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.

doi:10.1016/j.antiviral.2010.02.460

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Nucleoside Diphosphate Prodrugs of Antivirally Active Nucleosides

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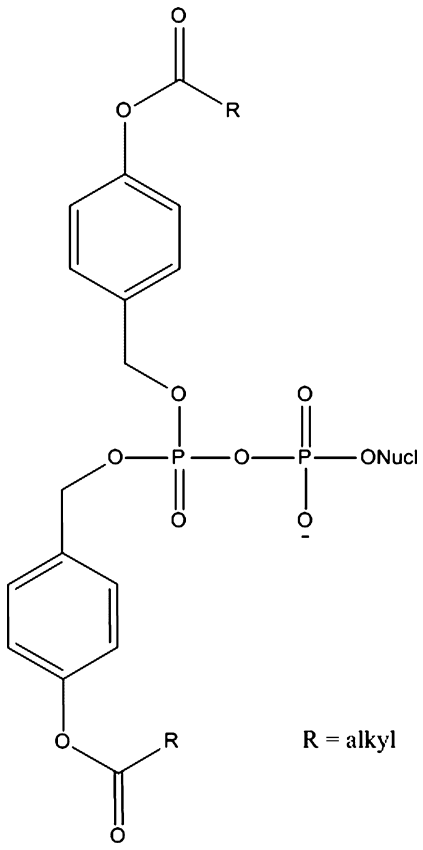
Analogs of natural nucleosides that can be modified either in the glycon or the aglycon are widely used in antiviral and anticancer therapy. Because of these modifications these nucleoside analogs can act as competitive inhibitors of DNA polymerase or as chain termination inhibitors of DNA synthesis. 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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doi:10.1016/j.antiviral.2010.02.461

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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