CMX001 therapeutic dosing and the maximum delay in treatment that can afford protection from lethal disease.

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119
Synthesis of P–O–C-linked Foscarnet–Peptide Conjugates and Sensitive Methods to Detect the Released Drug in Biological Samples

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The trisodium salt of phosphonoformic acid (PFA), foscarnet, is an analogue of pyrophosphate that inhibits a broad spectrum of viruses. A long-recognized limitation of foscarnet has been its very low oral bioavailability, which is due to the ionization of PFA at physiological pH. As a result, in the clinic it can only be administered intravenously. Here, we report the synthesis of a series of novel PFA prodrugs, created by incorporation of toxicologically benign amino acids or small peptides to abate the anionic state of the drug. Previous work done in our laboratory demonstrated the synthesis of P–N linked PFA-amino acid conjugates, which cleanly release the parent drug at physiological pH. In this work, conjugates of PFA monosalts were esterified by the alcohol side-chain group of serine using Mitsunobu chemistry to create the P–O–C link. The detection and analysis of foscarnet is made difficult by its lack of a visible–UV chromophore, therefore we have also sought improved methods to determine the parent drug released from the conjugates. Two new approaches (UV detection via formation of a Yb^{3+}/pyrocatechol violet complex, and fluorescence detection by formation of a 9,10-bis[(2,2′-dipicolylamino)methyl] anthracene zinc complex) will be compared with detection using LC–MS/MS.

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120
In Vivo Efficacy of CMX001 Against Herpes Simplex Virus Types 1 and 2

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CMX001, or HDP-cidofovir, has been previously reported to have excellent activity both in vitro and in vivo against vaccinia virus (VV), cowpox virus (CV) and human cytomegalovirus (HCMV). In the current studies, CMX001 was synthesized as a free acid form instead of the salt forms used previously and evaluated in murine models of herpes encephalitis and neonatal herpes. Compound was suspended in 0.4% carboxymethylcellulose to yield desired dosages in a 0.2 ml volume. Mice were lethally infected intranasally with herpes simplex virus (HSV), type I, E-377, MB-1 or HSV-2, strain MS and treatments were delayed until 24 h post viral infection. CMX001 was administered orally once daily at 2.5, 5 or 10 mg/kg beginning 24 h post HSV infection and continued for 7 days. CMX001 exhibited some toxicity at the 10 mg/kg dosages in uninfected and infected mice. Treatment with CMX001 significantly reduced mortality of HSV-1 infected mice at 5 and 2.5 mg/kg doses ($P < 0.001$). Also, CMX001 significantly reduced mortality in HSV-2 infected mice at 5 and 2.5 mg/kg doses ($P < 0.001$). Acyclovir (ACV) was administered twice daily beginning 24 h post HSV infection as a positive control at 30, 60, or 120 mg/kg. ACV was effective in reducing or eliminating mortality at all doses evaluated ($P < 0.001$). In these studies, CMX001 was as efficacious as ACV at non-toxic doses of 5 and 2.5 mg/kg. Additional evaluation of CMX001 will be required in order to assess its potential for the treatment of serious HSV types 1 and 2 infections in humans.

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121
A Synthetic Strategy to Different Cyclopentenyl-Nucleosides

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Carbocyclic nucleosides are compounds in which the furan ring has been replaced by a cyclopentane system. They possess increased metabolic stability against nucleoside phosphorylases as well a higher conformational flexibility. In the past, carbocyclic nucleoside analogues like abacavir showed very interesting antiviral properties *in vitro* and *in vivo*. Abacavir was approved as a HIV-drug for clinical application. Therefore, we were interested in a short and efficient stereoselective access to this class of compounds. As starting material 3-benzyloxymethylcyclopent-3-enol was chosen, that can be prepared from cyclopentadiene after deprotonation and alkylation using benzylchloromethylether to give symmetric benzyloxymethylcyclopentadiene. This compound isomerizes into two thermodynamically more stable benzyloxymethylcyclopentadienes. This mixture of compounds material can be used as precursor for the synthesis of different 3′,4′-cyclopentenyl-nucleosides. This material can be oxided to 3-benzyloxymethylcyclopent-3-enone. The β,γ-unsaturated ketone undergoes isomerization into 3-benzyloxymethylcyclopent-2-enone. After reduction, the resulting 3-benzyloxymethylcyclopent-2-enol can be used as precursor for the synthesis of different 4′,6′-cyclopentenyl-nucleosides. Moreover, this strategy offers the possibility for the synthesis of new carbocyclic nucleosides because the double bond can be functionalized before or after introduction of the nucleobase. The synthesized carbocyclic nucleosides were converted into their monophosphates by

hydrolysis of the corresponding *cyclo*Sal-phosphatetriesters. The nucleosides and the triesters were subjected to antiviral evaluation.

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122
Cyclopentenylcytosine (CPE-C) Inhibits Adenovirus Replication in the Ad5/NZW Rabbit Ocular Model

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Purpose: Presently, there is no FDA approved antiviral therapy for the treatment of adenovirus (Ad) ocular infections. The goal of the current study was to determine the antiviral efficacy of topical cyclopentenylcytosine (CPE-C), a nucleoside analog of cytosine, on acute Ad replication in the Ad5/NZW rabbit ocular model.
Method: 40 NZW rabbits were topically inoculated in both eyes, following corneal scarification, with 1.5×0^6 pfu/eye of Ad5. On day 1, the rabbits were divided into 4 topical treatment groups ($n = 10$/group): (I) 3% CPE-C, QID × 7 days; (II) 3% CPE-C, BID × 7 days; (III) 0.5% Cidofovir (CDV), BID × 7 days; (IV) Control (saline), QID × 7 days. Both eyes of the rabbits were similarly treated. All eyes were cultured for virus on days 0, 1, 3, 4, 5, 7, 9, 11, and 14.
Results:

Group	+ Cultures/total (days 1–14)	Duration of shedding (D)	Mean titer (days 1–5)	Mean titer (days 7–14)
CPE-C (QID)	24/160 (15%)*	1.3 ± 1.6*	$3.5 \pm 18.1 \times 10^{1}$*	$3.6 \pm 25.2 \times 10^{0}$*
CPE-C (BID)	31/160 (19%)*	2.5 ± 2.3*	$1.6 \pm 4.8 \times 10^{1}$*	$1.0 \pm 4.4 \times 10^{0}$*
CDV	27/160 (17%)*	2.0 ± 1.8*	$6.7 \pm 12.7 \times 10^{0}$*	$0.3 \pm 2.2 \times 10^{0}$*
Control	97/160 (61%)	8.1 ± 3.5	$1.0 \pm 3.0 \times 10^{2}$	$2.2 \pm 9.2 \times 10^{1}$

* $p \leq 0.011$ compared to the Control.

Topical 3% CPE-C QID and BID and 0.5% Cidofovir BID were significantly more effective than the control in reducing positive cultures/total (days 1–14), duration of shedding, mean titer (days 1–5), and mean titer (days 7–14) in the Ad5/NZW rabbit ocular model. There were no significant differences between the two topical regimens of 3% CPE-C and both were as effective as the positive antiviral control, 0.5% Cidofovir.
Conclusions: CPE-C demonstrated potent anti-adenoviral activity in the Ad5/NZW rabbit ocular model. Additional studies are warranted to establish the clinical potential of CPE-C as a topical antiviral treatment for adenovirus ocular infections.

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123
Synthesis and Properties of Glycosyl-functionalised *Cyclo*Sal–Pronucleotides

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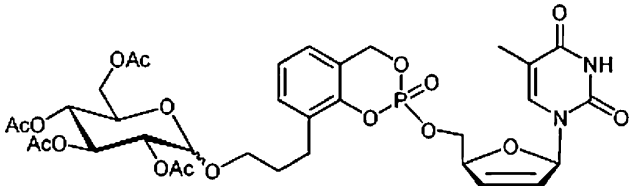
Nucleoside analogues are widely used in antiviral and cancer chemotherapy. The antiviral activity of nucleoside analogues like 2′,3′-dideoxy-2′,3′-didehydrothymidine (d4T) depends on their conversion to the ultimately bioactive 5′-*O*-triphosphates. The intracellular phosphorylation via mono- and diphosphates is catalysed by host cell enzymes and is often the metabolism-limiting step. The use of nucleotides as therapeutic agents is impossible because they are inable to penetrate the cellular membrane due to high polarity. In addition they are catabolised in blood by unspecific nucleotidases. The *cyclo*Sal-pronucleotide approach circumvents these hurdles. These lipophilic prodrugs penetrate the cell membrane and the nucleotide is released by a pH-driven chemical hydrolysis. Due to passive diffusion this could occur inside or outside the cellular membrane possibly leading to a decreased amount of nucleotide in the cytoplasm. Improvement of intracellular delivery might be achieved by using cellular transporters and/or recognition mechanisms, e.g. glucose transporter. In a first attempt, *cyclo*Sal–nucleotide–sugar conjugates (Fig. 1) were synthesized and their biological properties were investigated concerning pH-stability, cell extract stability, cytotoxicity and antiviral activity in CEM/0 and TK-deficient CEM cells.



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