Oral Session VII: Herpesvirus II, Papillomavirus Infections

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Inhibition of Herpes Simplex Virus (HSV) Entry by a Non-Homologous Peptide

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Peptides that inhibit protein-protein interactions are receiving greater attention as antivirals or lead compounds for further development. During HSV infection, following attachment to heparan sulfate proteoglycans, a poorly understood fusion event involving the viral glycoproteins gB, gD, and a complex of gH and gL allows viral entry. One of four possible cellular co-receptors is also involved. Peptides that specifically inhibit entry (fusion step) could be useful probes for dissection the process. We have identified a peptide that specifically inhibits entry. The peptide (EB1) consists of the leader sequence of the FGF4 precursor protein with 4 positively charged amino acids added to improve solubility. The peptide has no homologies to any HSV-encoded proteins. In various assays, EB1 inhibited plaque formation or viral yields with an IC_{50} of 1-10μM. A control peptide with a scramble sequence (EBX) was 10-100-fold less active. EB1 up to 100μM did not inactivate purified virions. Cytopathic effects in uninfected cells were first observed at 100μM EB1 (therapeutic index 10-100). EB1 was most effective when present 1 hour prior to and during infection. When added after viral adsorption, EB1 was 5-fold less effective and primarily inhibited cell-to-cell spreading (plaque size). EB1 inhibited a gC null virus, as well as a gC positive revertant. Furthermore, EB1 did not inhibit but actually enhanced binding of ^{32}P-labelled virions to cells at 4°C. Following viral attachment at 4°C, EB1 blocked viral infection only during a 2 hour period following a temperature switch to 37°C and became ineffective thereafter. Thus, EB1 blocks entry. This peptide will serve as a useful tool for probing HSV entry and may serve as a lead compound for further development of antivirals.

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Particular Characteristics of the Anti-Herpesvirus Activity of (1'*S*,2'*R*)-9-[[1',2'-bis(hydroxymethyl)cycloprop-1'-yl]methyl]guanine (A-5021)

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The novel nucleoside analog (1'*S*,2'*R*)-9-[[1',2'-bis(hydroxymethyl)cycloprop-1'-yl]methyl]guanine (A-5021) was shown to be a potent inhibitor of the replication of herpesviruses, both in vitro and in vivo (J. Med. Chem. 41,1284-1298; Antimicrob. Agents Chemother. 42:1666-1670). In a first set of experiments we compared the antiviral efficacy of A-5021 versus acyclovir (ACV) in protecting against HSV-1 induced mortality in SCID mice when the start of treatment was delayed. The compounds, (both at 50 mg/kg/day) were administered by subcutaneous injection for four consecutive days, starting either at 2 hr, 1 day or 2 days post infection. When administered from day 0 till day 4 p.i., A-5021 conferred complete protection against the infection (as assessed at 22 days p.i.) whereas ACV only delayed virus induced mortality by 7 days. When treatment was initiated at 24 hr p.i., 80% of the A-5021 treated animals survived the infection, whereas ACV only delayed mortality by 4 days. When treatment was initiated at 48 hr p.i., A-5021 delayed virus-induced mortality by 10 days, as compared to 3 days by A-5021. We next studied whether the novel immunosuppressive agent mycophenolate mofetil potentiates the antiviral activity of A-5021. We previously demonstrated that MPA enhances the anti-herpesvirus activity of ACV, penciclovir (PCV), lobucavir (LBV) and H2G [(*R*)-9-[4-hydroxy-2-(hydroxyme-thyl)butyl]guanine (Antimicrob. Agents Chemother. 42:216-222). We found that MPA also markedly potentiates the antiherpesvirus activity of A-5021. For example, at a concentration of 1 μg/ml, MPA potentiated the anti-HSV-1, anti-HSV-2 and anti TK$^-$ HSV-1 activity of A-5021 by 130-, 14- and ≥18-fold, respectively. Exogenously added guanosine reversed this potentiating action, indicating that a depletion of endogenous dGTP pools may favor the inhibitory effect of the triphoshate of A-5021 on the viral DNA polymerase. The combination of topically applied MMF (5%) with 0.05% A-5021 (a suboptimal dose) resulted in complete protection against HSV-1-induced cutaneous lesions in hairless mice, whereas therapy with either compound used singly had no protective effect at all.