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.