and weight was stable throughout the 7 day dosing period and the 7 day drug washout even in the 30 mg/kg group. Of the three new compounds tested, ODE-HPMPDAP appears to have no acute oral toxicity and should be evaluated further in view of its 20 nM EC50 against vaccinia virus in vitro.

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Cidofovir: Intratympanic Delivery and Hearing Loss

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Congenital Cytomegalovirus (CMV) is the leading cause of infectious-related sensioneural hearing loss (SNHL) worldwide. Approximately 90% of newborns infected with CMV are asymptomatic at birth, of these 20% exhibit SNHL. Clinicians are developing novel ways to treat SNHL caused by CMV infection. Our lab is exploring the intratympanic route (IT) for delivery of established antivirals to treat CMV related SNHL with promising preliminary results. IT injections provide an advantage over systemic delivery because IT delivery shields the patient from serious side effects. Moreover, the similarities in the anatomy and physiology of the guinea pig (GP) and human ear allows this to be a relevant model to study. Accordingly, viral kinetics studies and auditory brainstem responses (ABR) have shown that direct inoculation of guinea pig CMV (GPCMV) into the bulla of a GP is a consistent and reliable model for CMV infection. Studies are ongoing for IT injections of cidofovir (CDV) for the treatment of GPCMV related hearing loss. Administering CDV at different time points post viral inoculation is proving significant. The viral kinetics show replication begins at day 6 post-surgical inoculation. IT injection of CDV administered at day 7 shows the most impact in hearing improvement. ABR, *realtime* PCR, and histological data confirms that CDV given IT inhibits viral replication and improves hearing without manifesting any side effects. This data demonstrates that CDV given IT prevents the virus from replicating and shows an improvement in hearing loss by day 21. The lab is also exploring a unique application of drug-delivery, a temperature sensitive copolymer used as a transporter of antivirals. *In vitro* data shows that CDV in a temperature sensitive copolymer extends the effective life of the drug. To document the location and migration of these gels *in vivo*, MRI images of guinea pigs ears were taken at different time points post-IT injection of copolymer gels. The MRI data has shown that the gel can be injected IT and is located at the round window. Ongoing *in vivo* studies that include hearing and kinetic data will determine if the temperature sensitive copolymer gel can be used as a transporter for time controlled drug release into the inner ear.

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Toward A More Complete Anti-Herpesvirus SAR for 2nd Generation Methylenecyclopropane Nucleosides

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The discovery of methylenecyclopropane nucleosides (MCPNs) has lead to a large number of promising analogs with potent antiherpesvirus activity. One factor potentially limiting the further development of the MCPNs, however, is a stereocenter included in the first generation compounds (1). In an attempt to synthesize compounds without the problematic stereocenter, Zemlicka developed a second generation of methylenecyclopropane nucleosides that included an additional hydroxymethyl substituent (2). Unlike the thoroughly explored first generation MCPNs, only a few representatives of the second generation MCPNs were synthesized, including the guanine analog ZSM-I-62 (cyclopropavir, CPV), which is now in preclinical development for HCMV. The potent antiherpes activity of CPV and the other second generation MCPNs that were synthesized prompted us to further explore the structure, and elaborate the SAR for this series. Herein, we report the results of our investigations toward expanding the structure–activity relationship within the second generation of MCPNs. Several of the new analogs demonstrated low micromolar activity against HCMV and EBV, and some of the compounds also have moderate activity against HHV-8. We will discuss the relationships between amine, ether, and thioether analogs and the anti-herpes activities thereof.

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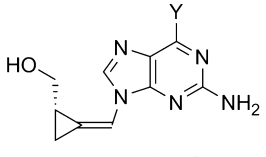
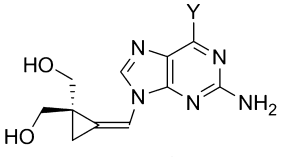


Y = NHR, OR, SR

1, first generation MCPN



Y = NHR, OR, SR

2, second generation MCPN

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Stereoselective Synthetic Strategy to Potentially Antiviral Active Carbocyclic L-Nucleosides and L-Nucleotides

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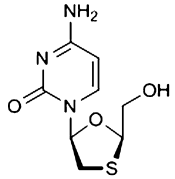
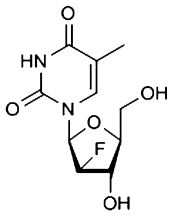
Since L-nucleosides like 3TC **1** and L-FMAU **2** show potent activity against HBV-replication it would be of interest if this also applies to their carbocyclic analogues. In addition to a higher stability towards phosphorylases the carbocyclic moiety prefers a specific conformation depending on the substituents on the cyclopentane system. This leads to a different structure–activity–relationship (SAR) for carbocyclic compounds which may effect their biological properties in comparison with natural nucleosides.

The aim of our work is to prepare new antivirally active carbocyclic L-nucleoside analogues with the help of an efficient and stereoselective synthesis. Starting from enantiomerically pure 2-benzyloxymethylcyclopent-3-enol it was possible to synthesize numerous analogues. Using the Mitsunobu coupling reaction for a convergent synthetic strategy modified nucleobases or functionalized carbocyclic moieties could be introduced. By this means a comprehensive library of carbocyclic compounds was synthesized (Jessel et al., 2007) as well as the corresponding *cyclo*Sal-pronucleotides to improve the activity of the carbocyclic nucleosides (Meier, 2006). Furthermore, different nucleotides were prepared using nucleoside triesters as starting material (Warnecke and Meier, 2008). For antiviral evaluation carbocyclic nucleosides and *cyclo*Sal-nucleoside triesters were used.



3TC, Lamivudine **1**



L-FMAU, Clevudine **2**

Reference

Jessel, S., Hense, E., Meier, C., 2007. Nucleosides, Nucleotides Nucleic Acids 26, 1181–1184.
Meier, C., 2006. Eur. J. Org. Chem., 1081–1102.
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A Novel Fullerene-Based Antiviral Active Against *Herpes Simplex* Virus *In Vitro* and *In Vivo*

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Background: Herpesviruses represent a group of human pathogens causing diseases ranging from *herpes labialis* to fatal encephalitis with immunocompromized persons as the main target. Infections transmitted by these viruses take the second place (15.8%) after influenza as a reason of death due to viral infection. Herpesviruses are therefore a serious challenge for medicinal science and health care. The purpose of the present study was to evaluate anti-viral activity of newly synthesized water-soluble derivative of fullerene, fullerene-polyaminocaproic acid (FPAC), against *Herpes simplex* virus type I.

Materials and methods: Toxicity of FPAC was determined by MTT. *Herpes simplex* virus type I (HSV-I) was propagated in Vero cells. FPAC was serially diluted in medium and added to cells 1 h before the virus inoculation. Virus titer was determined for each concentration of FPAC based on the study of virus-induced cell destruction after 72 h of cultivation by MTT. Virus titer was then plotted against FPAC concentration, and EC_{50} was calculated. For *in vivo* experiments mice were inoculated intracranially with HSV-1 and treated with FPAC intraperitoneally once a day. Animals were then monitored for 14 days for mortality. On days 3 and 7 post-inoculation brains of five mice from each group were studied for virus titer and virus-induced lesions. Acyclovir was used as reference compound.

Results: Based on the data of *in vitro* experiments, CTD_{50} and EC_{50} of FPAC were estimated as >1000 and 2 μg/mL, respectively, that gives a selectivity index >500. Application of FPAC to infected mice resulted in decreasing of mortality (68 and 90% in the group of treated and non-treated mice, respectively) and increasing of mean day of death (8.3 and 5.7 days). Virus titer in brain tissue of treated animals was slightly lower (4.1 against 5.1 $\log_{10}$ $TCID_{50}$/20 mg tissue in control). These values, nevertheless, were lower that those for acyclovir (mortality 50%, MDD 10.9 days, virus titer 3.0 $\log_{10}$ $TCID_{50}$/20 mg tissue). Morphological signs of infection in the brain, such as neuronal death, gliosis and cell infiltration were less manifested than in control animals.

Conclusion: Taken together, these data suggest that a novel fullerene derivative might be prospective anti-herpetic drug and should be further developed.

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Overview on Clinical Trials and Resistance Breaking Activity of the Anti-Cytomegalovirus Compound AIC246

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Background: CMV remains an important pathogen in immunocompromised individuals including transplant recipients. To date, all available drugs for HCMV infection/disease target the viral DNA-polymerase. Disadvantages of current therapies include toxicity and emergence of drug resistance. Hence, safe and improved antivirals with different molecular targets are urgently needed. Here we report on the results of clinical trials with AIC246, which belongs to a novel class of anti-CMV agents with a different mode of action compared to available drugs.

Methods: Anti-CMV activity of AIC246 was evaluated in vitro using laboratory strains and clinical isolates incl. drug resistant viruses. AIC246 was tested in phase I trials in over 200 healthy subjects and in a phase IIa trial, which enrolled 27 transplant recipients under a 14 days pre-emptive treatment strategy in comparison to observational treatment. DNA PCR was used as biomarker for HCMV.

Results: In vitro AIC246 exhibited excellent inhibitory activity against CMV including Ganciclovir resistant virus strains. In all clinical trials AIC246 was generally well tolerated. Within the limits of the small sample size of the phase II trial, reduction in viral markers were similar in all treatment groups. AIC246 reduced DNA PCR in a transplant patient who had developed a multiresistant CMV to the limit of detection.

Conclusion: AIC246 represents a novel CMV inhibitor with potent antiviral activity and which acts via a different mode of action compared to Ganciclovir. AIC246 was generally well tolerated in over 200 healthy subjects and in transplant patients with CMV viremia and also showed activity against a multidrug-resistant CMV strain.

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