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Increase of the Effect of Anti-Enteroviral Chemotherapy Used in Experimental Neurotropic Coxsackievirus B1 Infection in Newborn Mice when a Triple Combination of Antivirals is Administered in a Consecutive Treatment Course

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Our previous study showed that the triple combination of disoxaril, guanidine hydrochloride and oxoglaucine, applied in a new manner—consecutive administration of the partners, was quite effective when given to newborn mice with neurotropic coxsackievirus B1 infection, achieving around 50% survival rate. This new consecutive administration approach is especially suitable for treating enteroviral infections, in which the development of resistance is very rapid due to the extremely high viral mutation rate. The approach aims to restrict the resistance development in experiments *in vivo*, using antivirals with proved high efficiency in experiments in cell cultures. The partners in the combination are applied consecutively every third day and the treatment course begins on the day of the viral inoculation. In this study we have optimized this scheme of administration of the combination, examining the influence of the chronology of the application of the partners. The combination must start with disoxaril, followed by guanidine hydrochloride and oxoglaucine. The combinations which start with other antivirals – e.g. oxoglaucine or guanidine hydrochloride – have proved to be ineffective. Brain samples were taken daily from day 4th p.i. onwards in order to characterize the viral population in the brains of the treated mice during the treatment course. The isolates were purified by threefold cloning and then we studied the virus sensitivity to the inhibitors-partners by determining the IC_{50} values of each compound in FL cell line using the plaque-inhibition test of Hermann–Siminoff.

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Prodrugs of Antiviral Nucleosides Cleavable by Dipeptidyl-Peptidase-IV (CD26)

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We have recently (Balzarini et al., 2004) described a novel enzyme-based prodrug approach that provides conjugates of therapeutic agents with a peptidic moiety as a carrier wherein the conjugate [peptide]–[drug] is specifically cleavable by the endogenous dipeptidyl-peptidase IV enzyme (DPP-IV) present on the surface of certain cells or in plasma. The lymphocyte surface glycoprotein DPP-IV, also known as CD26, belongs to a group of atypical serine proteases preferentially cleaving X-Pro (or X-Ala) dipeptides from the N-terminus of a variety of natural peptides. For proof of the concept, we focused on the anti-HIV-1 lipophilic TSAO compounds, since this retrovirus mainly infect lymphocytes or macrophages that abundantly express DPPIV/CD26 enzyme in their membrane. [(Xaa-Pro)$_n$]–[TSAO-T] conjugates bearing di- and tetrapeptides sequences of different nature were prepared and studied. In all cases, DPPIV/CD26 was able to efficiently hydrolyse those "artificial substrates" different from natural peptides. Moreover, it was possible to modulate the hydrolisis rate (half-life) and physicochemical properties of the compounds by modifying the nature and length of the peptide (García-Aparicio et al., 2006, 2007). Once validated the prodrug strategy with TSAO derivatives (the peptidic sequence was linked to a primary amino group which is bound on an aliphatic alkyl chain), we now study if the prodrug strategy is feasible in primary amino groups bound to purine or pyrimidine rings. The synthesis of conjugates of several antiviral purine or pyrimidine nucleoside drugs bearing different di- and tetrapeptide sequences, their ability to act as efficient substrates of DPPIV/CD26 enzyme and their human or bovine serum hydrolisis profiles will be herein described.

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Biochemical Evaluation of a New Potential Antiviral Drug HPMP-5-Azacytosine

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(*S*)-1-[3-Hydroxy-2-(phosphonomethoxy)propyl]-5-azacytosine (HPMPazaC) is a new 5-aza analogue of a successful antiviral compound HPMPC (cidofovir, Vistide®), which has been used for the treatment of cytomegalovirus (CMV) retinitis in AIDS patients. While the initial screening tests indicated that the antiviral effects of HPMPazaC might be superior to those of HPMPC, we have investigated the intracellular metabolism of a (^{3}H)-labeled HPMPazaC in the CCRF-CEM cells. Nine major metabolites have been found: HPMPazaCp-choline (which may serve as an intracellular depot of HPMPazaCpp, an active form of the drug), HPMPazaC, HPMPazaU, HPMPazaCp, HPMPazaUp, HPMPazaCpp, HPMPazaUpp and two unknowns–most likely diphosphates

with open triazine ring. Interestingly, formation of HPMPazaU (by deamination of HPMPazaC) has not been observed with HPMPC (Cihlář et al., 1992) suggesting that the metabolic profile of HPMPazaC is more complex. We have also studied the effect of HPMPazaC on human telomerase expression since it is believed that inhibition of telomerase activity could result in toxicity to normal cells which express telomerase (Tendian and Parker, 2000) (contrary to the anticancer treatment where inhibiting telomerase in cancer cells is a part of therapeutic effect). Moreover, inhibition of telomerase activity has been previously observed with a number of other acyclic nucleoside phosphonates (Hájek et al., 2005). Here we demonstrate that HPMPazaC, however, is only a weak inhibitor of human telomerase expression ($IC_{50} > 500\ \mu M$). HPMPazaC is virtually free of cytotoxicity at relevant doses ($GIC_{50} > 1$ mM HL-60 cells, 72 h). The concentration corresponding to the GIC_{50} value causes a cell cycle arrest in S-phase indicating interference with DNAreplication. Further studies will be conducted to clarify the interactions of HPMPazaC with cellular replicative polymerases.

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A Microbicide Transmission and Sterilization Assay (MTSA) Defines the Effective Concentration of Topical HIV Microbicides Required to Suppress Virus Transmission

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An effective topical anti-HIV microbicide must prevent the transmission of virus to target cells in the vaginal epithelium by both cell-free virus and cell-to-cell transmission of virus from infected lymphocytes in the semen. We have previously reported the development of a microbicide transmission and sterilization assay (MTSA) which defines the effective concentration of a microbicide required to totally suppress the transmission of HIV. When compared with results obtained from standard virus transmission inhibition assays, the MTSA may be more predictive in determining the concentration of a candidate microbicide which must be employed to totally suppress virus transmission in the clinical setting. We have evaluated the efficacy of several approved drugs, potential microbicide candidates representing multiple mechanisms of action, and a group of structurally similar pyrimidinediones with equivalent activity in the standard transmission assays. Our data would suggest that the MTSA is robust enough to segregate transmission inhibitory capability between compounds that have highly similar chemical structures and biological anti-HIV activities and mechanisms of action. The results also suggest that the EC_{50} concentration defined in standard transmission assays can vary greatly from that determined in the MTSA. In some cases (SJ-3339, UC781 and Efavirenz), the EC_{99} concentration defined in standard transmission inhibition assays closely approximates the sterilizing concentration defined in the MTSA; in most cases the two concentrations are very different. The MTSA has also been optimized to better mimic the sexual transmission of HIV to include variables such as the effects of viral MOI, the relative effects of using a cell-free or cell-associated virus inoculum, the timing of microbicide application, the ability to inhibit drug-resistant viruses, and activity in the presence of semen or vaginal fluids. We believe the MTSA will address the critical issue of defining the concentration of a microbicide that will need to be utilized in human clinical trials and allow direct comparison of the relative transmission inhibition ability of different microbicide candidates.

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Stereospecificity, Substrate, and Inhibitory Properties of *P*-Borano Nucleoside Diphosphates for Creatine, Pyruvate, and NDP Kinases

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Pyruvate kinase (PK) and creatine kinase (CK) are potentially responsible for the last phosphorylation step of antiviral nucleoside diphosphates (NDPs) to their nucleoside triphosphates (NTPs). NTP analogs with Rp α-*P*-borano modification have proven to be better chain terminators for viral reverse transcriptases than their parent compounds. A borane group is isoelectronic with O in normal phosphate, isolobal with S in thiophosphates, and isosteric with CH_3 in methylphosphonates. The low electronegativity of the boron atom may be a reason why the α-*P*-borano group accelerates the incorporation of chain terminators into viral DNA, which in turn increases the potency of these drugs. Here, effects of nucleobase, ribose, and α-*P* substitution on the substrate specificities of CK, PK, and NDP kinase (NDPK) are evaluated. CK and PK show opposite stereospecificity to α-*P* substitution and may serve as a means for activation of antiviral α-*P*-borano substituted NDPs. Direct binding and TSAC binding affinities of the substrate analogs were determined. CK and PK showed two separate binding modes and negative cooperativity for binding of the second substrate molecule. In steady-state kinetics, the Sp-ADPαB isomer was a 70-fold better substrate for CK than the Rp-isomer, whereas PK showed a preference for the Rp-isomer. Although Rp-ADPαB isomer is minimally phosphorylated by CK or PK, it does not significantly inhibit either of these important enzymes.