147

Efficacy of 5-Halogenated 2′-Deoxyuridines on Vaccinia Virus Thymidine Kinase Positive and Negative Strains, and Influence of Cell Type on Antiviral Potency

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Thymidine kinase (TK) from orthopoxviruses plays an important role in the antiviral activity of certain thymidine analogs (Antiviral Res. 2006;71:1–6), since TK+ virus was more potently inhibited than TK− virus. To investigate this further we evaluated TK+ (wild-type) and TK− forms of vaccinia (WR strain) virus for inhibition by 5-fluoro (5-F), 5-chloro (5-Cl), 5-bromo (5-Br), and 5-iodo (5-I) derivatives of 2′-deoxyuridine (dUrd). 5-F-dUrd was non-selective in its action, inhibiting both TK+ and TK− viruses equally well (EC50's 0.01–0.03 μM), and exhibited considerable toxicity to rapidly dividing uninfected cells (IC50's 0.02–0.08 μM). The other compounds were nearly equal in antiviral potency, and were not toxic to cells at 1000 μM. They preferentially inhibited TK+ virus (EC50's 2–4 μM) over TK− virus (EC50's 55–90 μM) in monkey kidney cells. However, in mouse cells both TK+ and TK− viruses were inhibited at nearly the same concentrations (1–2 μM). To help explain these effects, cells were labeled with [3H]5-Br-dUrd, [3H]5-I-dUrd, or [3H]thymidine for 12 h. SAX HPLC results indicated that the intracellular amount of nucleoside triphosphate (NTP) was greatly increased in monkey kidney cells infected with TK+ virus compared to TK− virus. But similar amounts of NTP were produced in mouse cells infected with these viruses. These results explain why TK+ virus was more potently inhibited than TK− virus in monkey cells but not in mouse cells. Thus, the contributory role of viral TK to antiviral activity of thymidine analogs depends upon cell type.

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148

Nucleosidic Fusion Inhibitors

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The chemistry, safety and pharmacology of nucleosidic drugs are all well-understood, as are their antiviral mechanisms. Whereas they target viral DNA replication, non-nucleosidic drugs target functions such as fusion. Fusion involves binding and conformational changes to bring virion envelopes and cell membranes into close apposition. Proper amphipathic compounds may be able to inhibit fusion by targeting the virion envelope to prevent such apposition. We screened nucleosides 5-derivatized to aryl-ethynyl/-propargylethers. Treated herpes simplex virus (HSV) virions were adsorbed onto cells and washed. The propargylether linker enhanced inhibition of infectivity (3-fold), as did replacing the sugar, or increasing the lypophilicity of the 5-aryl (2000-fold). The most potent compound, dUY11, had an IC_{50} of 20 or 49 nM (HSV-1 or -2), was not cytotoxic (<4% non-viable cells; SI > 1000), and just marginally cytostatic at 70 μM (3500xIC_{50}).

dUY11 did not inhibit HSV DNA replication, gene expression or virion release. HSV virions treated with 7 μM dUY11 (350xIC_{50}) were adsorbed onto cells at 4 °C and washed. dUY11 had no effect on binding (7% bound in dUY11 or no drug; 0.5% in heparin). Expression of fluorescent reporters driven by HSV-inducible promoters indicates entry. There was no fluorescence when virions were treated with 2 μM dUY11. The only function between binding and entry is fusion between two lipid bilayers, virion envelopes and cell membranes. Whereas envelopes are mostly for fusion, cell membranes are selective barriers; their lipids, proteins, curvatures and fluidities also differ. Untreated virions were adsorbed onto treated cells, or treated virions onto untreated cells. Virion treatment blocked infectivity (IC_{50} 20 nM), whereas that of cells only reduced it by 75% (IC_{50} 5.4 μM). dUY11 inhibited infectivity of vesicular stomatitis or Sindbis virus (IC_{50} 6 or 58 nM, respectively), which bind to unrelated receptors to fuse to endosomes. dUY11 targets are thus conserved among otherwise unrelated enveloped viruses.

Amphipathic nucleoside derivatives inhibit entry by preventing fusion between virion and cell membranes, providing novel scaffolds for broad-spectrum fusion inhibitors.

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149

Enhanced Potency and Efficacy of 29-mer shRNAs in Inhibition of Enterovirus 71

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Enterovirus 71 (EV71) is the main causative agent of hand, foot, and mouth disease (HFMD) in young children. It has been associated with severe neurological complications and has caused significant mortalities in large-scale outbreaks in Asia. In this study, we demonstrated an enhanced silencing of EV71 through the use of chemically synthesized 29-mer shRNAs. The 29-mer shRNAs were designed to target three highly conserved regions of EV71 genome. Transfection of rhabdomyosarcoma (RD) cells with the 29-mer shRNAs significantly inhibited EV71 replication in a dose dependent manner as demonstrated by reduction of viral RNA, VP1 protein and plaque forming units. The inhibitory effects were more potent and were achieved at 10-fold lower concentrations when compared to 19-mer siRNAs reported previously (Sim et al., 2005). The viral inhibitory effects lasted