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TIME-DEPENDENT ACTION OF 5-PROPYL DEOXYURIDINE AS ANTIHERPES SIMPLEX VIRUS TYPE 1 AND TYPE 2 AGENTS

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Abstract—The most effective period of 5-propyl deoxyuridine action was 3–6 hr post-infection in both herpes simplex virus type 1- and type 2-infected HeLa BU cells. This antiviral activity could not be reversed by removing the drug from the culture medium. A correlation exists between inhibition of virus growth and inhibition of induction of virus specific DNase and DNA polymerase. No inhibition of induction of virus specific thymidine kinase was observed when virus replication was inhibited by 5-propyl deoxyuridine.

5-Propyl deoxyuridine (PdUrd) has been shown to have selective effects against the proliferation of herpes simplex virus (HSV) in culture [1, 2]. The PdUrd selectivity stems from the fact that it is a much poorer substrate than thymidine for human cytoplasmic and mitochondrial thymidine kinases (TK) [3–5]. In contrast, PdUrd and thymidine (dThd) are equally good substrates for HSV-1 and HSV-2 specific TK. PdUrd, once phosphorylated in HSV-infected or -transformed cells, can exert its adverse effects on DNA synthesis. In contrast, cells which do not have HSV specific TK can not phosphorylate PdUrd and, therefore, the compound has no toxicity toward them [1]. Recently, other investigators have found this "selective alternative substrate" approach to be the basis of the selectivity of some other specific antiherpes viral agents [6–10]. PdUrd was added to infected cultures as part of a program to explore the possible use of this drug as an effective HSV-1 and HSV-2 agent *in vivo*, and its mechanism of action. PdUrd was added to infected cultures and removed at different time intervals to study the reversibility of drug action and to determine the critical period of drug exposure. In addition, the effects of PdUrd on viral enzyme induction were also examined. The results of these studies are reported in this communication.

MATERIALS AND METHODS

Cells and viruses. The stock viruses, HSV-1 (Strain KOS) and HSV-2 (Strain 333), were given to us by W. Munyon and R. Hughes of this Insitute. They were propagated in CV-1 monolayer culture as described previously [1].

HeLa BU, a TK^- subline of HeLa, was used because this cell line allowed us to examine the effects of PdUrd on the ability of the virus to induce TK activity without the complication of host TK.

Confluent HeLa BU cells (25 cm^2) were exposed to a 1-hr adsorption of virus, with 5–10 plaque-forming units (PFU)/cell. Unadsorbed virus was removed by rinsing the cell layers twice with phosphate buffered saline. The cells were incubated in 4 ml of Eagle's minimum essential medium supplemented with 5% calf serum for a total period of 28 hr at 37°.

PdUrd (25 μM) was added at the designated times and removed by replacement with drug-free medium. The untreated infected cultures had the same medium changes as the treated infected cultures. At the end of the incubation the flasks were stored at −70° until titration.

Materials. PdUrd was provided by Dr. D. Bergstrom from the University of California at Davis. The other materials are the same as described previously [1].

Enzyme assays. Preparation of the crude homogenate for enzyme assays and the methods for assaying TK and DNase were described previously [11, 12].

The DNA polymerase assay was a modification of Weissbach *et al.* [13] in that 150 mM KCl was substituted for $(NH_4)_2SO_4$.

RESULTS

Antiviral activity of PdUrd at different times post-infection. PdUrd (25 μM) was added to virus-infected cell cultures at different times post-infection. Its effectiveness on the growth of HSV-1 and HSV-2 in cultured cells is depicted in Fig. 1, panels A and B respectively. When PdUrd was added at a zero time of 2 hr post-infection with either virus, a strong inhibition of growth of the respective virus was observed. The effectiveness decreased when PdUrd was added at later times post-infection. The numbers of infectious viruses synthesized when the drug was added 8 and 11 hr post-infection are the same as those synthesized without addition of drug.

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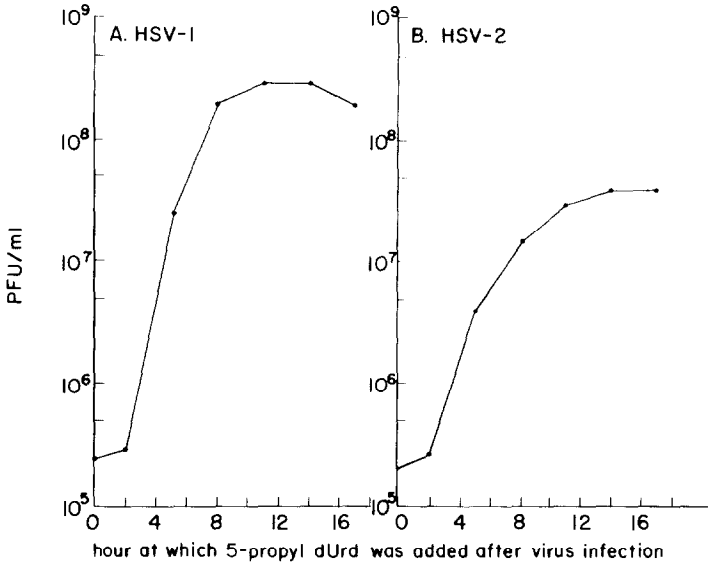

Fig. 1. Effects of PdUrd on the growth of HSV-1 and HSV-2 in cultured cells. HeLa Bu cells were infected with HSV-1 or HSV-2. PdUrd (25 μM) was added at different times as indicated by post-infections. Twenty-eight hr post-infection, cells were frozen with the medium at −70° until the virus titration was performed.

Reversibility of PdUrd antiviral action. PdUrd (25 μM) was added to the cultures at zero time, and was removed at designated times post-infection by changing the medium. The PFU/ml in the drug-treated infected cultures were compared with those in untreated infected cultures after a 28-hr incubation (Table 1). There was no difference in the effectiveness of PdUrd as an antiviral agent when the drug was removed at 6, 9 or 12 hr post-infection. A difference in the antiviral efficacy of PdUrd was observed in infected cultures when the drug was removed at 3 hr post-infection.

Critical period of the antiviral action of PdUrd. Virus-infected cultures were exposed to PdUrd (25 μM) at different times post-infection, for 3 hr intervals. At the end of a 28-hr incubation, the PFU/ml in each culture were determined and compared with the proper controls (Table 2); the virus-infected cells are most sensitive to PdUrd during the 3–6 hr interval. This is consistent with the observations described in Fig. 1 and Table 1.

Effects of PdUrd on virus specific enzyme induction. The effects of PdUrd on the induction of three virus-specific enzymes, TK, DNA polymerase and DNase, in HSV-1 infected cells were studied (Fig. 2). When PdUrd (25 μM) was given immediately after infection, there was an increase in the activity of TK but a decrease in the activity of DNA polymerase and DNase. The drug was added to virus-infected cells at different 3-hr intervals post-infection during an 18-hr incubation; then the enzyme activity of these cells was compared with that of the untreated controls (Table 3). The TK activity of virus-infected cells treated with PdUrd was not drastically different during any 3-hr interval. The DNA polymerase and DNase activities in the infected cells treated with PdUrd from 0–3 and 3–6 hr periods post-infection were less than those in cells treated with drug from 6–9 and 9–12 hr periods post-infection.

Table 1. Relationship of antiviral activity of 5-Propyl dUrd to the time of exposure of HSV-infected cells to the drug*

Period of exposure after viral infection (hr)	% Control PFU	
	HSV-1	HSV-2
0–3	10	12.5
0–6	0.5	5.0
0–9	0.5	2.0
0–12	0.25	1.0

*Concentration of 5-propyl-dUrd used was 25 μM.

DISCUSSION

PdUrd was demonstrated previously to be a selective antiherpes simplex agent [1,2]. The selectivity is due to the induction of HSV-1 or HSV-2 specific TK in virus-infected cells [5], but the mechanism of action is still unclear.

Table 2. Relationship of antiviral activity of 5-Propyl dUrd to the post-infection time before exposure of HSV-infected cells to the drug*

Period of exposure after viral infection (hr)	% Control PFU	
	HSV-1	HSV-2
0–3	10	12.5
3–6	0.5	3.0
6–9	67	50
9–12	100	60
12–15	100	58

*Concentration of 5-propyl dUrd used was 25 μM.

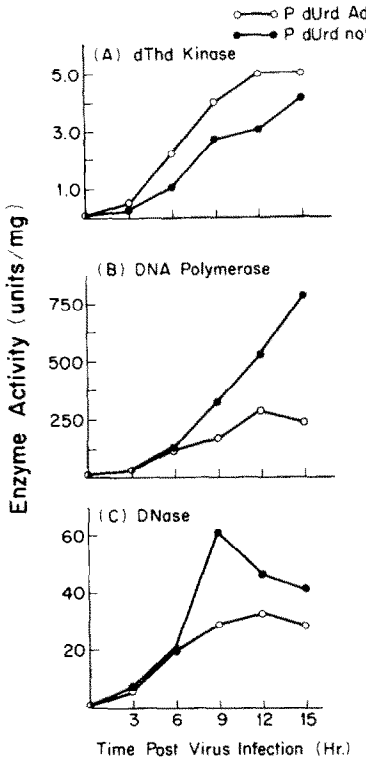

Fig. 2. Effects of PdUrd on viral enzyme induction in cells infected with HSV-1 (KOS). HeLa Bu cells were infected with HSV-1 (KOS) with multiplicity of 5–10 PFU/cell. They were incubated in the regular culture medium with or without 25 μM PdUrd, as described. At designated times post-infection, cells were harvested and processed as described in Materials and Methods.

Studies reported in the above sections clearly demonstrate that the antiviral action of PdUrd is irreversible. PdUrd exerted its action even in the first 3 hr post-infection, when the virally induced TK has just started to appear in infected cells [12]. The most effective period of drug action is between 3 and 6 hr post-infection. The rate of PdUrd incorporation into viral DNA is faster in the period of 6–9 hr than 3–6 hr (unpublished results); however, when PdUrd was given at 6 hr or later, the drug was no longer effective.

This raises the question of whether the antiviral action of PdUrd is due simply to the incorporation of the drug into viral DNA. The DNA synthesis in virus-infected cells does not reach its peak until 8 hr post-infection [14], and all enzymes required for PdUrd incorporation into viral DNA are present after 6 hr. In addition, the PdUrd-containing virion is as effective an infectious agent as virion containing no PdUrd (unpublished results in collaboration with Dr. D. Bergstrom). These observations strongly suggest that the antiviral action of PdUrd cannot be explained on the basis of its incorporation into viral DNA, which is packed into the virion. This does not rule out the possibility that PdUrd may have its effect in an initial program of viral DNA synthesis, or in an early event which could affect later DNA synthesis, or in the induction of a viral enzyme required for viral DNA synthesis.

In summary, the effects of PdUrd on the induction of three virus enzymes were examined. The induction of viral TK was not inhibited by the drug. The higher activity of TK in drug-treated cells in comparison with the control may be be due to the fact that TK was protected against inactivation in cells by PdUrd. In contrast, the induction of both viral DNA polymerase and DNase was inhibited by PdUrd, and this inhibition is not reversible. Since viral DNA polymerase is required for viral DNA synthesis, it is possible that the action of PdUrd is through some mechanism by which the induction of viral DNA polymerase and DNase is inhibited. In turn, this leads to the inhibition of viral DNA synthesis and proliferation. We are currently investigating this hypothesis.

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Table 3. Effects of the 5-Propyl dUrd exposure period on enzyme induction in HSV-1 infected cells*

Time of exposure to drug post virus infection (hr)	dThd kinase	DNA polymerase (units/mg)	DNase
0–3	3.94	185	27
3–6	4.57	239	36
6–9	4.63	540	69
9–12	4.81	540	73
–†	0.05	0.7	0.45
–‡	4.27	740	75

* HeLa Bu cells were infected with HSV-1 (KOS) with multiplicity of 5–10 PFU/cell. 5-Propyl dUrd (25 μM) was added at different times post-infection, then it was removed at the designated times by substituting the drug containing culture medium with fresh medium. The rest is the same as described in the text.

†Mock infected HeLa Bu cells.

‡HSV-1 infected HeLa Bu cells without 5-propyl dUrd addition.

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