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Design and Biochemical Properties of Orally Active Inhibitors of Herpes Simplex Virus Thymidine Kinase

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An important feature of herpes simplex virus (HSV) is its ability to establish latency in neuronal ganglia from which reactivation can cause severe recurrent clinical episodes. HSV encodes a thymidine kinase (TK) which is important in viral pathogenesis. The viral enzyme differs from its cellular counterpart in its ability to phosphorylate a wide range of nucleoside analogues. *In vivo* studies with TK deficient mutants suggests that the virally encoded enzyme is necessary for virus reactivation and replication in neuronal tissue. We have designed and developed potent (sub-nanomolar) and highly selective inhibitors of HSV TK that are orally active in a range of murine models of HSV infection. Such compounds have the potential to prevent recurrent genital herpes.

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Pharmacological properties of orally active inhibitors of Herpes simplex virus (HSV) thymidine kinase

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Both HSV-1 and HSV-2 encode for a thymidine kinase (TK) enzyme which differs from cellular TK in substrate specificity. Viral TK enables the virus to replicate within cells which lack cellular TK, namely those of the neuronal ganglia where the virus periodically reactivates from a latent state. In female BALB/c mice, following a HSV-2 infection via the ear pinna, replicating virus can be detected within isolated dorsal root (DR) ganglia by plaque assay on day 3 post infection. Viral titre in the DR ganglia peaks on day 5 post infection, before declining to undetectable levels as seen in the latent state of the HSV life cycle. ACV dosed orally at 5 mg/kg twice daily (12 hours apart) reduced the observed peak viral titre in the DR ganglia by approximately 60%. The replication of the herpes virus was suppressed by a specific inhibitor of viral TK, Ro 32-1520, which was administered continuously using osmotic mini-pumps. Overall a 97 - 99 % reduction in peak viral titre was observed, and a reduction in the number of ganglia in which replicating HSV was detected. A second specific inhibitor, Ro 32-4397, dosed orally four times daily (6 hours apart) at 150 mg/kg resulted in an 88% reduction in DR viral titre on day 4 post infection. The study confirms that thymidine kinase inhibitors can suppress the replication of HSV *in-vivo*, and so imply that TK inhibitors may inhibit reactivation of the virus from latency if used therapeutically in recurrent HSV infection.

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MYCOPHENOLATE MOFETIL MARKEDLY ENHANCES THE ANTIVIRAL ACTIVITY OF ACYCLOVIR, GANCICLOVIR AND PENCICLOVIR AGAINST HERPES SIMPLEX VIRUS (WILD TYPE AND TK⁻ STRAINS) AND HUMAN CYTOMEGALOVIRUS.

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Mycophenolate Mofetil, the oral prodrug of mycophenolic acid, a potent inhibitor of inosine monophosphate dehydrogenase, is used as an immunosuppressive agent in kidney transplant recipients. Since these patients are susceptible to opportunistic herpesvirus infections, they may be treated simultaneously with Mofetil for immunosuppression and acyclovir (ACV), ganciclovir (GCV) or penciclovir (PCV) for intercurrent herpesvirus infections. Inhibition of IMP dehydrogenase results in (1) depletion of intracellular dGTP pools which may favor the competitive effect of ACV-, GCV- or PCV-triphosphate for the viral DNA polymerase and (2) increased IMP pools, that may, as a phosphate donor, increase the 5'-nucleotidase-catalyzed phosphorylation of ACV, GCV and PCV. We therefore evaluated the anti-herpes activity of mycophenolic acid (MPA) and Mofetil in combination with acyclovir, ganciclovir or penciclovir. When used alone, neither MPA nor Mofetil proved active against HSV-1 [wild type and thymidine kinase deficient (TK⁻) strains] and HSV-2 at concentrations up to 50 µg/ml. When combined with either acyclovir, ganciclovir or penciclovir, MPA or Mofetil (at concentrations ranging from 0.25 to 10 µg/ml and 1 to 50 µg/ml, respectively) markedly increased (20- to 100-fold) the antiviral activity of these compounds. In combination with MPA or Mofetil, TK⁻ HSV strains became sensitive to the action of ACV, GCV and PCV. In combination with ganciclovir, MPA also had a marked synergistic effect on the replication of HCMV. However, one should also be cautious for a potential increase in the side effects of ganciclovir if combined with Mofetil.

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Anti-Herpes Activities of 2-Thio-Pyrimidine Analogues. S. Shigeta, T. Kira, S. Mori and M. Saneyoshi. Fukushima Medical College, Fukushima and Teikyo University of Science, Yamanashi, Japan.

We synthesized novel 2-thio-pyrimidine nucleoside analogues and assessed their anti-herpes activities. Among fifteen 2-thio-thymine and cytosine nucleoside analogues, 9 compounds showed selective anti-HSV and VZV activities. As a result of investigation of structure-activity relationship, pyrimidine base is active in order of 2S-5-halogenocytosine, 2S-5-halogenothymine, 2S-thymine, 2S-cytosine and sugar base is active in order of arabinose, deoxyribose, dideoxyribose. 2S-5-fluoro-cytidinearabinoside (2S-FAC) has emerged as a most potent and selective compound against HSV-1, 2 and VZV whereas did not show inhibitory activity against TK⁻ HSV and CMV. Its EC50 to HSV-1 (Kos), 2 (Lyon) and VZV (CaQu) were o.o4, o.15 and 12 ug/ml respectively. Its CC50 to RPMI8226 cells (host cells) was >500 ug/ml. Thus selectivity index to HSV-1 (Kos) exceeded 10,000. 2-Thio-pyrimidine nucleosides are potent inhibitor of HSV and VZV and are worth of investigating for development as anti-herpes drugs.