Biochemical Pharmacology, Vol. 28, pp. 3483-3486.

0006-2952/79/1201-3483 $02.00/0

PRELIMINARY COMMUNICATIONS

SYNERGISTIC EFFECT OF 5'-AMINO-5'-DEOXYTHYMIDINE AND 5-IODO-2'-DEOXYURIDINE AGAINST HERPES SIMPLEX VIRUS INFECTIONS IN VITRO

Paul H. Fischer, Jung J. Lee, Ming S. Chen, Tai-Shun Lin and William H. Prusoff

Department of Pharmacology, Yale University School of Medicine, New Haven, CT 06510, U.S.A.

(Received 4 September 1979; accepted 27 September 1979)

5-Iodo-2'-deoxyuridine (IdUrd), first synthesized in this laboratory [1], is an effective inhibitor of herpes simplex virus type 1 (HSV-1) replication [2] and a clinically effective drug in the treatment of herpes keratitis [3]. However, the systemic use of this agent is limited by bone marrow toxicity [4,5]. In contrast, 5'-amino-5'-deoxythymidine (5'-AdThd), first synthesized by Horwitz et al. [6], is a highly selective, but not particularly potent, antiherpes agent [7]. An effort has been made to reduce the toxicity of IdUrd and yet retain its high degree of therapeutic efficacy. We postulated that selective inhibition of drug activation in uninfected tissues with retention of antiviral activity in virally infected cells could be achieved by using these thymidine analogs in combination. The target of this approach is the host tissue thymidine kinase, the enzyme which catalyzes the phosphorylation of IdUrd to the 5'-monophosphate, IdUMP [8]. We hypothesized that 5'-AdThd should block the activation of IdUrd in normal cells, whereas both compounds would be phosphorylated in HSV-1 infected cells for the following reasons: (1) the 5'-amino derivative of IdUrd is phosphorylated only in HSV-1 infected cells [9], (2) 5'-AdThd is a good inhibitor of mammalian cell thymidine kinase [10] and (3) high levels of the virally specified thymidine kinase, the enzyme responsible for the phosphorylation of thymidine analogs in HSV-1 infected cells, are usually induced in HSV-1 infected cells [11,12].

In these experiments, Vero cells, grown as a monolayer in Dulbecco's modified minimum essential medium supplemented with 10% fetal calf serum, were used for the toxicity studies and for the propagation and assay of virus [7]. The effects of IdUrd and 5'-AdThd, alone and in combination, on the replication of HSV-1 (strain C1-101) were determined using a yield reduction assay. Confluent Vero cells were infected with HSV-1 at a multiplicity of infection of 10 for 1 hr and then washed with phosphate-buffered saline. Medium, with or without drug(s), was added and the incubation continued for 24 hr. The flasks were then stored at -70° until the virus yield was determined by plaque formation on Vero cells. Toxicity to the uninfected cells was assessed by determining the effects of these compounds on the replication of exponentially growing Vero cells during a 72 hr exposure period.

Uptake of [6-^{3}H]IdUrd (Moravek Biochemicals, City of Industry, CA) was measured as the accumulation of acid-soluble radioactivity in Vero cells. Nearly confluent cells were exposed to 5'-AdThd for 30 min before the addition of [6-^{3}H]IdUrd. At various times thereafter, the cells were washed three times with ice-cold phosphate-buffered saline and then extracted with 0.5 M $HClO_4$ for 15 min.

The ability of 5'-AdThd to prevent the toxicity of IdUrd to uninfected Vero cells is shown in Fig. 1. The dose-response curve is shifted to the right, indicating protection. 5'-AdThd could totally prevent the inhibition of cell growth caused by 25 μM IdUrd. Thus, IdUrd can be used in combination with 5'-AdThd at higher concentrations than would be possible as a single agent and still be non-toxic to the uninfected host cells.

In contrast, the antiviral activity of the two agents in combination is enhanced. The effects of adding IdUrd (1 μM) to increasing concentrations of 5'-AdThd on the replication of HSV-1 are shown in Fig. 2. All of the combinations were more effective than either agent alone. Nearly three log reductions in virus titer have been achieved using higher concentrations of these analogs (25 μM IdUrd and 1 mM 5'-AdThd) in the absence of host cell toxicity.

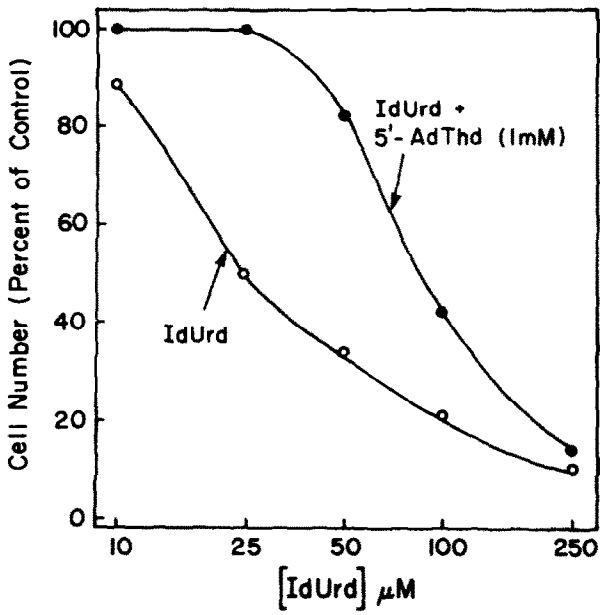

Fig. 1. Effect of 5'-AdThd on the cytotoxicity of IdUrd. Vero cells, growing exponentially in monolayer, were exposed for 72 hr to the indicated concentrations of IdUrd in the presence (●) or absence (o) of 5'-AdThd (1 mM).

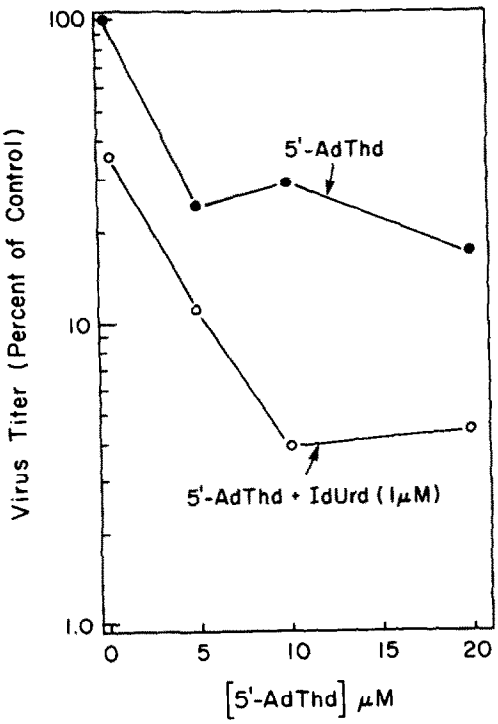

Fig. 2. Effect of 5'-AdThd and IdUrd on the replication of herpes simplex virus. HSV-1 infected Vero cells were exposed to either 5'-AdThd at the indicated concentrations (●) or varying concentrations of 5'-AdThd in combination with 1 μM IdUrd (o).

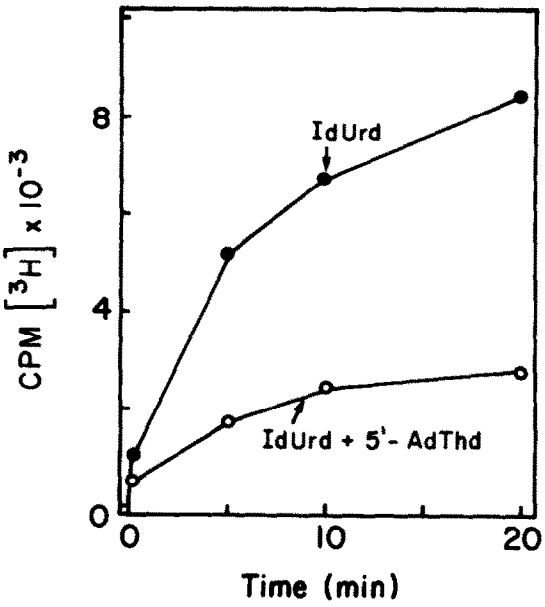

Fig. 3. Effect of 5'-AdThd on the uptake of [^{3}H]IdUrd into acid-soluble metabolites in Vero cells. Confluent monolayers of Vero cells were exposed to [^{3}H]IdUrd (1 μM) (●) or to [^{3}H]IdUrd (1 μM) and 5'-AdThd (300 μM) (o). The amount of perchloric acid-extractable radioactivity retained in the cells after three washes with ice-cold phosphate-buffered saline was determined after various times of incubation at 37°.

The uptake of IdUrd in uninfected cells should be reduced by 5'-AdThd if thymidine kinase is, indeed, being inhibited. The data in Fig. 3 show this effect. 5'-AdThd (300 μM) reduced IdUrd (1 μM) uptake by about 65 percent. Uptake, which reflects the intracellular phosphorylation and, thus, trapping of the free nucleoside, could also be reduced by interference with the transport of IdUrd. 5'-AdThd did not inhibit [^{3}H]uridine uptake, which is presumably transported by a similar mechanism [13] but phosphorylated by different enzymes than IdUrd. These results suggest that phosphorylation rather than transport is probably involved.

Thus the preliminary data, if confirmed in animal systems, suggest that appropriate combinations may have clinical value in viral chemotherapy. This therapeutic synergism may be accounted for by inhibition of phosphorylation of IdUrd in normal cells, but with retention of antiviral activity in HSV-1 infected cells. However, much more data are required to rigorously test this hypothesis, to determine the effectiveness of this combination against a variety of herpes virus strains, and to establish which biochemical parameters determine drug sensitivity.

Acknowledgements—This investigation was supported by the United States Public Health Service Grant CA-05262 from the National Cancer Institute. P.H.F. is supported by Public Health Service Postdoctoral Fellowship CA-05604 from the National Cancer Institute and T.S.L. is supported, in part, by a grant from the Bristol-Myers Co.

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