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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