utilized in order to reduce the numbers of false positive hits. Both targeted and general small molecule libraries were screened for inhibitors of SARS-CoV entry, and a number of compounds were identified that inhibit SARS-CoV entry and replication. In particular, we took advantage of our previous findings that cathepsin L in target cells is required for activation of SARS-CoV Spike, in order to focus on libraries of cysteine protease inhibitors. 16 positive "hits" with 95% inhibition or higher in the primary screen were further studied for drug dose–response, cell toxicity, and the ability to inhibit coronavirus 229E, Ebola and live SARS-CoV. Three related compounds, exhibiting potent antiviral activity ($IC_{50} < 10^{-4}$ μM) were selected for small animal studies.

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Escaping Development of Drug-Resistant Mutants: Basis for Effective Chemotherapy of Enterovirus Infections

Angel S. Galabov[1,*], Ralitsa Vassileva-Pencheva[1], Ivanka Nikolova[1], Boris Atanasov[2]

[1] *The Stephan Angeloff Institute of Microbiology, Bulg. Acad. Sci., Sofia, Bulgaria;* [2] *Institute of Organic Chemistry, Bulg. Acad. Sci., Sofia, Bulgaria*

Enteroviruses are causative agents of more than 50 various diseases, including meningitis, encephalitis, pleurodinia, myocarditis, pericarditis, insulin-dependent diabetes mellitus, etc. Most of enteroviruses induce several clinical syndromes, a phenomenon unique in the infectious pathology. The great majority of these infections are unapparent ones or have a subclinical course. Prevailing role in the strategy of counteraction of enteroviral infections is the use of anti-enteroviral chemotherapeutic agents administered during disease latency period—urgent prophylaxis. It might reduce to a minimum the risk of enterovirus induced myocarditis, acquired diabetes in infant age and other enterovirus infections with severe course. The main obstacle of the development of effective anti-enteroviral chemotherapy is the development of drug-resistance, phenomenon based on the unusually high level of mutation rate (10^{-3}). We carried out systematic study of the drug-resistance on the model of coxsackievirus B1 neuroinfection in mice treated with disoxaril, WIN compounds, binding to the hydrophobic pocket of enteroviral VP1 protein. In parallel, disoxaril-resistant and disoxaril-dependent Cox B1 mutants have been developed in vitro, in FL cells. Phenotypic characteristics, VP1 genome sequencing and VP1 protein sequence deduction of disoxaril mutants have been determined. Sequence changes gave satisfactory explanation for mutant resistance and on the unusual effect of inhibitor-dependence. Combination effects of anti-enteroviral agents with different modes of action have been carried out in cell culture experiments and a series of synergistic combinations have been selected. Administration of antivirals in synergistic combinations could be considered as a prospective approach to decrease the level of drug-resistance and to improve the chemotherapy efficacy. A new scheme of application of the partners in the synergistic combinations was developed on the model of experimental coxsackievirus B1 infection in newborn mice. The maximum protective effect was reached with the combination disoxaril/guanidine.HCl/oxoglaucine.

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Novel Small Molecule Inhibitors of Dengue Virus Replication

Chelsea Byrd*, Jessica Page, Chris Harver, Doug Grosenbach, Aklile Berhanu, Dennis Hruby, Robert Jordan

SIGA Technologies, Corvallis, USA

There is an urgent need for new antivirals for both treatment and control of dengue virus, given that over 50 million people are infected worldwide every year and there are no approved vaccines or antiviral drugs available. An antiviral drug that inhibits viral replication without increasing the risk for antibody-dependent enhancement (ADE) of infection would be extremely valuable for public health by providing a means to control outbreaks, as well as to travelers to endemic regions. The goal of the SIGA dengue program is to develop a small molecule therapeutic for the treatment and/or prevention of disease caused by dengue virus, with a final drug product that will be a safe, effective, and orally administered antiviral compound. A sensitive and specific high throughput screening (HTS) assay has been developed to evaluate compounds from the SIGA chemical library for inhibitory activity against dengue-2 (DEN-2) virus replication. Hits have been identified that are potent (EC50 < 5 μM) and selective (CC50 > 50 μM), with initial structure activity relationship in several series of related compounds. Early hits have structures that are chemically tractable, in that they possess chemically stable functionalities and have potential drug-like qualities. Lead series have been identified with activity against all four serotypes of dengue virus which are being defined by spectrum of activity, mechanism of action, preliminary absorption, distribution, metabolism, and excretion (ADME) profiles, and pharmacokinetic (PK) evaluations. One of these series has shown proof-of-concept efficacy in a murine model of disease. The identification and characterization of early stage dengue virus inhibitors with activity in a murine model of dengue virus infection represents a compelling start toward our goal.

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Escaping Development of Drug-Resistant Mutants: Basis for Effective Chemotherapy of Enterovirus Infections

Angel S. Galabov[1,*], Ralitsa Vassileva-Pencheva[1], Ivanka Nikolova[1], Boris Atanassov[2]

[1] *Institute of Microbiology, Bulg. Acad. Sci., Sofia, Bulgaria;* [2] *Institute of Organic Chemistry, Bulg. Acad. Sci., Sofia, Bulgaria*

Enteroviruses are causative agents of more than 50 various diseases, including meningitis, encephalitis, pleurodinia, myocarditis, pericarditis, insulin-dependent diabetes mellitus, etc. Most enteroviruses induce several clinical syndromes, a phenomenon unique in the infectious pathology. The great majority of these infections are unapparent ones or have a subclinical course. Prevailing role in the strategy of counteraction of enteroviral infections is the use of anti-enteroviral chemotherapeutic agents administered during disease latency period—urgent prophylaxis. It might reduce to a minimum the risk of enterovirus induced myocarditis, acquired diabetes in infant age and other enterovirus infections with severe course. The main obstacle for the development of effective anti-enteroviral chemotherapy is the development of drug-resistance, phenomenon based on the unusually high level of mutation rate (10^{-3}). We carried out a systematic study of the drug-resistance on the model of coxsackievirus B1 neuroinfection in mice treated with disoxaril, WIN compounds, binding to the hydrophobic pocket of enteroviral VP1 protein. In parallel, disoxaril-resistant

and disoxaril-dependent Cox B1 mutants have been developed in vitro, in FL cells. Phenotypic characteristics, VP1 genome sequencing and VP1 protein sequence deduction of disoxaril mutants have been determined. Sequence changes gave satisfactory explanation for mutant resistance and on the unusual effect of inhibitor-dependence. Combination effects of anti-enteroviral agents with different modes of action have been carried out in cell culture experiments and a series of synergistic combinations have been selected. Administration of antivirals in synergistic combinations could be considered as a prospective approach to decrease the level of drug-resistance and to improve the chemotherapy efficacy. A new scheme of application of the partners in the synergistic combinations was developed on the model of experimental coxsackievirus B1 infection in newborn mice. The maximum protective effect was reached with the combination disoxaril/guanidine.HCl/oxoglaucine.

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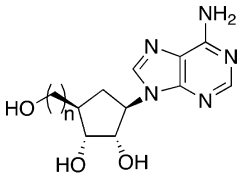
Arenaviral Inhibitory Activity of MY-24, a Novel Aristeromycin Derivative

Brain Gowen[1,*], Kie-Hoon Jung[1], Wei Ye[2], Min-Hui Wang[1], Deanna Larson[1], Donald Smee[1], John Morrey[1], Stewart Schneller[2]

[1] *Institute for Antiviral Research and Department of Animal, Dairy, and Veterinary Sciences, Utah State University, Logan, USA;* [2] *Chemistry & Biochemistry Department, Auburn University, Auburn, USA*

Several arenaviruses are known to cause viral hemorrhagic fever (HF), a syndrome often associated with a fatal outcome. The only option for antiviral intervention is ribavirin, a drug that has had mixed success in treating severe arenaviral HF, and has significant toxicity. Inhibitors of S-adenosyl-L-homocysteine hydrolase such as Aristeromycin (1) have shown promise as antivirals by disrupting essential viral macromolecular methylation processes. A search for new lead compounds based on 1, where structural variation occurred at the 5'-center, led to the 5'-homo derivative 2 (MY-24). Developing an efficacious synthesis of MY-24 allowed for evaluation of antiviral activity in cell culture systems based on infection with Pichinde, Tacaribe, or Junin arenaviruses, and in vivo against Pichinde virus (PICV) infection in hamsters. By virus yield reduction assay, the 90% inhibitory concentration against these viruses ranged from 0.64 to 3.54 μM, with therapeutic indexes ranging from 10 to 42. MY-24 was well-tolerated in hamsters up to the highest tested dose of 175 mg/kg/day. In PICV-challenged hamsters, MY-24 repeatedly extended survival and significantly reduced liver disease as measured by serum alanine aminotransferase, but no remarkable reduction in viral load was observed. Activity in cell culture against several arenaviruses and limited efficacy in treating hamsters infected with PICV may warrant further exploration of related Aristeromycin derivatives that may have improved activity in vivo. Funded by NO1-AI-15435, NO1-AI-30048, NO1-AI-30063, and U19-AI-56540, Virology Branch, NIAID, NIH.



1, X = CH_2, n = 1
2, X = CH_2, n = 2

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Pyrazine Derivative Treatment of Phleboviral Infection in Cell Culture and Rodent Model Systems

Brian Gowen[1,*], MIn-Hui Wong[1], Kie-Hoon Jung[1], Donald Smee[1], John Morrey[1], Yousuke Furuta[2]

[1] *Utah State University, Logan, USA;* [2] *Research Laboratories, Toyama Chemical Company Ltd., Toyama, Japan*

Several pyrazine derivatives have been reported to be effective in treating viral disease in mouse and hamster models. Remarkably, T-1106 has been found to be more effective than T-705 in treating yellow fever virus infection in hamsters. Based on these findings, we hypothesized that T-1106 may be a better choice for treating hepatotropic viral disease such as that caused by Punta Toro virus (PTV), a phlebovirus related to the highly pathogenic Rift Valley fever virus (RVFV). T-705 and T-1106 efficacy against several phleboviruses was evaluated in cell culture by cytopathic effect and virus yield reduction assays. Both compounds were also tested in the mouse and hamster PTV infection models. For additional comparison, T-705 and T-1106 were evaluated in the hamster Pichinde arenavirus infection model, as disease is based on diffuse pantropic infection. The compounds were administered orally, twice daily ranging from 25 to 100 mg/kg/day, for 5–7 days. In cell culture, the inhibitory effects of T-705 and T-1106 against PTV, sandfly fever virus, and RVFV (vaccine strain) were comparable with 50% and 90% inhibitory concentration ranges from 3 to 55 μM for T-705 and 8-75 μM for T-1106. In PTV-challenged hamsters, a model that generally presents with high liver viral burden, T-1106 was more effective at reducing mortality than T-705 when compared on a molar basis. In contrast, T-705 had better activity than T-1106 in preventing mortality in a Pichinde arenavirus infection in hamsters. Both compounds were equally effective in treating mice infected with PTV. The data support the idea that T-1106 may be a more effective treatment for severe viral diseases that predominantly target the liver. Supported by contracts NO1-AI-15435, NO1-AI-30048, and NO1-AI-30063 from the Virology Branch, NIAID, NIH.

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FGI-104: A Broad-Spectrum Small Molecule Inhibitor of Viral Infection

Michael Kinch[1,*], Abdul Yunus[1], Hanwen Mao[1], Calli Lear[2], Guangxiang Luo[3], Michael Murray[4], Zhuhui Huang[4], Zena Fesseha[1], Hanson Chen[1], Gene Olinger[2], Michael Goldblatt[1]

[1] *Functional Genetics, Inc., Gaithersburg, USA;* [2] *USA Army Medical Research Institute of Infectious Diseases, Fort Detrick, USA;* [3] *Department of Microbiology Immunology and Molecular Genetics, Lexington, USA;* [4] *Hepatitis Research Program, Frederick, USA*

The treatment of viral diseases remains an intractable problem facing the medical community. Most conventional antivirals focus upon selective targeting of virus-encoded targets. However, the plasticity of viral nucleic acid mutation, coupled with the large number of progeny that can emerge from a single infected cells, often conspire to render conventional antiviral ineffective as resistant variants emerge. Compounding this, new viral pathogens are increasingly recognized and it is highly improbable that conventional approaches could address emerging pathogens in a timely manner. Our laboratories have adopted an orthogonal approach to combat viral disease: Target the host to deny the pathogen the ability to cause disease. The advantages of this novel approach are many-fold, including the potential to identify host pathways that