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5-Propyl-2'-Deoxyuridine: a Specific Anti-Herpes Agent

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In both primary rabbit kidney cells and human skin fibroblasts, 5-propyl-2'-deoxyuridine proved inhibitory to herpes simplex virus at a concentration as low as 1 μg/ml, whereas concentrations higher than 200 μg/ml were required to inhibit vaccinia virus replication or normal cell metabolism.

Several deoxythymidine (TdR) analogs, namely, 5-methylamino-2'-deoxyuridine (18), 5-methoxymethyl-2'-deoxyuridine (2), 5-methylmercapto-2'-deoxyuridine (12), 5-bromo- and 5-iodo-2'-deoxycytidine (9, 15, 16, 17), 5-iodo-5'-amino-2'-5'-dideoxyuridine (4, 13, 14), 1-β-D-arabinofuranosylthymine and 1-β-D-arabinofuranosyl-5-methylcytosine (1, 8, 11), and 5-ethyl-, 5-vinyl-, 5-propyl- and 5-allyl-2'-deoxyuridine (3, 7, 10), have been shown to inhibit the replication of herpes simplex virus (or varicella-zoster virus [9]) at doses which did not adversely affect the growth of the (uninfected) host cells. Whereas 5-iodo-2'-deoxycytidine, 5-bromo-2'-deoxycytidine, 5-ethyl-2'-deoxyuridine, and 1-β-D-arabinofuranosylthymine were also reported as being active against vaccinia virus (7, 8, 15, 16), other TdR analogs such as 5-iodo-5'-amino-2'-5'-dideoxyuridine (14), 5-methylamino- and 5-methoxymethyl-2'-deoxyuridine (2, 18) proved specifically active against herpes simplex virus.

Here we report that 5-propyl-2'-deoxyuridine (PrUdR) inhibits the replication of herpes simplex type 1 virus (strain KOS) at a concentration that is at least 200 times lower than that required to inhibit other deoxyribonucleic acid (DNA) viruses such as vaccinia virus and to suppress cellular DNA synthesis. Accordingly, PrUdR, the antiviral properties of which were first mentioned by Gauri and Malorny (10), could be considered as a selective inhibitor of herpes simplex virus.

PrUdR was synthesized in a manner analogous to that of 5-ethyl-2'-deoxyuridine (EtUdR), previously described (19), starting with 5-propyluracil kindly supplied by B. Fiszer. The product was recrystallized from ethanol in the form of needles, mp 169°C (uncorrected). Its ultraviolet absorption spectrum in aqueous medium at neutral and alkaline pH was similar to that of thymidine and EtUdR. The same compound has been prepared by Cheng et al. (3) by catalytic hydrogenation of 5-allyl-2'-deoxyuridine on 5% palladium-charcoal in methanol. We are also indebted to E. Mauz (Robugen GMBH) for a sample of PrUdR. The following nucleoside derivatives served as reference materials: EtUdR (7, 19), 5-iodo-2'-deoxyuridine (IUdR) (Ludeco, Brussels), 1-β-D-arabinofuranosylcytosine (ara-C) (Upjohn; Puurs, Belgium) and 9-β-D-arabinofuranosyladenine ([ara-A] courtesy of R. Wolf, Parke, Davis Clinical Research Western Europe, Munich).

Antiviral activity was determined as inhibition of cytopathogenicity induced by herpex simplex virus, vaccinia, or vesicular stomatitis virus in primary rabbit kidney (PRK) cells or human skin fibroblasts ([HSF] strain VGS) (5, 6). Antimetabolic activity was based on inhibition of incorporation of [*methyl*-^{3}H]2'-TdR or [2-^{14}C]2'-deoxyuridine (UdR) into host cell DNA. The incorporation of [*methyl*-^{3}H]TdR and [2-^{14}C]UdR into cellular DNA was monitored by a microplate assay described previously (5). [*methyl*-^{3}H]TdR was obtained from the Institute of Radio-elements (Fleurus, Belgium) and [2-^{14}C]UdR was obtained from the Radiochemical Centre (Amersham). The specific radioactivity of [*methyl*-^{3}H]TdR was 12 Ci/mmol, and the specific radioactivity of [2-^{14}C]UdR was 57 mCi/mmol.

For EtUdR, IUdR, and ara-C, the minimum effective doses required to inhibit herpes simplex virus did not differ from those which were found inhibitory to vaccinia virus. For ara-A, a 10-fold-higher dose was needed to inhibit herpes simplex than to inhibit vaccinia virus replication (Table 1). PrUdR, however, was selectively active against herpes simplex. It inhibited herpes simplex type 1 virus (strain KOS) at 1 μg/ml but failed to inhibit vaccinia virus at concentrations up to 200 μg/ml (Table 1). PrUdR proved also inhibitory to herpes simplex virus strains other than the KOS strain, e.g., herpes simplex type 1 (strain LYONS) and herpes simplex type 2 (strain 196) albeit at a somewhat higher concen-

TABLE 1. *Antiviral and antimetabolic activities of PrUdR as compared with the antiviral and antimetabolic activities of related compounds with established antiviral properties*

Assay system	Minimum inhibitory concentration[a] (μg/ml)				
	PrUdR	EtUdR	IUdR	Ara-C	Ara-A
Antiviral activity					
Vaccinia/PRK	≥200	1	0.2	0.04	0.4
Herpes simplex type 1 (strain KOS)/PRK	1	1	0.2	0.04	4
Vesicular stomatitis/PRK	>200	>200	>200	40	30
Herpes simplex type 1 (strain KOS)/HSF	1	1	0.1	0.02	1
Herpes simplex type 1 (strain LYONS)/HSF	10	2	1	0.1	4
Herpes simplex type 2 (strain 196)/HSF	7	0.7	0.7	0.04	4
Antimetabolic activity					
[*methyl*-^{3}H]TdR/PRK	300	150	2.5	0.1	25
[2-^{14}C]UdR/PRK	300	30	1.2	0.05	20

[a] Concentration required to inhibit virus-induced cytopathogenicity, or [*methyl*-^{3}H]TdR or [2-^{14}C]UdR incorporation by 50%. The compounds were added immediately after virus infection or together with the radiolabeled precursors. Virus input: 100 50% cell culture infecting dose. Input of radiolabeled precursors: 0.12 μCi/0.01 nmol/10^5 cells for [*methyl*-^{3}H]TdR and 14 μCi/250 nmol/10^5 cells for [2-^{14}C]UdR.

tration (10 μg/ml) than that required to inhibit herpes simplex type 1 KOS (Table 1). PrUdR did not inhibit the replication of ribonucleic acid viruses such as vesicular stomatitis virus (Table 1), polio type 1, coxsackie B4, and measles virus (data not shown).

PrUdR did not show evidence of microscopic toxicity for PRK cells at 200 μg/ml and did not impair cellular DNA synthesis, as monitored by either [*methyl*-^{3}H]TdR or [2-^{14}C]UdR incorporation, unless doses as high as 300 μg/ml were employed (Table 1). In contrast to PrUdR, the reference materials IUdR, ara-C, and ara-A inhibited cellular DNA synthesis at concentrations that were not far in excess of those required to inhibit virus replication (Table 1).

To ascertain that the inhibitory effects of PrUdR on viral cytopathogenicity actually reflected an inhibition of viral multiplication, vaccinia and herpes simplex virus growth were determined in cell cultures that had been exposed to PrUdR immediately after virus inoculation (Fig. 1). Like IUdR, PrUdR effectively suppressed the growth of herpes simplex virus (strain KOS). Unlike IUdR, PrUdR failed to affect the multiplication of vaccinia virus (Fig. 1).

Antiviral indexes, computed by dividing the minimum toxic dose (causing 50% inhibition of cellular DNA synthesis, as determined by either TdR or UdR incorporation) by the minimum effective dose (causing 50% inhibition of the cytopathogenicity of herpes simplex virus type 1, strain KOS) (Table 1), were as follows: 300 for PrUdR, 30 to 150 for EtUdR, 6 to 12 for IUdR, 1 to 2 for ara-C, and 5 to 6 for ara-A. If the minimum toxic dose was defined as the dose

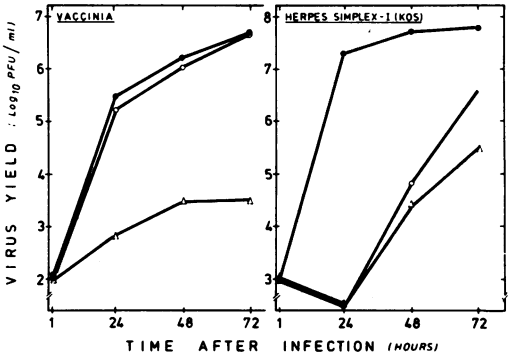

FIG. 1. *Effect of PrUdR and IUdR on the growth of vaccinia virus and herpes simplex type 1 (KOS) virus in PRK cell cultures. The compounds were added immediately after virus adsorption. Virus input: 4.5 $\log_{10}$ plaque-forming units per ml. Virus yields were determined by plaque formation in either PRK (vaccinia) or VERO (herpes simplex) cell cultures. Symbols: ●, control; ○, PrUdR (100 μg/ml); △, IUdR (10 μg/ml).*

causing 30% inhibition of (PRK) cell proliferation, the antiviral indexes were: >300 for PrUdR, 100 for EtUdR, and 20 for IUdR (E. De Clercq, J. Descamps, P. F. Torrence, E. Krajewska, and D. Shugar, 10th Int. Cong. of Chemother., Zürich, Switzerland, September 18–23, Abstr. no. 450, 1977). Thus, PrUdR appears to exhibit a significantly greater safety margin (at least in PRK cell cultures) than the established antiviral drugs IUdR, ara-C, and ara-A.

The minimum concentration at which PrUdR was found effective in inhibiting herpes simplex type 1 (strain KOS) in PRK and HSF cell cultures (1 μg/ml) closely corresponds to the mini-

mum concentration (~3 μM) at which PrUdR inhibited herpes simplex virus replication in HeLa cells (3). However, in these previous studies (3) it was not assessed whether PrUdR was effective against viruses other than herpes simplex.

The mechanism by which PrUdR exerts its selective anti-herpes action remains to be unravelled. PrUdR may specifically inhibit one or another enzyme that is coded for by the virus. As noted previously for other TdR analogs (6), the anti-herpes activity of PrUdR depends on the presence of a virus-induced TdR kinase in the infected cell. PrUdR was efficacious against herpes simplex type 1 strain KOS, a virus that induces TdR kinase activity in both PRK and HSF cell cultures (6). However, PrUdR did not prove efficacious against herpes simplex type 2 strain 333, a virus that does not induce TdR kinase activity in PRK and HSF cells (6). The latter observations point to the necessity of a specific virus-induced TdR kinase for PrUdR to be effective as an anti-herpes agent. It does not necessarily imply that PrUdR acts at the TdR kinase level. After it has been converted to its 5'-monophosphate, PrUdR may interfere at various steps that lead to the synthesis of viral DNA, including the DNA polymerization step.

It is noteworthy that the anti-herpes activity of PrUdR was readily reversed by TdR: 0.4 μg/ml of TdR sufficed to partially block the antiviral effect of 10 μg/ml of PrUdR. The anti-herpes activity of IUdR could also be reversed by TdR, but only if the concentration of TdR equalled that of IUdR. The latter results may reflect differences in the mode of action of IUdR and PrUdR. This possibility is now being examined.

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