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Selective Inhibition of Herpes Simplex Virus by 5'-Amino-2',5'-Dideoxy-5-Iodouridine

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5'-Amino-2',5'-dideoxy-5-iodouridine, a new thymidine analogue, is a potent inhibitor of herpes simplex virus type 1 replication. In contrast to most other nucleoside analogues which possess antiviral activity, 5'-amino-2',5'-dideoxy-5-iodouridine exhibits little, if any, cellular toxicity. Preliminary evidence suggests that 5'-amino-2',5'-dideoxy-5-iodouridine selectively inhibits viral-specific DNA synthesis.

Herpesviruses are responsible for many disease entities in man such as herpetic keratitis, herpes genitalis, herpes encephalitis, and herpes zoster. Recent evidence also suggests a strong correlation between herpes infection and the incidence of certain human malignancies, including cervical carcinoma (2). Considerable effort has been directed, therefore, toward the synthesis of compounds which inhibit viral growth without adversely affecting normal cell replication. Although several nucleoside analogues (5-iodo-2'-deoxyuridine [IdUrd], 5-trifluoromethyl-2'-deoxyuridine [F_3dThd], cytosine arabinoside [ara-C], and adenine arabinoside [ara-A]) have been shown to possess antiviral activity, they also induce moderate to severe cytotoxicity (1). In this report the antiviral and cytotoxic properties of a new thymidine analogue, 5'-amino-2',5'-dideoxy-5-iodouridine (AIU) (Y. C. Cheng, B. Goz, J. P. Neenan, D. C. Ward, and W. H. Prusoff, Ann. N.Y. Acad. Sci., in press), are described and compared with those of IdUrd, F_3dThd, ara-C, and ara-A. In contrast to the latter compounds, AIU exhibits significant antiviral activity in the absence of detectable cytotoxicity.

Vero cells were grown to confluency in 25-cm^2 Falcon flasks using Dulbecco medium supplemented with 10% fetal calf serum. The cells were then infected with herpes simplex virus type 1 (HSV-1) (CL-101, obtained from Wilma Summers, who originally received the virus from Saul Kit) at a multiplicity of infection of 10. After a 1-h absorption period at 37 C, the viral inoculum was removed, and the flask was washed once with phosphate-buffered saline. Medium, drug free or containing the drug concentrations indicated in Fig. 1, was then added. The infected cultures were incubated at 37 C for 40 h and then frozen until virus titrations were performed. Virus was released by freezing and thawing the media-cell suspension three times. The cell lysates were diluted directly, and the virus yield was assayed by plaque formation on Vero cells.

The percentage of PFU of virus in the drug-treated cultures relative to that found in the drug-free condition is depicted in Fig. 1A. IdUrd, F_3dThd, and ara-C have essentially the same potency of antiviral activity, although ara-C may be slightly less active, and result in a 100-fold decrease in HSV titers when present at approximately 50 μM. Although AIU is less potent than IdUrd, F_3dThd, or ara-C, on a molar basis it possesses greater activity than ara-A. Whereas 200 μM AIU produced about a 2 log decrease in virus yield, a similar concentration of ara-A reduced the virus titer by only 1 log.

The cytotoxicity of the various drugs was determined initially by measuring their effect on the growth of Vero cells. At 50 μM F_3dThd, IdUrd, or ara-C, cell growth is completely inhibited, whereas 200 μM ara-A causes a moderate, but appreciable, inhibition. In contrast, AIU produces no growth inhibition in concentrations up to at least 200 μM (Fig. 1B). Similar inhibition studies (data not shown) have shown that a continuous 3- to 4-day exposure to 400 μM AIU does not inhibit the growth rate of any of the following cell lines: murine sarcoma 180, Ehrlich ascites, BHK-21, HeLa, secondary chicken embryo fibroblasts, A-9, and L cells. In addition, AIU (at 200 μM) neither detectably alters the morphological

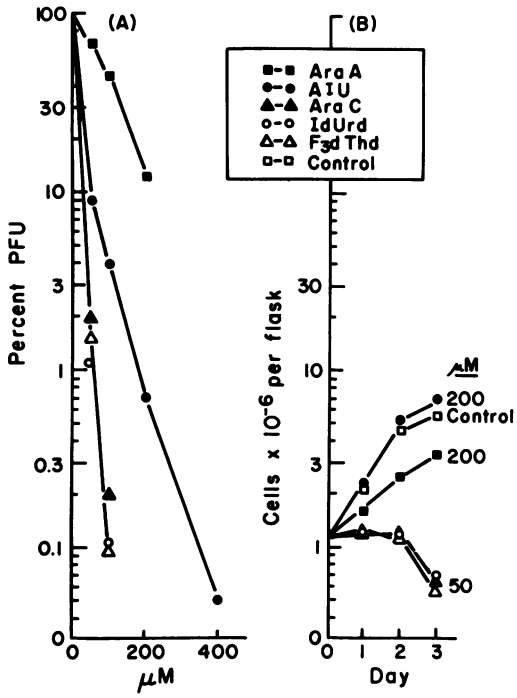

FIG. 1. *The effect of 5'-amino-2',5'-dideoxy-5-iodouridine on the replication of HSV-1 and Vero cells relative to several known antiviral agents. (A) Inhibition of HSV as a function of drug concentration (micromolar); (B) growth of Vero cells in the presence of the indicated micromolar concentration of various antiviral agents.*

characteristics nor inhibits the normal rate of DNA and RNA synthesis of uninfected Vero cells.

Although the metabolism of AIU has yet to be examined, it is unlikely that the observed biological properties result from extensive deiodination or deamination. Loss of the iodine atom would generate 5'-amino-2',5'-dideoxyuridine, a compound which is totally inactive against HSV-1 (J. P. Neenan, Y. C. Cheng, and W. H. Prusoff, Biochim. Biophys. Acta, in press). Similarily, considerable cellular cytotoxicity would be expected if IdUrd were being produced by hydrolytic deamination. The presence of the 5'-amino group may, indeed, account for the fact that AIU exhibits little, if any, cytotoxicity. Preliminary data suggests that AIU, unlike the other antiviral analogues of thymidine, is not a substrate in vitro for thymidine kinase derived from herpes-infected Vero cells. Should AIU prove incapable of being phosphorylated and thus prevented from entering the deoxynucleotide pools in vivo, then it could circumvent potential adverse cytotoxic effects, e.g., carcinogenesis or mutagenesis, which often arise by incorporation of nucleoside analogues into host cell DNA. Since cells infected with HSV-1 have an altered deoxynucleoside metabolism (Y. C. Cheng, B. Goz, and W. H. Prusoff, submitted for publication) and contain several enzymes believed to be specified by the viral genome (3, 4), the apparent selective inhibition of herpes simplex by AIU may result from direct interference with a viral-specific function.

Initial studies on the mechanism of action of AIU have led to the following observations. (i) AIU affects an intracellular event in virus replication. Preincubation of HSV with AIU prior to infection does not decrease virion infectivity. When AIU is present in the media only during the adsorption process no inhibition of virus production occurs. In contrast, addition of AIU (200 μM) 4 to 6 h postinfection markedly reduces the yield of progeny virus. (ii) AIU does not inhibit the rate of RNA or protein synthesis in either uninfected or HSV-infected Vero cells. (iii) AIU markedly inhibits the uptake of [^{14}C]thymidine into the DNA of HSV-infected Vero cells; however, no inhibition of DNA synthesis is observed in uninfected Vero cells. Although AIU appears to selectively inhibit DNA synthesis in HSV-infected Vero cells, it does not inhibit the in vitro activity of a purified HSV-1 DNA polymerase (the gift of A. Weissbach), even at a concentration of 2 mM.

The mechanism by which AIU exerts its antiviral activity and the potential use of AIU in viral chemotherapy are under current investigation.

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