 diastereomeric mixture. In some cases it is possible to separate the diastereomers by semipreparative HPLC. Chemical hydrolysis studies show a difference in the chemical stability of the stereoisomers. The R_P-configurated stereoisomers were found to be antivirally more potent and less inhibitory to BChE than S_P-stereoisomers (e.g. 3-Me-*cyclo*Sal-d4T, shown below).



Unfortunately, it is not possible to separate all *cyclo*Sal-triesters into their diastereomers by semipreparative HPLC. Therefore, a synthesis leading to the single isomers would be of great interest.

We will present a synthetic route to phosphoramidates based on a 2-substituted pyrrolidine moiety with high diastereomeric excess. These amidates may be converted into isomerically pure phosphate triesters.

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Novel "Lock-in" Modified *cyclo*Sal Nucleotides (I): Variations of the Linker Moiety

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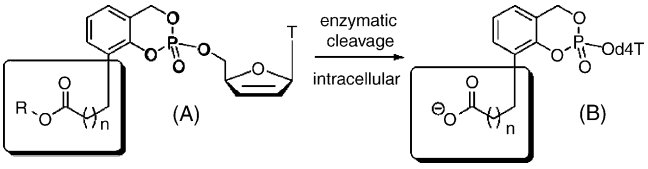
We present the second generation of *cyclo*Sal pronucleotides, the so-called 'lock-in' modified *cyclo*Sal-NMPs. The *cyclo*Sal-concept has already been applied successfully to several nucleoside analogues. The most remarkable advantages of the prototype *cyclo*Sal-NMPs is a purely pH-induced delivery of the nucleotides. However, due to the lipophilic nature of the *cyclo*Sal-triesters, it cannot be excluded that a concentration drug equilibrium is formed between the culture medium and the cell content. Therefore, the intention was to modify the *cyclo*Sal-triesters in order to trap them inside the cells. To achieve this goal, phosphate triesters bearing esterase-cleavable sites in the *cyclo*Sal-moiety **A** were synthesized.



The modification was attached to the *cyclo*Sal-moiety via an alkyl-linker. The ester group was introduced in the 3-position of the masking unit. After enzymatic cleavage the much more polar *cyclo*Sal-NMP-carboxylate **B** or -alcohol could be released. The increased polarity should prevent the efflux of the compound and enable a 'lock-in'. Our previous work revealed that esters from *cyclo*Sal-alcohols with C2-linkers were enzymatically cleaved. Surprisingly, the inverted esters that should give the carboxylate showed no enzymatic cleavage. So, the aim was to increase the linker length to improve substrate properties. The synthesis and anti-HIV activity data will be presented and related to the results of the drug stability studies in chemical media and cell extracts.

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*Cyclo*Amb Nucleoside Phosphonates: Nucleoside Phosphonate Prodrugs Based on the *cyclo*Sal Concept

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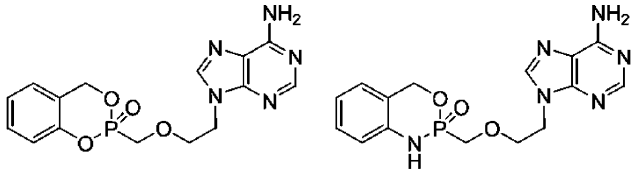
The *cyclo*Sal pronucleotides efficiently deliver therapeutically active nucleoside monophosphates in cells. The nucleoside phosphonates PMEA, PMPA and HPMPC show very broad antiviral activity against a couple of DNA- and retroviruses. Because of the interesting properties of these nucleoside phosphonates as nucleotide mimics that have a stabile P–C bond, lipophilic prodrugs of PMEA based on the *cyclo*Sal concept were synthesized. These compounds showed very low hydrolysis stability leading to antiviral activities that were only slightly better than the non-masked nucleoside phosphonates.



*cyclo*Sal-PMEA *cyclo*Amb-PMEA

Because of lack of inhibition of human BChE and selective drug delivery in all cases we decided to design more stabile nucleoside phosphonates. Higher stability was achieved by substitution of the phenolic O-atom by the less electronegative N-atom. The synthesis, characterization and in vitro antiviral evaluation of *cyclo*Amb PMEA will be presented. These new compounds show ideal stability, were not inhibitory to BChE and selectively deliver PMEA.

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Use of Biolabile Constructs for Mononucleotide Delivery

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In an attempt to improve the therapeutic potential of nucleoside analogs, various mononucleotide prodrugs (pronucleotides) have been reported during the last decade. Two main approaches have been developed requiring either structural modifications or introduction of transient groups. Usually, such groups are constituted by two components (double prodrug concept) and involve in their decomposition an enzyme-mediated process. Our work in this topic started with mononucleoside symmetrical phosphotriesters bearing SATE (*S*-acyl-2-thioethyl) groups as biolabile phosphate protection. Herein, we will present new series of mononucleoside mixed SATE phosphoesters involving two different enzymatic systems in their decomposition process. The ability of the studied mononucleoside SATE aryl phosphotriesters (Peyrottes et al., 2003), SATE phosphoramidate diesters (Egron et al., 2003) and SATE glucosyl phosphorothiolates to act as mononucleotide prodrugs will be demonstrated in cell culture experiments.

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tBuSATE (Dipeptidyl) Phosphotriesters as Potential Pronucleotides

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The use of pronucleotides appeared as a valuable strategy to overcome cellular limitations associated to the first phosphorylation step of nucleoside analogues. At that time, our research interest has focused on the study of mixed pronucleotides carrying two different biolabile phosphate protecting groups. In this respect, we designed the series of tBuSATE (aryl) phosphotriester derivatives of 3′-azido-2′,3′-dideoxythymidine (AZT) and shown that such kinds of derivatives can act as mononucleotide prodrugs, with liberation of the corresponding 5′-monophosphate following two successive enzymatic activities due to esterases and phosphodiesterases, respectively (Schlienger et al., 2000; Peyrottes et al., 2003).

Herein, we will describe synthetic approaches and stability studies of tBuSATE (aryl) phosphotriester derivatives of AZT where the aryl counterpart is a dipeptidyl residue.

References

Peyrottes, S., Coussot, G., Lefebvre, I., Imbach, J.-L., Gosselin, G., Aubertin, A.-M., Pèrigaud, C., 2003. J. Med. Chem. 46, 782–793.

Schlienger, N., Peyrottes, S., Kassem, T., Imbach, J.-L., Gosselin, G., Aubertin, A.-M., Pèrigaud, C., 2000. J. Med. Chem. 43, 4570–4574.

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Phosphoramidate Prodrugs of the Most Potent and Selective Anti-VZV Bicyclic Pyrimidine Nucleosides

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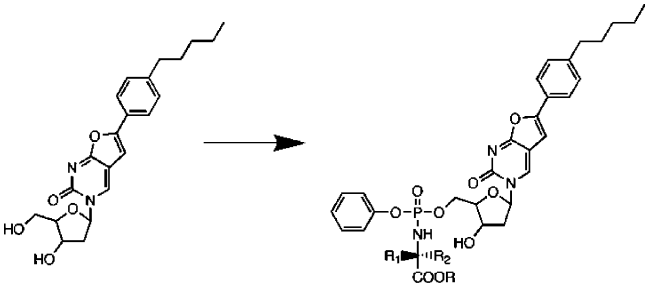
The phosphoramidate technology has been conceived as a means to improve cellular penetration of nucleotides and to bypass the first step of kinase-mediated activation of nucleosides. This technology has recently been applied to the highly potent anti-VZV bicyclic furo-pyrimidine BCNA CF1743. According to this, a new series of phosphoramidates has been planned. A study has been made, changing the ester moiety and using several amino acids. To identify any structure–activity relationships and mechanism of action. The synthesis involves the coupling of the phenyl dichloro phosphate with an esterificated amino acid salt. The products of this reaction, phosphochloridates, are then coupled with CF1743 in the presence of *N*-methyl-imidazole to give the desired products. A total of 12 compounds were synthesized and evaluated for antiviral activity against VZV. The results received so far are inconclusive as to any SARs, but show that some of these prodrugs are active at concentrations less than 0.01 μM. Full antiviral data will be presented at the conference.



CF1743

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Synthesis of Nucleoside Boranophosphoramidates Conjugated with Amino Acids as a New Class of Promising Prodrugs

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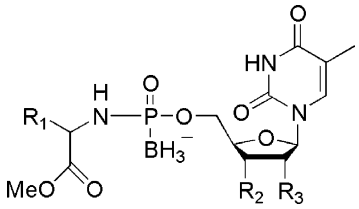
Nucleoside boranophosphates and nucleoside amino acid phosphoramidates have shown potent antiviral activity with the potential to act as nucleotide prodrugs. A combination of these two types of compounds results in a boranophosphoramidate linkage between the nucleoside and amino acid. This new class of potential prodrugs is expected to possess advantages conferred by both types of parent compounds. We developed two new strategies for the synthesis of nucleoside boranophosphoramidates conjugated with amino acids. One of them involves H-phosphonate chemistry, whose application was limited due to the low yields and purification difficulty. The oxathiaphospholane approach proved to be a better method to obtain the boranophosphoramidates. Based on an ‘adjacent’ type mechanism, the tentative assignment of configurations for both *P*-diastereomers was carried out through molecular modeling and proton spectra, in which the results were all in accordance with each other. The two *P*-diastereomers of nucleoside amino acid boranophosphoramidates are expected to have different substrate properties toward phosphoramidases and should be useful for investigating the roles of phosphate and metal ions in biological processes to elucidate the stereochemical and metal requirements of the enzymatic reactions involving phosphoramidases. Moreover, due to the presence of a borane group, the boranophosphoramidates are expected to have increased lipophilicity relative to their parent phosphoramidate compounds, which would facilitate the delivery of prodrugs containing antiviral nucleosides. All of these advantages, in combination with the potential utility as a carrier of ^{10}B in BNCT, make the nucleoside boranophosphoramidate conjugated with amino acid a useful compound and biochemical tool in antiviral drug research.



New Antivirals

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An Overview of Antimicrobial Peptides and Their Therapeutic Potential as Antiviral Drugs

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Antimicrobial peptides (AMPs) are an essential part of innate immunity that evolved in most living organisms over 2.6 billion years to combat microbial challenge. These small cationic peptides are multi-functional effectors of innate immunity on skin and mucosal surfaces and have demonstrated direct antimicrobial activity against various viruses (HSV-1 and -2, CMV, VSV, HIV, influenza virus, adenovirus, and vaccinia virus), bacteria, fungi, and parasites. Our laboratory has demonstrated direct antiviral inhibitory activity in vitro by human alpha and beta defensins and cathelicidin against HSV-1 and various adenovirus serotypes. The general mechanisms of rapid killing by AMPs are attributed to perturbation of lipid membranes, blockage of virus entry into the cell, and other undefined mechanisms. Commercial development of engineered antimicrobial peptides as systemic and topical therapies for various diseases has progressed through clinical trials. We will summarize their progress to date as well as critically evaluate AMPs clinical promise and practical limitations as novel anti-infective drugs. The potential role of customized antimicrobial peptides as single therapeutic agents, as adjuncts to conventional antivirals/antibiotics, and as immunostimulators of natural innate immunity will be discussed.

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Antiviral Activity of 1,2-Dithiol-3-propylsulfonat Sodium In Vitro and In Vivo

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Influenza and herpes viruses attract the attention of clinicists and researchers around the world, because they initiate massive acute and chronic diseases. According to World Health Organization data, almost 70% of the Earth human population is infected by herpes viruses. The diseases caused by herpes virus held the second place (15.8%) after influenza with 35.8% as the cause of viral infections' lethality. That is why, the elaboration of the methods and means of such infections' treatment are the actual task for health care system. The scope of the present work was to study antiviral efficacy of 1,2-dithiol-3-propylsulfonat sodium (DPS) towards influenza A, B strains viruses and herpes simplex virus (HSV). We have had to study the antiviral activity of said preparation against the Newcastle disease virus (NDV). We have studied influence on the proteolytic processes of virus membranes interaction on DPS.

Antiviral activity of DPS was studied in vitro on the model of influenza virus strains A/Hong Kong/1/68(H3N2), A/PR/8/34(H1N1), B/Leningrad/17/86 and on NDV strain La Sota replication on the tissue culture of 11–14-days chicken embryos' choryoallantoic membranes (CAM). Anti-herpetic activity of DPS in vitro was studied in primarily tripsinized culture of chick embryos cell (CEC) against the HSV-1 (strain US). The anti-influenza activity DPS in vivo was evaluated on the ground of the animals' lethality reduction during the 14 days after infection.

The results of the present study allow us to state that 1,2-dithiol-3-propylsulfonat sodium demonstrates sufficient antiviral activity both in vitro and in vivo. The preparation taken in dose 1 mkg/ml has inhibited the reproduction of influenza A and B viruses as well as NDV in the tissue culture CAM. DPS in dose 2.5–5 mkg/ml has inhibited HSV-1 reproduction in primarily tripsinized culture of CEC. Prophylactic and therapeutic application of DPS was demonstrated through considerably decreased mortality of animals infected with influenza virus. Preparation inhibits the proteolysis increase that takes place during virus–membrane interaction.

The results of the present study allow us to state that 1,2-dithiol-3-propylsulfonat sodium demonstrates significant antiviral activity and could be recommended for clinical trial.

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Structurally Unrelated Pharmacological CDK Inhibitors (PCIs) Target Initiation of Transcription from Viral Genomes, A Novel Target for Antiviral Drugs

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Antiviral drugs are commonly designed to target viral proteins. Although very successful, this approach has certain limitations. For example, drugs that target viral proteins tend to be active against only one or a few closely related viruses and to quickly select for drug-resistant strains. Because of such limitations, cellular proteins may be considered as potential targets for novel antivirals. This approach is especially attractive because many inhibitors of cellular proteins have been developed as drugs against cancer or metabolic diseases. Cyclin-dependent kinases (CDKs) are arguably the cellular proteins best studied as potential targets for antiviral drugs. Specific pharmacological CDK inhibitors (PCIs) have been developed, such as roscovitine, which appear to be

well tolerated in clinical trials against cancer. Several PCIs, such as roscovitine and flavopiridol, have antiviral activity in vitro against wild-type or drug-resistant strains of HIV, HCMV, HSV-1, HSV-2, EBV, VZV, KSHV, HTLV, and other viruses. PCIs are scheduled to enter clinical trials as antivirals in 2005. However, the antiviral mechanisms of different PCIs remain incompletely characterized. PCIs such as roscovitine and flavopiridol have different molecular specificities and have been shown to inhibit different functions of different viruses. To evaluate whether PCIs may also target a common viral function, we analyzed the antiviral activities of roscovitine and flavopiridol, structurally unrelated PCIs that preferentially inhibit different subsets of CDKs. Herein, we show that unrelated PCIs prevented initiation of HSV-1 transcription. These antiviral effects required no viral proteins or promoters but were specific for viral genomes. The more specific PCIs, such as roscovitine, were more specific for viral genomes. These results show that PCIs have a common antiviral target and identify a novel functional target for antiviral drugs, initiation of transcription from viral genomes. Drugs that target such function could be less prone to select for drug-resistant mutants than conventional antiviral drugs.

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Synthesis and Study of New Conformationally Restricted Nucleoside Analogues

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Over several decades, a large number of nucleoside analogues have been synthesized and some of them have been shown to present potent antiviral or antitumoral activities. In order to discover new nucleoside derivatives endowed with antiviral activities, modifications of the base and/or sugar moiety of natural nucleosides can be attempted. For our part, we chose to introduce modifications on the sugar capable of restricting the dynamic equilibrium between the northern-type and southern-type geometry that normally characterize the sugar moiety of standard nucleosides in solution. In this respect, we have synthesized new conformationally locked nucleoside analogues built on a 2-oxabicyclo[3.1.0.]hexane system bearing pyrimidine bases. Assuming that the conformation and puckering of the glycon moiety of nucleosides play a critical role in modulating biological activity, such new conformationally restricted nucleoside analogues, could be used to obtain further information regarding the correlation between sugar ring conformation and biological activity. Herein, we will report on the synthesis, as well as the results of some antiviral evaluations against a broad range of viruses.

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Effects of Interferon Alpha on Human Hepatoma Cell Lines: DNA Microarrays Analysis and Evaluation of Cell Proliferation

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Aim of this study was the investigation of IFNα effects on gene expression profile and proliferation of human hepatoma cells. Microarray analysis using slides containing 21,500 human oligonucleotides was performed in human HepG2, Hep3B and HuH7 hepatoma cell lines treated with 1000 UI/ml IFNα for 4, 8, 24, and 48 h. Analysis of cell viability and cell cycle was performed in cells treated with different doses of IFNα at the same time points. Analysis of microarray experiments demonstrated that a total of 73 genes were induced and 19 were repressed in HepG2 cells whereas in HuH7 cells 57 genes were induced and a total of 116 genes were repressed while in Hep3B cells 72 genes were over-expressed and 50 genes were under-expressed at least twofolds with respect to untreated cells at all time points. Induced genes included gene previously implicated as IFNα-inducible genes (e.g., *OAS1*, *IFITM1*, *DCK*, *IFIT4*, *STATs*), but also potassium channels (e.g., *KCNJ1*, *KCNE2*), transcription factors (e.g., *TFDP2*, *NR2E3*), G protein-coupled receptors (e.g., *GPR43*, *NPR2*), and receptor co-factors (e.g., *TRIP6*, *RAMP1*). Inhibited genes included mainly those involved in cell metabolism (e.g., *CYP2B7*), cell proliferation (growth factors and growth factor receptors), and viral infection (virus receptors, viral oncogenes, cell factors interacting with viral proteins). Moreover, hierarchical cluster analysis identified genes up-regulated within the first hours of IFNα treatment or after 24–48 h. Short-term treatment with high doses IFNα inhibited hepatoma cell proliferation and modified cell cycle, as demonstrated by MTT assay and flow cytometry. In conclusion, we characterized IFNα-modulated genes in human hepatoma cell lines and demonstrated that IFNα inhibits expression of genes involved in viral replication and cell proliferation, thus supporting its use as antiviral and anti-cancer agent.

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Development of Highly Potent Pyrimidinedione Inhibitors as Topical Microbicides

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Topical microbicides to prevent the sexual transmission of HIV are urgently needed, especially in developing countries. Efforts to develop topical microbicides have received increasing attention, recognizing the unmet need for a safe and effective, easy to use and inexpensive means to reduce the rates

of HIV transmission. At present a wide variety of therapeutic agents are being evaluated as topical microbicides, most especially substances, which act to prevent virus attachment to target cells. In addition to these entry inhibitors, highly potent nonnucleoside RT inhibitors have also been evaluated. We began development of pyrimidinediones as topical microbicides based on their unique dual mechanism of anti-HIV action that includes highly potent NNRTI activity as well as the ability to inhibit virus entry at a conformational target formed upon interaction of virus and cells. The series of compounds has thus far been exemplified by the lead molecule SJ-3366 [1-(3-cyclopenten-1-yl)methyl-6-(3,5-dimethylbenzoyl)-5-ethyl-2,4-pyrimidinedione] which inhibits the replication of all tested clinical strains of HIV-1 and HIV-2 at sub-nanomolar concentrations in fresh human PBMCs. A wide variety of congeners of SJ-336 were evaluated for their ability to inhibit virus attachment and reverse transcription and the compounds were ranked based on their activity. Several highly active entry inhibitors with therapeutic indices ranging from 0.5 to 4 million were identified and evaluated as potential topical microbicides in various anti-HIV assays, including activity in PBMCs, in CD4-dependent and CD4-independent cell based assays, combination anti-HIV activity assays, assays at various MOIs and in the presence of high concentrations of mucin/polysaccharide. The compounds were also evaluated in an ex vivo cervical epithelial cell based tissue assay which measures the inhibitory activity of the SJ compounds in a relevant tissue based assay. The results of the in vitro and ex vivo assays suggest that the pyrimidinones are highly effective topical microbicides that combine the ability to inhibit both virus entry and reverse transcription. SAR evaluations to define the molecular features responsible for the relative potency of the molecules in the inhibition of attachment and RT have been performed and will be presented.

First Author Index—Abstract Number

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Invitation to the Nineteenth International Conference on Antiviral Research
San Juan, Puerto Rico
May 7–11, 2006

The 19th International Conference on Antiviral Research, preceded by a Clinical Symposium, will be held in San Juan, Puerto Rico. The conference will begin on Sunday, May 7, 2006 and will end at 4 p.m. on Thursday, May 11, 2006. All scientific sessions will be held at the Caribe Hilton in San Juan.

The purpose of the International Conference on Antiviral Research is to provide an interdisciplinary forum where investigators involved in basic, applied, and clinical research worldwide can meet to review recent developments in all areas of antiviral research. Specific topics to be covered in the program include synthesis and chemistry, biochemistry and mechanism of action, molecular biology and drug targeting, in vitro evaluation, animal models, pharmacokinetics, toxicology, and clinical trials. Within these areas of interest, there will be invited overview speakers, oral presentations, and poster presentations.

San Juan offers an excellent site for our Conference. It offers wonderful conference facilities, and an excellent atmosphere as well. It is a unique tropical island where you can find local exotic hideaways, miles of white sandy beaches, mountains and valleys, a rain forest and other natural wonders all within reach surrounded by a warm friendly people.

If you are fortunate enough to have some time before or after the Conference, it is an ideal site for lovers of the outdoors who can hike in El Yunque, an unbelievable rain forest 40 km from San Juan, that provides rare wildlife and over 240 species of flora. San Juan provides a diver's paradise for scuba and snorkeling with warm water temperatures and underwater visibility of 60–75 feet allowing excellent viewing of rich coral reefs and gardens. Alternatively, you may elect to spend some time on the miles of beautiful beaches, such as Isla Verde or on the picturesque golf courses.

The old town provides great local color and museums of note. The Old City is inviting with its narrow, steep streets paved with cobbles of adoquine, a blue stone cast from furnace slag, a 7-square block area of charming 16th and 17th century Spanish colonial buildings and many plazas. Spectacular attractions are plentiful in San Juan, including the incredible El Morro, a six-level fortress situated 140 feet above the Atlantic. The views of San Juan Bay from El Morro are breath taking, and the fort is open to the public daily.

The historic intermingling of Spaniards, Africans, Italians, French, German, Lebanese, and Cubans has produced a very unique and diverse culture in Puerto Rico. The food in Puerto Rico is an exotic blend of Spanish, African, Taino and American influences. The locals call their cooking *Cocina criolla,* meaning Creole cooking, and it is similar to Spanish and Mexican cuisine. Puerto Rico is not a wine producer but it has great beers and, of course, a large variety of its national drink, rum. It is the worlds leading rum producer dating back to the sugar cane brought by Columbus in 1493.

We invite you to take advantage of this once-in-a-lifetime opportunity to combine an important learning experience with a magnificent travel experience and join us in San Juan, Puerto Rico for the 19th International Conference on Antiviral Research.

Future Conferences

2006: May 7–11, San Juan, Puerto Rico

2007: April 29–May 3, Palm Springs, California