pitulate the pathogenesis of the natural disease and the host's response to it. In the case of evaluation of orthopoxvirus antivirals, the non-human primate model employs an intravenous (IV) challenge, which bypasses the natural infection in the respiratory tract and the primary viremia. Furthermore, certain rabbit/rabbitpox models utilize an intradermal route of infection that, like the IV route, removes the seeding and early stages of viral replication in the respiratory tract. Here we compare the effect of infectious routes on pathogenesis of, and the host response to, ectromelia (ECTV) infections of the C57BL/6 mouse. ECTV is the etiological agent of mousepox, and is arguably the best small animal model for smallpox. The ECTV/mousepox model presents with similar route-dependent disease outcomes as are observed in humans infected with VARV and monkeypox virus. Intranasal infections with ECTV have a low LD_{50} (100 PFU/mouse) and result in a highly fulminant disease with time to death of 7 to 12 days. IV infections also result in a fulminant disease with a shorter time to death of 3 to 8 days and an intermediate LD_{50} value (13,000 PFU/mouse). Conversely, infections via subcutaneous or footpad route result in a milder, non-lethal, illness and have a high LD_{50} value (>9000 PFU/mouse). Here we present data that show the temporal and reactive responses of the immune system varies according to route, and discuss these finding in light of non-respiratory tract animal models for the evaluation of orthopoxvirus antivirals.

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Nucleoside Diphosphate Prodrugs of Antivirally Active Nucleosides

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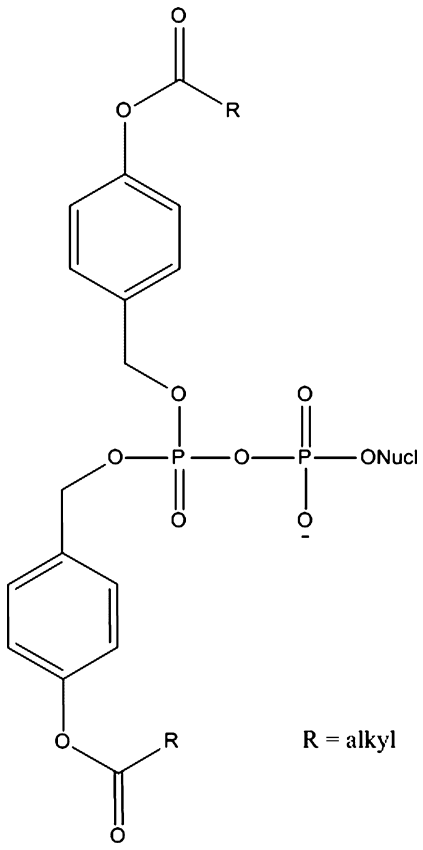
Analogs of natural nucleosides that can be modified either in the glycon or the aglycon are widely used in antiviral and anticancer therapy. Because of these modifications these nucleoside analogs can act as competitive inhibitors of DNA polymerase or as chain termination inhibitors of DNA synthesis. In order to possess antiviral activity these compounds need to be phosphorylated to their biologically active triphosphates. Due to the substrate specificity of kinases that catalyze the stepwise phosphorylation these reactions can be hindered resulting in a low antiviral activity. Recently, we reported on the first efficient prodrug concept for the intracellular delivery of nucleoside diphosphates to circumvent these metabolic restrictions (Jessen et al., 2008a,b). For this purpose we turned to 4-acyloxybenzyl moieties to compensate two of the negative charges of the nucleoside diphosphate leaving the α-phosphate unprotected. Intracellularly, the corresponding nucleoside diphosphate is then released selectively by hydrolysis of the acyl ester bond and subsequent 1,4-elimination.

Having applied this prodrug concept successfully to several nucleoside analogs e.g. 2′,3′-dideoxy-2′,3′-didehydroythymidine (d4T) and 3′-azidothymidine (AZT) we turned to other nucleosides with known antiviral activity of their corresponding triphosphates against HIV but which show no or poor activity in their nucleoside and nucleotide form. Here, we report on the synthesis and properties of these promising potential nucleoside diphosphate prodrugs.



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Evaluations of Combinations of CMX001 and Ganciclovir Against Cytomegalovirus Infections Using Real Time PCR

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CMX001 (HDP-cidofovir) has been reported previously to inhibit the replication of human cytomegalovirus (HCMV) both *In Vitro* and *In Vivo*. Since CMX001is a monophosphate analog, it does not require initial phosphorylation by the HCMV UL97 kinase; therefore, it is highly active against most ganciclovir (GCV) resistant strains, and should be useful in the treatment of resistant-virus infections. We investigated the antiviral activity of CMX001 in combination with GCV *In Vitro* to evaluate the efficacy and safety of this combination. Human foreskin fibroblast cells were infected with HCMV at a multiplicity of infection of 0.01 PFU/cell and serial concentrations of CMX001 and GCV alone or in combination were added to either uninfected or infected cells. Total DNA was harvested following a 7 day incubation and the copy number of viral DNA was determined by real time PCR. As expected, CMX001 was highly active against HCMV and reduced the quantity of viral DNA by 10-fold at concentrations less than 1 nanomolar, and 1000-fold at 10 nanomolar. The efficacy of GCV was comparatively modest

and reduced the accumulation of viral DNA by less than 10-fold at 10 μM. Combinations of CMX001 and GCV were synergistic, when concentrations of CMX001 as low as 3 picomolar were added to GCV. No significant changes in cytotoxicity were observed for any of the concentrations tested confirming that the combination was not toxic. The exceptional potency of CMX001 observed in these assays was confirmed in a quantitative real-time RT-PCR-based array that determined levels of all viral transcripts. Reductions in the levels of viral transcripts were consistent with the reductions in genome copy number and reflected the marked inhibition of viral replication in vitro relative to GCV. These results clearly indicated that combinations using suboptimal concentrations of CMX001 with GCV are synergistic in vitro. In vivo studies should be performed to further explore this combination, especially in drug resistant HCMV.

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In Vivo Efficacy of Twice Daily Oral Treatment with N-MCT Against Herpes Simplex Virus Type 2 in Balb/C Mice

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N-MCT has been previously reported to have excellent activity both in vitro and in vivo against human herpesviruses (HSV). Mice were lethally infected intranasally with herpes simplex virus (HSV), type 2, strain MS and treatments were initiated 24, 48 or 72 h post-viral infection. Compound was suspended in 0.4% carboxymethylcellulose to yield desired dosages in a 0.2 ml volume. N-MCT was administered orally twice daily at 100, 50 or 25 mg/kg and continued for 7 days. Acyclovir (ACV) was given similarly at 100 mg/kg as a positive control. No toxicity was observed in uninfected mice treated with N-MCT. All dosages of N-MCT were highly effective ($p < 0.001$) in reducing mortality when treatments were initiated 24, 48 or 72 h post-viral infection. Pathogenesis studies were performed to determine the effect on viral replication in target organs of lung, liver, spleen, kidney and sections of brain. Treatment with N-MCT significantly reduced viral replication of HSV-2 better than ACV in all organs, particularly within the CNS. In order to determine the lowest effective dose, additional studies using lower doses were performed with N-MCT from 25 to 0.01 mg/kg given twice daily beginning 24 h post-viral inoculation. All doses of N-MCT greater than 0.03 mg/kg significantly reduced mortality ($p < 0.001$). The lowest dose evaluated was 0.01 mg/kg which also reduced mortality to 40% ($p = 0.001$) and increased the mean day to death from 7.5 to 10 days ($p = 0.001$). N-MCT is a potent antiviral for herpesvirus and has potential for the treatment of serious HSV type 1 and 2 infections in humans.

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Enhanced Efficacy Using Combinations of CMX001 with Acyclovir Against Herpes Simplex Virus Infections *In Vitro* and In Mice

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Previous studies have shown that either CMX001 (HDP-cidofovir) or acyclovir (ACV) is effective in vitro against herpes simplex virus (HSV) isolates and in preventing mortality of mice infected intranasally with HSV-1or 2. Evaluation of efficacy using suboptimal doses of these two agents in combination has not been previously reported. In cell culture, CMX001 was evaluated against a panel of both wild-type and ACV-resistant isolates of HSV-1 and HSV-2 and found to be highly effective with EC_{50} values ranging from 0.008 to 0.03 μM. These same virus isolates were also inhibited by concentrations of ACV ranging from 2.0 to >100 μM. Using various concentrations of CMX001 and ACV in combination in tissue culture, we demonstrated synergistic efficacy without an increase in toxicity in cell culture experiments. To determine if this combination would result in enhanced efficacy in an animal model, CMX001 was given once daily at 1.25, 0.42 or 0.125 mg/kg with or without ACV to mice infected intranasally with HSV-2. ACV was given twice daily at 30, 10 or 3 mg/kg. Treatments were initiated 72 h post-viral infection by oral gavage for 7 days. As expected from previous work where 5 mg/kg was an optimal dose of CMX001 in this model, CMX001 as a single therapy at 1.25, 0.42 or 0.125 mg/kg did not significantly improve survival or increase the mean day to death (MDD). ACV alone improved survival at 30 mg/kg ($p = 0.06$) and significantly increased the mean day to death at 30 or 10 mg/kg ($p < 0.01$), but not at 3 mg/kg. Suboptimal doses of CMX001 and ACV given together significantly enhanced protection from mortality or increased the MDD when compared with either drug alone in 8 of 9 combination groups. No additive toxicity was detected. Our results indicate that low dose combinations of these two agents act synergistically in vitro and in vivo and should be considered for use in herpesvirus infections in humans.

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First Diastereoselective Synthesis of Pronucleotides

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Pronucleotides represent a promising alternative to improve the biological activity of nucleoside analogs in antiviral and cancer chemotherapy. Two of the most successful prodrug-systems are the *cyclo*Sal-pronucleotides (Meier, 2002) as well as the nucleoside arylphosphoramidates (Congiatu et al., 2006). As the first approach is based on a selective chemical hydrolysis, the second technology requires an enzyme-mediated activation. Due to their synthesis pathways, so far derivatives belonging to these two classes of pronucleotides were obtained as 1:1 mixtures of diastereomers