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In Vitro Investigation of Cytotoxic Action of Hemocyanins on Cell Cultures

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Molluscan hemocyanins have particular interest due to their significant immunostimulatory properties. Besides, anti HSV-1 activity of these preparations have been shown recently (Pagano and Gershburg, 2005). Epstein Barr virus (family *Herpesveridae*) is essential for different medicine spheres, but the drugs that have antiEBV activity are limited by Gancyclovir and Acyclovir (Velkova et al., 2009). The purpose of our work is study of the native hemocyanins from *Rapana venosa* (RvH) and *Helix vulgaris* (HvH) and their isoforms as substances with feasible antiEBV activity. Cytotoxic action of hemocyanins (HvH, RvH) on cell cultures was investigated in vitro. The following cultures of cells have been used in the work: B95-8 – leukocytes of monkeys-marmaset, transformed by EBV, Raji – human B-lymphocytes, which produce only separate early antigenes, but not virus particles, Namalwa – human B-lymphocytes. Influence of hemocyanins on viability and proliferative activity of lymphoblastoid cells was characterized by cytomorphological and colorimetric methods. Viability of cell cultures was determined by staining of them with 0.4% tripan blue ("Sigma", USA) which was used for revealing dead cells. Proliferative activity of lymphoblastoid Raji cells was studied with use of MTT-assay ("Sigma", USA). Analysis was carried out within concentrations from 2000 μg/ml to100 μg/ml of RvH, HvH and structural subunits RvH1, RvH2, HvH1 and HvH2. Concentrations of the investigated substances which caused 50% inhibition of viability of cells that are CC_{50} are submitted in the table. Table—Cytotoxic concentration of tested hemocyanins in different cultures of lymphoblastoid cells.

Cells	CC_{50} (μg/ml)			
	RvH1	RvH2	HvH1	HvH2
Raji	720	700	260	50
Namalwa	1358	1000	185	355
B95-8	1352	709	255	705

Thus, cytotoxicity of hemocyanins in several cell cultures of B-phenotype was defined. Low toxic hemocyanins are selected for investigation of anti EBV activity.

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The Antienteroviral Effect of Oxoglaucine and Phenotypic Characterization of the Oxoglaucine Resistant Mutant of Coxsackievirus B1

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The medicinal and economic impact of enteroviral infections imposes the search of safe and specific inhibitors of enterovirus replication. Oxoglaucine (OG), an aporphinoid alkaloid isolated from Glaucium flavum Crantz, which can also be obtained synthetically, possesses a promising antiviral effect against the replication of a panel of 15 tested enteroviruses. The selectivity index (SI), defined as the ratio between the 50% inhibitory concentration (IC_{50}) and the 50% cytotoxic concentration (CC_{50}), both determined in the CPE-inhibition test, ranges from 20 to above 200 depending on the virus. Time of addition study in the one-step virus growth cycle set-up reveals strong inhibition during the early periods of virus replication. Since growth of resistant virus is considered as an indicator of specific antiviral activity, OG-resistant progeny was developed *in vitro* for poliovirus 1 and the six coxsackieviruses B. Viruses develop rapidly phenotypic signs of resistance. A correlation is established between the sensitivity to OG and the necessary number of serial passages for the selection of resistant mutants. The more sensitive the virus to the antiviral effect, the faster the selection of resistant progeny. The phenotypic characteristics of the OG-resistant mutant of coxsackievirus B1 (CV-B1), selected after 20 consecutive passages in increasing concentrations of OG, are determined. The resistant CV-B1 possesses a lower infectious titer in comparison to the ancestral strain. The resistance index, defined as the ratio between the 90% effective concentration (EC_{90}) of OG for the resistant virus and EC_{90} for the ancestral strain, both determined in the virus yield reduction essay, as well as the sensitivity index, which is the titer in the presence of drug divided by that in its absence, and the IC_{50} for the virus from each consecutive passage are determined and the gradual increase of the rate of the relative resistance is established. The selected OG-resistant CV-B1 progeny will further serve as a tool for understanding the mechanism of antiviral action of OG.

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The Disease Course and Host's Response to Mousepox is Dependent on Inoculation Route

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The pathogenesis of an infectious agent is greatly affected by its route of infection. Variola virus (VARV) causes a systemic, fulminant disease following a respiratory tract infection with a case-fatality rate of 10–30%. In contrast, infection through the skin results in a systemic infection, but with a milder disease course and case-fatality rate of <1%. Therefore, it is important that the route of infection and the challenge virus used to evaluate antivirals reca-

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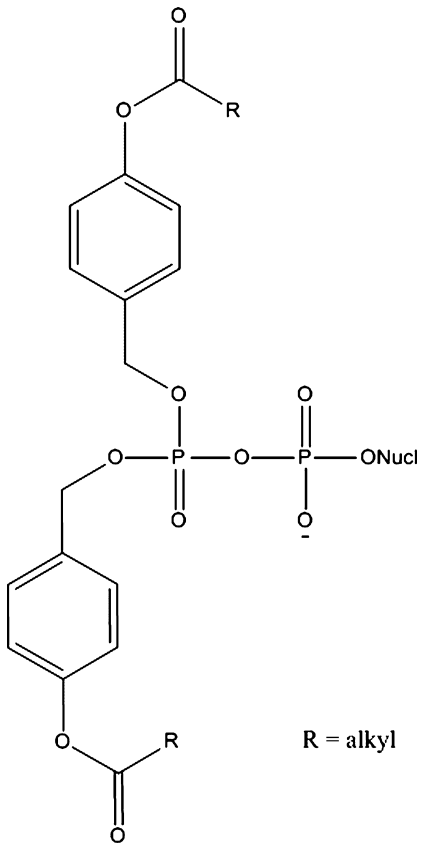
Analogs of natural nucleosides that can be modified either in the glycon or the aglycon are widely used in antiviral and anticancer therapy. Because of these modifications these nucleoside analogs can act as competitive inhibitors of DNA polymerase or as chain termination inhibitors of DNA synthesis.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