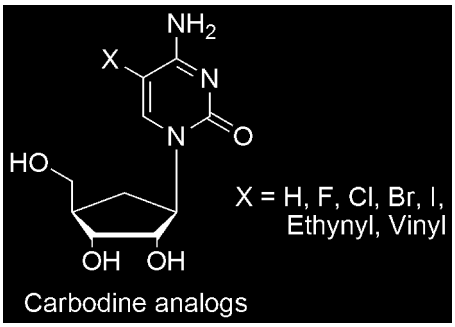


doi:10.1016/j.antiviral.2009.02.150

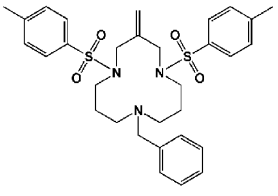
146

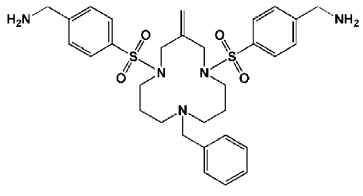
Synthesis of CADA Analog Prodrugs Designed as Novel Down-modulators of the CD4 Receptor

Emily Scarbrough[a,*], Sreenivasa Anugu[a], Kurt Vermeire[b], Dominique Schols[b], Thomas Bell[a]

[a] *Department of Chemistry, University of Nevada, Reno, USA;* [b] *Rega Institute for Medical Research, Department of Microbiology and Immunology, Katholieke Universiteit Leuven, Leuven, Belgium*

Cyclotriazadisulfonamide (CADA) inhibits HIV replication by specifically down-modulating expression of the of the CD4 receptor protein on host cells. Many analogs of CADA have been synthesized in order to enhance potency, reduce toxicity, and improve physical properties, especially solubility and cell permeability (Bell et al., 2006). These analogs have also been used to develop a three-dimensional, quantitative structure–activity relationship (3D-QSAR) computer model. Current studies are aimed at developing a pro-drug approach involving novel CADA analog ES02. This compound is expected to have a CD4 down-modulation potency that is similar to that of CADA, according to our 3D-QSAR model. ES02 is the parent compound for prodrugs bearing dipeptide chains that are covalently bonded to the two amino groups of the aminomethylbenzenesulfonyl side arms. Cleavage of these chains by dipeptidyl-peptidase IV (Garcia-Aparicio et al., 2006) is expected to convert the prodrugs into ES02. The synthesis of ES02 involves a new macrocyclization method using palladium as a catalyst. This technique avoids large solvent volumes, long reaction times, and polymer side products associated with the conventional, Richman–Atkins macrocyclization method. The anti-HIV and CD4 down-modulation activities of the novel CADA compounds will be presented.





Reference

Bell, et al., 2006. J. Med. Chem. 49, 1291–1312.
Garcia-Aparicio, et al., 2006. J. Med. Chem. 49, 5339–5351.

doi:10.1016/j.antiviral.2009.02.151

147

Bioreversible Protection of Nucleosidediphosphates—Synthesis and Properties

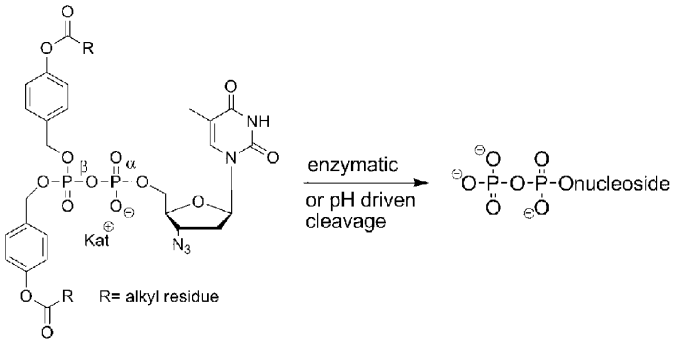
Tilmann Schulz[a,*], Henning J. Jessen[a], Jan Balzarini[b], Chris Meier[a]

[a] *Institue of Organic Chemistry, Department of Chemistry, Faculty of Science, University of Hamburg, Martin-Luther-King Platz 6, D-20146 Hamburg, Germany;* [b] *Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroederstraat 10, B-3000 Leuven, Belgium*

Nucleoside analogs are widely applied in antiviral and antitumor therapy. A severe limitation of these compounds arises from the need of biotransformation to the eventually active nucleoside triphosphates (NTP) by stepwise addition of phosphate groups by kinases which often proceeds insufficient. Prodrugs (e.g. the *cyclo*Sal- or phosphoamidate-approach) can possibly enhance the antiviral or antitumor activity of nucleotide analogs, enabling the nucleotide analogs to penetrate cellular membranes and protecting them from degradation by unspecific plasma phosphatases. They bypass the limited bioactivation of nucleoside kinases, hence rising the intracellular level of nucleoside monophosphates. However the subject of nucleosidediphosphate prodrugs has been addressed very rarely and unsuccessful.

This is remarkable, considering that, e.g. 3′-azido-3′-deoxythymidine (AZT) is only very slowly diphosphorylated by thymidylate kinase resulting in the loss of antiviral activity and unwanted side effects. For these reasons we turned our interest on the bioreversible protection of nucleoside diphosphates. This way of protection might also be very desirable for other nucleotide analogs.

We will present the synthesis and biological properties (pH- and cell extract stability, cytotoxicity, antiviral activity) of newly designed bis-(4-acyloxybenzyl)nucleoside-diphosphates which should act as nucleoside diphosphate prodrugs. Once insight the cell, the pyrophosphate protecting groups should be cleaved by enzymes resulting in a spontaneous release of the nucleotide.



doi:10.1016/j.antiviral.2009.02.152

148

Multivalent Synthetic Lectin Polymers Against HIV

Julie Jay[a,*], Bonnie Lai[b], Patrick Kiser[a]

[a] *University of Utah, Department of Bioengineering, Salt Lake City, USA;* [b] *Duke University, Department of Biomedical Engineering, Durham, USA*

While Phase III trials of microbicides proceed there still remain critical gaps in the therapeutic pipeline. Specifically there is a lack of affordable, and broadly efficacious entry inhibitors that target the HIV ENV complex, and are safe for repeated topical delivery. Cyanovirin-N, one of the most potent anti-HIV agents known, inac-

tivates HIV by binding to glycan residues on gp120. However, the prohibitive cost of mass producing and isolating this protein negate its deployment in the developing world. By exploiting the multivalency affect we have developed a synthetic lectin capable of binding to gp120. We present surface Plasmon resonance binding of small molecules to recombinant gp120 HIV BaL as a function of pH. Based on affinity and ease of synthesis, one lead compound was chosen. Synthesis of a monomer and incorporation at different feed ratios yielded polymers at 25, 50 and 75 mol%. Antiviral activity was tested in two R5-tropic, well-characterized, reference strains of HIV-1 isolated from acute, sexually transmitted infections and grown in peripheral blood mononuclear cells: HIV-1 DU156 (Clade C) and HIV-1 TRO (Clade B) as well as a pseudotyped CXCR-4 topic HIV-1 WEAU (Clade B) produced in 293T/17 cells. The EC_{50} for the 75 and 50 mol% were in the 1 to 5 μg/mL range (∼10 nM). The 75 mol% exhibited slightly higher activity against DU156, while the activity for the 50 mol% was similar across all three strains. Cytotoxicity against the vaginal cell line, Vk2/E6E7 vaginal cell lines after 24 h suggests these compounds may be safe and warrant further investigation as a vaginal lumen active entry inhibitor.

doi:10.1016/j.antiviral.2009.02.153

149

Induction of HIV-1 Gene Expression in Human Monocytic Cells by the Failed Microbicide Carrageenan Occurs Through Activation of Toll-like Receptor 4 (TLR4)

Shawn Keogan*, Vanessa Pirrone, Shendra Passic, Brian Wigdahl, Fred Krebs

Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, USA

Microbicides are intended for use by women in order to reduce or eliminate the risk of human immunodeficiency virus type 1 (HIV-1) sexual transmission. Carrageenan is a polyanionic compound that was among the first agents to undergo clinical evaluations as a microbicide. Unfortunately, despite the potent *in vitro* antiviral activity of carrageenan and the documented *in vivo* safety of Carraguard (the topical vaginal formulation containing the active ingredient carrageenan), Phase III clinical trials of Carraguard concluded that it was ineffective in preventing HIV-1 transmission. These results have prompted investigations into the mechanisms that underlie the failure of carrageenan. Previous studies of carrageenan as a food additive demonstrated its ability to act as an agonist for toll-like receptor 4 (TLR4), a pattern recognition receptor involved in initiating innate immune responses during pathogen invasion. Based on these observations, we hypothesized that the anti-HIV-1 activity of carrageenan may be offset by its capacity for increasing HIV-1 gene expression through stimulation of TLR4. In experiments in which human monocytic U-937 cells were transiently transfected with an HIV-1 long terminal repeat (LTR) reporter vector, carrageenan induced LTR activity to a level similar to that of LPS, which is a natural ligand of TLR4. These results suggest that the presence of carrageenan may facilitate HIV-1 infection of uninfected cells or increase the magnitude of viral replication in cells already infected with HIV-1. Experiments are now underway to fully characterize carrageenan as a TLR4 agonist in the context of HIV-1 infection and the clinical failure of Carraguard.

doi:10.1016/j.antiviral.2009.02.154

150

SEVI and Semen Impair the Anti-HIV-1 Activity of Drugs and Microbicides

Kim Kyeong-Ae[a,*], Shibo Jiang[b], Frank Kirchhoff[a], Jan Muench[a]

[a] *Institiute of Virology, Ulm, Germany;* [b] *New York Blood Center, New York, USA*

Currently, an enormous effort is made to develop vaginal microbicides to prevent sexual transmission of HIV. Thus far, however,microbicides have generally failed to reduce the rate of HIV-1 transmission. We have recently shown that human semen potently enhances HIV-1 infection and demonstrated that fragments of the prostatic acid phosphatase form amyloid like aggregates (termed "SEVI") that contribute to this effect (Muench et al., 2007).

Since HIV-1 containing semen is the major vehicle for sexual HIV-1 transmission worldwide it is highly relevant whether it affects the efficacy of preventive agents. To test this we examined the effect of semen and SEVI on the antiviral potency of a large number of antiretrovirals and first generation microbicides with various modes of action. We found that SEVI and semen substantially reduced the susceptibility of HIV-1 to these agents. For example, concentrations of polyanion based microbicides usually sufficient to block untreated HIV-1 infection had little if any inhibitory effect on virus that was briefly exposed to semen or SEVI.

Our data demonstrate that many microbicides and antiretrovirals are ineffective in inhibiting semen treated HIV-1infection. Thus, to develop potent preventive drugs or microbicides it will be critical to identify those that are effective against the virus in the presence of semen—the major vector of HIV-1 transmission.

Reference

Muench, et al., 2007. Cell 131.

doi:10.1016/j.antiviral.2009.02.155

151

Nonoxynol-9 (N-9), After Repeated Applications, Does not Result in Cumulative Damage to the Murine Cervicovaginal Epithelium

Karissa Lozenski[a,*], Tina Kish-Catalone[b], Brian Wigdahl[a], Fred Krebs[a]

[a] *Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, USA;* [b] *Department of Natural Sciences, DeSales University, Center Valley, USA*

The disappointing clinical failures of four topical vaginal microbicides have provided new insights into factors that impact microbicide safety and efficacy. Specifically, the greater risk for HIV-1 acquisition in association with multiple uses of an N-9-containing product has highlighted the importance of application frequency as a variable during pre-clinical microbicide development, particularly in animal model studies. To evaluate an association between application frequency and N-9 safety, a mouse model of cervicovaginal microbicide safety was used. This model system, which is used to assess changes in epithelial integrity and immune cell recruitment following exposure to microbicidal agents, was shown to be predictive of clinical toxicity following a single N-9 exposure and concentration-dependent toxicity of the microbicide Savvy (C31G), which was also removed from clinical trials. In multiple exposure experiments using this model, the initial application of N-9 (aqueous, 1%) caused considerable damage to the cervical epithelium (as previously published). Subsequent daily exposures were characterized by diminished cervical toxicity relative to the