102

Inhibition of herpes simplex virus type 1 replication by a hybrid *E. coli* heat-labile enterotoxin B subunit

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The nine carboxyterminal aminoacids of herpes symplex virus ribonucleotide reductase small subunit are responsible for binding to the large subunit to form the dimeric active enzyme. Synthetic peptides corresponding to these aminoacids have been successfully used to inhibit the enzyme activity *in vitro* by disruption of the dimer. However, no activity was ever shown on virus-infected cells, possibly because of impaired intracellular access. To circumvent this problem the nine carboxyterminal aminoacids of the herpes simplex ribonucleotide reductase small subunit were fused to the C-terminus of *E. coli* heat-labile enterotoxin B subunit, the holotoxin moiety responsible for binding to eukaryotic cell surface ganglioside receptors. The fusion protein (EtxB-R2) has been shown to retain the ability to bind both the gangliosidic receptor and the major subunit of HSV ribonucleotide reductase. In addition HSV replication in VERO cells was specifically inhibited by treatment with this hybrid protein. Preincubation of EtxB-R2 with soluble ganglioside receptor abolished the antiviral effect, thus confirming the strict dependence of the above activity from EtxB-R2 binding to the target cell.

This hybrid protein could rapresent a novel approach for the treatment of viral infections as well as a useful probe for studying the internalization of *E. coli* heat-labile enterotoxin. Fusion of peptides to bacterial protein toxins could also provide a mean for peptide delivery into eukaryotic cells that covers a wide range of biological applications and is alternative to the use of self-replicating agents.

103

The N7-Isomeric Acyclic Nucleoside Analogue 2242 is a Potent and Selective Inhibitor of Cytomegalovirus Replication.

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We report on the *in vitro* anti-CMV activity of 2-amino-7-[1,3-dihydroxy-2-propoxy)methyl)purine (2242), the first known antivirally active nucleoside analogue with the side chain substituted at the N7 position of the purine ring. Compound 2242 inhibits the *in vitro* replication of both murine and human cytomegalovirus with EC_{50} values ranging from 0.02 (HCMV) to 1 μg/ml (MCMV). This is about 10 fold lower than the EC_{50} values for ganclovir and 100- to 1000-fold lower than the CC_{50} values of 2242 for cell growth. Unlike ganciclovir, compound 2242, when incubated with the infected cell cultures for a limited period of time (i.e. for the first 24-48 h post infection) afforded a long-lasting antiviral effect. Akin to ganciclovir, compound 2242 was shown to inhibit HCMV DNA synthesis [as monitored by CsCl gradient analysis] and the expression of late viral antigens with EC_{50} values of 0.18 and 0.06 μg/ml, respectively. This is close to the EC_{50} value of 2242 for inhibition of virus replication. Neither compound had any effect on the expression of immediate early viral antigens, a process that occurs before viral DNA synthesis. In a time of addition experiment, compound 2242 behaved like ganciclovir, i.e. addition of the drugs could be delayed until onset of viral DNA synthesis without loosing antiviral potency. Taken together these data indicate that compound 2242 exerts its anti-HCMV activity *via* specific inhibition of viral DNA synthesis.