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Peptide Disruption of Protein-Protein Complexes: a Novel Strategy for Antiviral Therapy

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The specificity of an antiviral compound depends on the inhibition of viral replication without affecting any other cellular function. With a better understanding of the molecular biology and biochemistry of human viruses it has become possible to screen for and detect inhibitors with activity against specific viral functions and to develop new approaches for the treatment of viral infections.

A novel strategy to inhibit viral replication is based on the specific disruption of viral protein-protein complexes. Protein-protein interactions which can be disrupted by peptides corresponding to one of the partners of the interaction can be divided in homologous interactions, in which both subunits are of the same origin (i. e. cellular or viral), and heterologous interactions involving proteins of different origin (i. e. one viral and the other cellular). Peptide-based inhibitors have a broad spectrum of potential targets. However, the evaluation and use of these peptides *in vivo* has been thwarted as they appear to be too large to enter eukaryotic cells unaided. Recently we overcame this difficulty by using the non-toxic B-subunit of *E. coli* heat-labile enterotoxin (EtxB) as a recombinant carrier for the receptor-mediated delivery of a peptide active in disrupting the ribonucleotide reductase of HSV. This discovery suggests that EtxB-mediated delivery of peptides into cells will have general application in assessing the intracellular biological efficacy of peptides with some *in vitro* activity. We are currently attempting the same strategy for the delivery of another inhibitory peptide directed to the homologous interaction between the DNA polymerase of HSV and its accessory protein UL42.

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Activity of Hydroxyurea (HU) in Combination with Several Antiviral Nucleosides Against *in vitro* Replication of Herpes Simplex Type 1 (HSV-1) and Type 2 (HSV-2) and Varicella-Zoster Virus (VZV)

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Hydroxyurea (HU) is known for its inhibitory effect on ribonucleotide reductase (RR), which is a key enzyme in the synthesis of DNA, catalyzing the conversion, at the 5'-diphosphate level, of ribonucleotides to deoxyribonucleotides. We report here the effect of the combination of HU with several antiviral drugs against wild-type (wt) and thymidine kinase-deficient (TK^-) strains of HSV-1, HSV-2 and VZV in human embryonic lung (HEL) fibroblasts. The antiviral activity of the different combinations was assessed by a plaque reduction assay (VZV) or inhibition of virus cytopathicity (HSV). The antiherpetic compounds included in the present study were acyclovir (ACV), ganciclovir (GCV), ribavirin, vidarabine (AraA) and the acyclic nucleoside phosphonates HPMPC, HPMPA, PMEA and PMEDAP. Up to concentrations of 50 μg/ml, HU did not show any effect on the *in vitro* replication of either HSV or VZV. However, HU significantly enhanced the activity of AraA against both wt and TK^- strains of HSV-1, HSV-2 and VZV [10- to 25-fold decrease in the 50% inhibitory concentration (IC_{50}) of AraA in the presence of HU]. The activity of ACV, HPMPC, ribavirin and PMEDAP was not significantly affected by HU against any of the virus strains tested. Whereas the activity of GCV, HPMPA and PMEA against wt and TK^- strains of VZV was potentiated by HU, this potentiating effect was not observed with the HSV strains, except for a slight decrease in the IC_{50} values of GCV and HPMPA for the TK^- HSV-2 strain. HU did not affect significantly the toxicity of the antiviral compounds under the conditions in which the antiviral assays were carried out, i.e. on confluent HEL cell monolayers. Whether the synergism in the antiviral action of HU and AraA, GCV, HPMPA and PMEA may be explained by the inhibitory effect of HU on RR activity or results from other properties of HU (e.g. the formation of a bidented ligand with zinc in metalloproteins) remains to be elucidated.