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Incorporation of (*E*)-5-(2-Iodovinyl)-2′-Deoxyuridine into Deoxyribonucleic Acids of Varicella-Zoster Virus (TK^+ and TK^- Strains)-Infected Cells

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SUMMARY

The incorporation of (*E*)-5-(2-iodovinyl)-2′-deoxyuridine (IVDU) into DNA of varicella-zoster virus (VZV)-infected human embryo fibroblasts was studied, using thymidine kinase-positive (TK^+) and thymidine kinase-negative (TK^-) VZV strains. [^{125}I]IVDU was taken up by cells infected with TK^+ VZV-, but not by TK^- VZV- or mock-infected cells. [^{125}I]IVDU was incorporated into both VZV DNA and cellular DNA of TK^+ VZV-infected cells. When the cells were exposed to 0.3 μM IVDU, a more marked shift was noted in the buoyant density of viral DNA than of host DNA. In contrast, the DNAs isolated from TK^- VZV- or mock-infected cells did not exhibit a detectable incorporation of [^{125}I]IVDU. [^{125}I]IVDU-labeled VZV DNA was purified from the viral nucleocapsids of TK^+ VZV-infected cells. Substitution of no more than 0.1–1% of the thymidine residues in the VZV DNA by IVDU seemed to suffice to inhibit the replication of VZV.

BVDU and IVDU are very potent and selective antiherpes agents (1–8). They inhibit HSV-1 and VZV replication in cell culture at a very low concentration with minimal toxicity for the host cells. The biochemical basis for the antiviral action of these nucleoside analogues is under investigation (9–18). The basis for the selective action of BVDU and IVDU against HSV-1 replication resides in their enhanced affinity for the virus-encoded TK, since phosphorylation of these drugs appears to be essential for antiviral activity (10, 12–18). The triphosphate derivatives (BVDUTP, IVDUTP) of BVDU and IVDU are competitive inhibitors of HSV-1 DNA polymerase with respect to the normal substrate, dTTP (9, 11). Moreover, BVDU and IVDU are incorporated into DNA of HSV-1-infected cells (12), and the degree of inhibition correlates well with the amount of BVDU substituted for dThd in HSV-1 DNA (19).

Although BVDU and IVDU are more inhibitory to VZV than HSV-1 (2, 8), the mechanism of action of these drugs against VZV replication has not been the subject of detailed investigation, except for the effect of BVDUTP in VZV DNA polymerase (20). BVDUTP proved considerably more inhibitory to the VZV DNA polymerase than cellular DNA polymerases.

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To further assess the effect of these nucleoside analogues on VZV DNA synthesis, we have now investigated the incorporation of [^{125}I]IVDU into viral and cellular DNAs of both TK^- and TK^- VZV-infected cells.

The present investigations differ in various aspects from previous studies (10, 12, 19) aimed at delineating the mechanism of antiviral action of BVDU and IVDU in that, in the present studies: (i) VZV was used, a virus which is significantly more sensitive to the antiviral activity of BVDU and IVDU than is HSV-1 (2, 8); (ii) a highly radiolabeled analogue of BVDU, namely, [^{125}I]IVDU, was employed to monitor its incorporation into viral and cellular DNA; (iii) incorporation of [^{125}I]IVDU into DNA was assessed in parallel for both TK^+ and TK^- VZV-infected cells; (iv) this incorporation was not only assessed by measuring radioactivity but also visualized morphologically by using autoradiography; (v) [^{125}I]IVDU-labeled VZV DNA was purified from viral nucleocapsids; and (vi) [^{125}I]IVDU-labeled VZV DNA and cell DNA were characterized by using hybridization with the appropriate DNA probes.

From the results of our studies it appears that substitution of no more than 0.1–1% of the thymidine residues of VZV DNA by IVDU sufficed to confer an inhibitory effect on VZV replication.

ABBREVIATIONS: BVDU, (*E*)-5-(2-bromovinyl)-2′-deoxyuridine; IVDU, (*E*)-5-(2-iodovinyl)-2′-deoxyuridine; HSV-1, herpes simplex virus type 1; VZV, varicella-zoster virus; TK, thymidine kinase; TK^+, thymidine kinase-positive, TK^-, thymidine kinase-negative; HEF, human embryo fibroblast; CPE, cytopathic effect; PBS, phosphate-buffered saline; FFU, focus-forming unit; PEI, polyethyleneimine; EDTA, ethylenediaminetetraacetate; C-IVDU, carbocyclic analogue of IVDU.

Materials and Methods

VZV. The parent strain (Kanno, TK^+ VZV) of VZV, which had been isolated from a patient with herpes zoster, was cloned and cultivated in HEF cells. The mutant strain (Kanno-Kohmura, TK^- VZV), which is resistant to BVDU and IVDU, was isolated by repeated passages of the parent VZV strain in the presence of 5-bromodeoxyuridine and 5-iododeoxyuridine. The details of the isolation procedures of the TK^- VZV strain have been described previously (21–23). To obtain cell-associated virus, HEF cell monolayers were infected with VZV by co-cultivation of VZV-infected HEF cells and uninfected cells at a ratio of 5:1. Seventy-two hr after infection, when more than 80% of cells showed a CPE, the cells were washed twice with PBS and treated with 0.01% trypsin in PBS. Trypsinized cells were then collected by centrifugation at 1500 rpm for 5 min, suspended in growth medium (Dulbecco's modified minimum essential medium supplemented with 10% fetal bovine serum) containing 10% dimethyl sulfoxide, and frozen at −80° until used. In the infectivity assays, FFUs were counted.

Chemicals. [^{125}I]IVDU (specific activity, 32.2 Ci/mmol) was synthesized as described previously (14). [*methyl*-1′,2′-^{3}H]Thymidine ([^{3}H]dThd) (specific activity, 130 Ci/mmol) was obtained from New England Nuclear Corp. Calf thymus DNA and λ phage DNA were obtained from Boehringer Mannheim Corp.

Quantitation of cellular uptake of [^{125}I]IVDU. HEF cells were grown to confluency in Falcon tissue culture trays (Six-well tray, 9.6 cm^2/well) or flask (75 cm^2/flask). The growth medium was removed, and the monolayer was infected with TK^+ VZV or TK^- VZV (3×10^3 FFUs/well or 5×10^4 FFUs/flask) or mock-infected. Each infection was performed in triplicate, and either 2 ml or 30 ml of fresh growth medium were added to each well or flask. The cultures were then incubated at 37°. After 24–36 hr incubation, when 40–50% of cells showed CPE, [^{125}I]IVDU was added to the cell cultures at the indicated concentration. Twelve hr after the addition of [^{125}I]IVDU the cells were detached by gentle scraping and harvested by low speed centrifugation. The harvested cells were washed five times with PBS. The cell pellets were then counted directly in an LKB gamma counter.

Acid-soluble and -insoluble fractions. The harvested cell pellets (10^6 cells) were suspended in 0.2 ml of 0.5 N perchloric acid. After 5 min incubation at 0°, the supernatant fraction was separated by centrifugation and evaluated for acid-soluble radioactivity. The residual pellet was washed twice with 0.2 ml of perchloric acid solution and evaluated for acid-insoluble radioactivity. The acid-soluble (supernatant) fraction was subjected to PEI-cellulose thin layer chromatography in a solvent system of 5 M lithium chloride/formic acid/water (1:1:8), as described previously by Cheng *et al.* (24).

Autoradiography and staining. VZV- and mock-infected cells were cultivated in the presence of [^{125}I]IVDU (0.1 μCi/ml) for 12 hr. The cells (on slide glass plates) were washed five times with PBS and fixed with Bouin's solution. After repeated washings with 70% alcohol in distilled water and one washing with distilled water, the slide glasses were taken out and air dried. Dipping autoradiography (25) was carried out with NR-M2 emulsion (Konishiroku Photo Industry Co., Tokyo), using a 3-day exposure period at 4°. The cells were stained with either hematoxylin-eosin or Giemsa solution.

VZV DNA. TK^+ VZV DNA and TK^- VZV DNA were purified from the nucleocapsids isolated from either TK^+ VZV- or TK^- VZV-infected cells, as described previously by Martin *et al.* (26) and Straus *et al.* (27).

Isolation and analysis of DNA by CsCl buoyant density gradients. HEF cells were grown in Falcon flasks (25 cm^2/flask). The monolayers were infected with 3×10^4 FFUs of TK^+ VZV or TK^- VZV. Infected HEF monolayers which displayed 50–60% CPE within 24–36 hr post-infection were labeled with 0.03 or 0.3 μM [^{125}I]IVDU or 0.05 μM [^{3}H]dThd. The radiolabeled precursor was added directly to the growth medium. Following a labeling period of 24 hr, the cell monolayers were washed twice with PBS, the cells were detached with 0.01% trypsin in PBS, and after centrifugation the cell pellets were washed twice with PBS. Mock-infected cells were processed in the same way. The washed cells were then digested at 37° in a solution containing 15 mM NaCl, 5 mM sodium citrate, pH 7.3, 20 mM EDTA, 1% Sarkosyl NL 30, and 2% Pronase. After digestion, DNA was extracted by a mixture of phenol/chloroform/isoamyl alcohol (25:24:1), designated as phenol mixture. Samples (0.2 ml) of DNA in 0.01 M Tris-HCl, pH 7.4, 0.001 M EDTA were mixed with a 3.5-ml solution consisting of 15 mM NaCl, 15 mM sodium citrate, pH 7.4, and 5.2 g of CsCl. The solution was centrifuged at 30,000 rpm for 70 hr; three-drop fractions were collected and spotted onto Whatman No. 1 paper discs. The discs were washed twice with 5% trichloroacetic acid, once with 95% ethanol, then air-dried, and the amount of radioactivity was determined.

Hybridization on membrane filter. We used a membrane filter technique for the detection of complementary DNA, as described previously by Denhardt (28).

Results

Dose response of cellular uptake of [^{125}I]IVDU. The 50% inhibitory dose (ID_{50}) of IVDU for VZV replication *in vitro* falls within the range of 0.003–0.03 μM (8). To measure uptake of [^{125}I]IVDU by VZV-infected cells, the compound was used over a wide range of concentrations (from 3×10^{-4} to 3 μM). HEF cells (4×10^7 cells) were mock-infected or infected with TK^+ VZV or TK^- VZV. [^{125}I]IVDU was added 24 hr after virus inoculation at the concentrations indicated in Fig. 1. Twelve hr after drug addition, the cells were harvested and washed five times with PBS. The cell pellets were then evaluated directly in an LKB gamma counter. No [^{125}I]IVDU was taken up by mock- or TK^- VZV-infected cells if incubated with [^{125}I]IVDU at concentrations up to 10^{-1} μM. In contrast, uptake of [^{125}I]IVDU by TK^+ VZV-infected cells was evident at a [^{125}I]IVDU concentration of 10^{-4} μM and increased proportionally with the concentration of drug added to the TK^+ VZV-infected cells.

Time response of cellular uptake of [^{125}I]IVDU. The

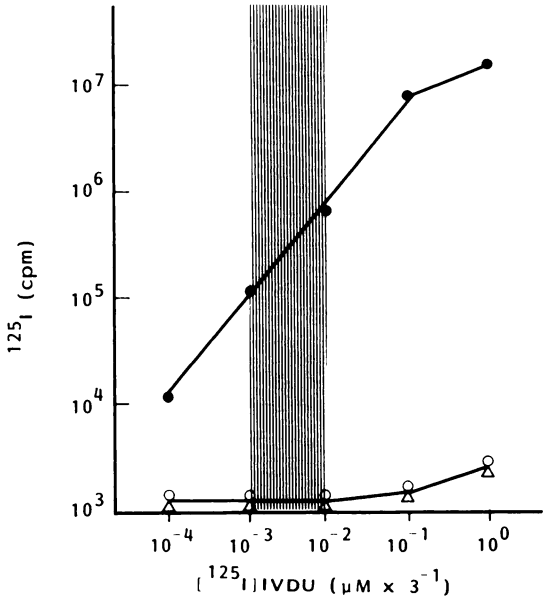

Fig. 1. Dose response of cellular uptake of [^{125}I]IVDU. HEF cells (4×10^7 cells) were mock-infected or infected with TK^+ VZV or TK^- VZV (5×10^4 FFUs). Twenty-four hr after infection, [^{125}I]IVDU was added at the indicated (final) concentration. Twelve hr after addition of [^{125}I]IVDU, cells were harvested and washed five times with PBS. Radioactivity of washed cells was determined directly in an LKB gamma counter. ■ corresponds to the ID_{50} range of IVDU for different VZV strains isolated from clinical materials. ●, TK^+ VZV-infected cells; ○, TK^- VZV-infected cells; △, mock-infected cells.

results of the time response for the cellular uptake of [^{125}I]IVDU are shown in Fig. 2. [^{125}I]IVDU was added at a final concentration of 0.03 μM (time zero). Virtually no [^{125}I]IVDU was taken up by mock-infected cells or TK$^-$ VZV-infected cells. In contrast, the intracellular concentration of [^{125}I]IVDU in TK$^+$ VZV-infected cells rose proportionally with the exposure time to [^{125}I]IVDU for the first 10 hr and leveled off thereafter. The amount of [^{125}I]IVDU taken up by TK$^+$ VZV-infected cells was at least 100 times greater than that taken up by mock- or TK$^-$ VZV-infected cells.

Incorporation into acid-soluble and -insoluble fraction. [^{125}I]IVDU-labeled cell pellets that were harvested from TK$^+$ VZV-infected cultures which had been incubated in the presence of 0.03 μM [^{125}I]IVDU were treated with perchloric acid and separated into acid-soluble and -insoluble fractions. The radioactivities in the acid-insoluble fractions were consistently higher than in the corresponding acid-soluble fractions. As shown in Fig. 3, [^{125}I]IVDU activity in the acid-insoluble fraction increased gradually with the exposure time of the cells to [^{125}I]IVDU. In contrast, radioactivity in the acid-soluble fractions peaked at 5 hr after [^{125}I]IVDU addition and then declined gradually with time. At 20 hr after the addition of [^{125}I]IVDU, the amount of radioactivity incorporated into the acid-insoluble fraction was almost 20 times greater than that in the acid-soluble fraction. The acid-soluble fraction collected at 10 hr after [^{125}I]IVDU addition was subjected to PEI-cellulose thin layer chromatography. Radioactivity associated with nucleoside mono-, di-, and triphosphate was 5%, 5%, and 85%, respectively.

Autoradiographic study. Selective uptake of [^{125}I]IVDU by TK$^+$ VZV-infected cells was confirmed morphologically by means of autoradiography. Mock- or VZV-infected HEF cells were exposed to growth medium containing [^{125}I]IVDU at a final concentration of 0.1 μCi/ml (0.03 μM). After exposure to [^{125}I]IVDU for 12 hr, the cells were washed five times with PBS and fixed. Autoradiograms of the cells are shown in Fig. 4. No [^{125}I]IVDU uptake was detected for TK$^-$ VZV- and mock-infected cells. Although TK$^-$ VZV-infected cells showed viral CPE, no uptake of [^{125}I]IVDU could be visualized. In contrast, marked incorporation of [^{125}I]IVDU was noted in TK$^+$ VZV-infected cells. [^{125}I]IVDU grains were detected mainly in the nucleus.

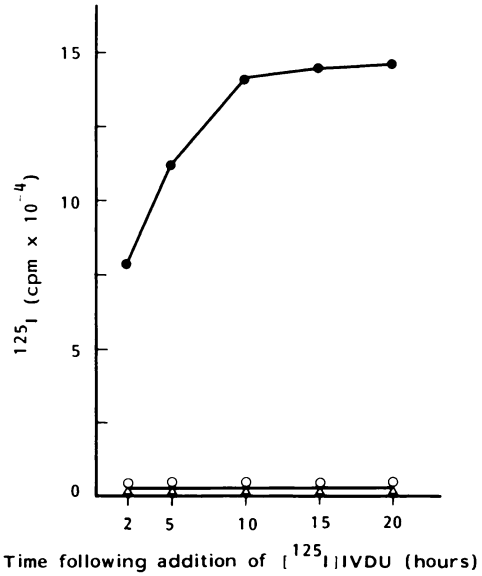

Fig. 2. Time response of cellular uptake of [^{125}I]IVDU. HEF cells (10^6 cells) were mock-infected or infected with TK$^+$ VZV or TK$^-$ VZV (3×10^3 FFUs). Twenty-four hr after infection, [^{125}I]IVDU was added at a final concentration of 0.03 μM (time zero). The cells were harvested at the indicated times and evaluated for radioactivity. ●, TK$^+$ VZV-infected cells; ○, TK$^-$ VZV-infected cells; △, mock-infected cells.

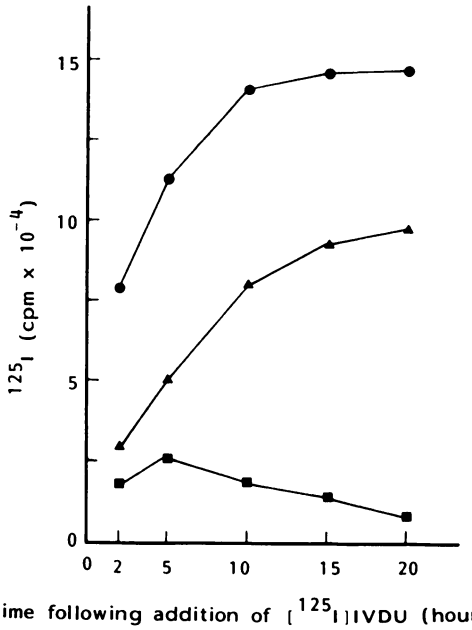

Fig. 3. Distribution of [^{125}I]IVDU in acid-soluble and -insoluble fractions. HEF cells (10^6 cells) were infected with TK$^+$ VZV. Twenty-four hr after infection, [^{125}I]IVDU was added at a final concentration of 0.03 μM. The cells were harvested at the indicated times. After perchloric acid treatment, radioactivity was determined in the acid-soluble and -insoluble fractions. ●, cell pellets before acid treatment, ■, acid-soluble fractions; ▲, acid-insoluble fractions.

Analysis of DNA by isopycnic centrifugation in CsCl gradients. TK$^+$ VZV-infected cells were labeled with [^{125}I]IVDU as described above. [^{125}I]IVDU-labeled cell pellets were directly treated with Pronase and Sarkosyl NL 30, and the DNA was extracted by the phenol mixture. The DNA was then separated by isopycnic centrifugation in CsCl gradients. As shown in Fig. 5, two peaks of radioactivity were demonstrated in the extracts from TK$^+$ VZV-infected cells cultivated in the presence of 0.3 μM [^{125}I]IVDU. The buoyant densities of the two peaks were 1.727 g/ml and 1.712 g/ml, respectively. As described below, the first peak from the bottom corresponded to VZV DNA, whereas the second corresponded to cellular DNA. A similar result was obtained if [^{125}I]IVDU was used at a concentration of 0.03 μM, although the buoyant densities of the peaks were now lower than if [^{125}I]IVDU was used at a concentration of 0.3 μM. The peak of cellular DNA detected following exposure of TK$^+$ VZV-infected cells to 0.03 μM [^{125}I]IVDU was much lower than that seen following exposure to 0.3 μM [^{125}I]IVDU.

Incorporation of [^{125}I]IVDU into VZV DNA. As [^{125}I]IVDU radioactivity was detected in the acid-insoluble fraction, we next examined whether [^{125}I]IVDU was incorporated into VZV DNA or cellular DNA. For this purpose, VZV DNA was purified from the nucleocapsids isolated from the cells infected with TK$^+$ VZV. As shown in Table 1, the radioactivity associated with the nucleocapsids was about 10–25% of the corre-

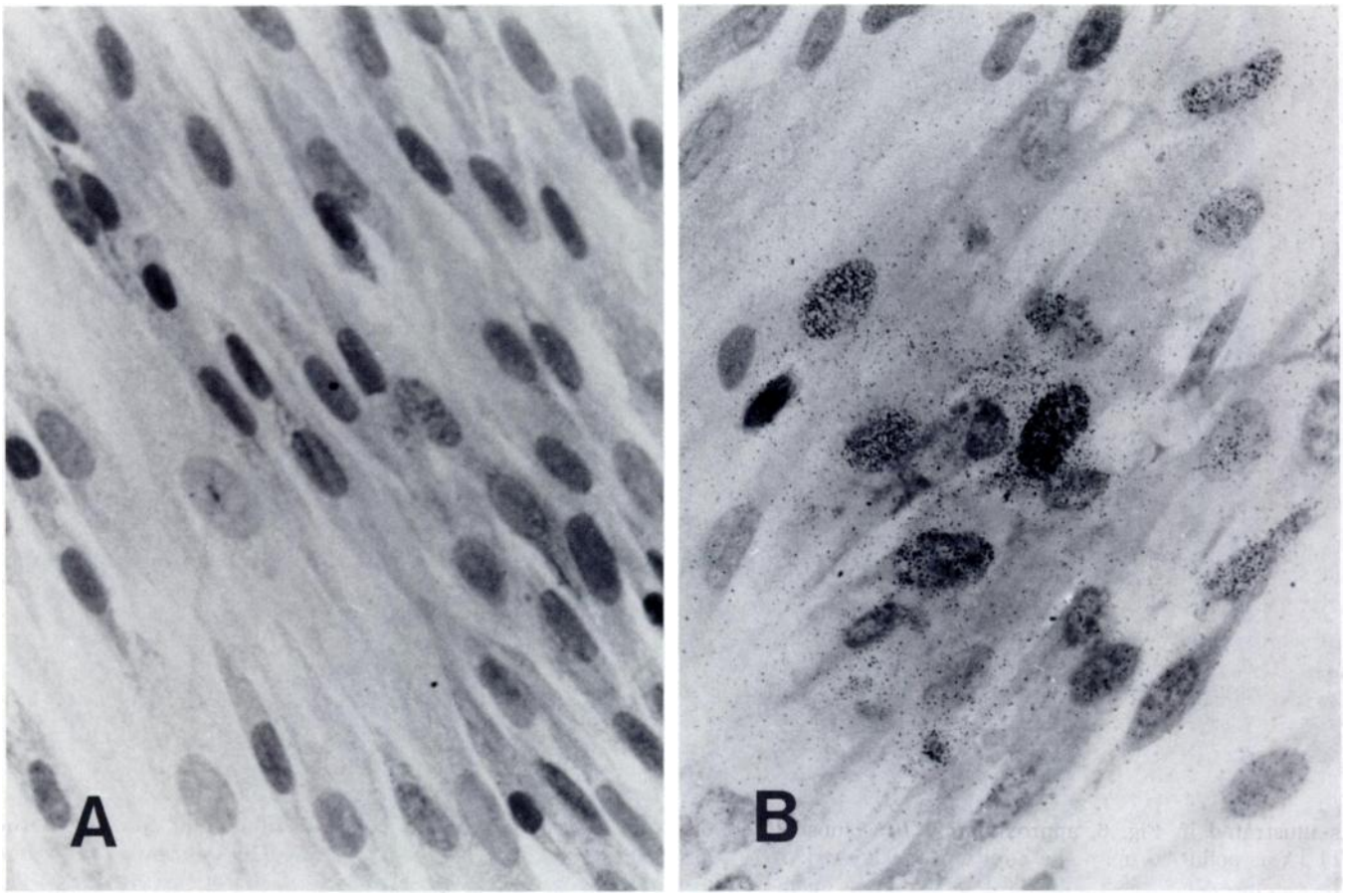

Fig. 4. Uptake of [^{125}I]IVDU by HEF cells. A. Mock-infected cells. B. TK$^+$ VZV-infected cells.

sponding cell-associated radioactivity. The amount of radioactive nucleocapsids increased with the concentration of [^{125}I]IVDU in the cell culture medium. When VZV DNA was purified from the nucleocapsids, 80–95% of the nucleocapsid-associated radioactivity appeared to reside in the DNA. The specific activity (cpm of [^{125}I]IVDU per μg of VZV DNA) increased proportionally with the concentration of [^{125}I]IVDU in the culture medium. No radioactive nucleocapsid or VZV DNA could be extracted from TK$^-$ VZV- or mock-infected cells. To confirm that [^{125}I]IVDUMP was as such incorporated into DNA, [^{125}I]IVDU-labeled DNA purified from the nucleocapsids was digested with deoxyribonuclease (EC 3.1.21.1), phosphodiesterase (EC 3.1.4.1), and alkaline phosphatase (EC 3.1.3.1). The ^{125}I-radioactivity originating from the DNA co-migrated with IVDU in PEI-cellulose thin layer chromatography.

When ^{125}I-labeled VZV DNA was subjected to agarose gel electrophoresis, the VZV DNA migrated as a single band, as identified by both ethidium stain gel and autoradiography. The VZV DNAs harvested from TK$^+$ VZV-infected cells which had been exposed to different [^{125}I]IVDU concentrations showed a similar migration pattern. The molecular weight of the DNAs could be estimated at approximately 50×10^6.

Hybridization test. By isopycnic centrifugation in CsCl gradients, two peaks of [^{125}I]IVDU-labeled DNA were detected (Fig. 5). To confirm that the DNAs with a buoyant density of 1.727 and 1.712 g/ml corresponded to VZV DNA and cellular DNA, respectively, they were analyzed by a DNA·DNA hybridization technique using nitrocellulose membrane filters. Cold and radiolabeled VZV DNA were purified from the nucleocapsids produced by TK$^+$ VZV-infected HEF cells incubated in the presence or absence of [^{125}I]IVDU. [^{125}I]IVDU-labeled DNAs were extracted from TK$^+$ VZV-infected HEF cells incubated in the presence of 0.3 μM [^{125}I]IVDU. Cell DNA was isolated from uninfected HEF cells. As shown in Table 2, [^{125}I]IVDU-labeled VZV DNA annealed more efficiently to the VZV DNA probe than to the HEF DNA. The DNA with a buoyant density of 1.727 g/ml annealed 5.5 times more efficiently to VZV DNA than to HEF DNA, whereas the DNA with a buoyant density of 1.712 g/ml annealed 3.8 times more efficiently to HEF DNA than to VZV DNA. Thus, the results of the hybridization experiments suggest that the DNA with a buoyant density of 1.727 is mainly VZV DNA, whereas the DNA with a buoyant density of 1.712 is mainly cellular DNA. Therefore, the DNAs isolated from TK$^+$ VZV-infected cells incubated in the presence of [^{125}I]IVDU exhibited a marked increase in the buoyant density of viral DNA (from 1.708 to 1.727 g/ml) and a less pronounced increase in the buoyant density of host cell DNA (from 1.700 to 1.712 g/ml). Moreover, [^{125}I]IVDU was incorporated in both viral and cellular DNA in the cell-infected TK$^+$ VZV, but was not incorporated in the viral and cellular DNAs in TK$^-$- or mock-infected cells.

The percentage of substitution of dTMP by IVDUMP. The extent of substitution of dTMP by IVDUMP was calculated from the specific radioactivity (cpm/μg of DNA) of [^{125}I]

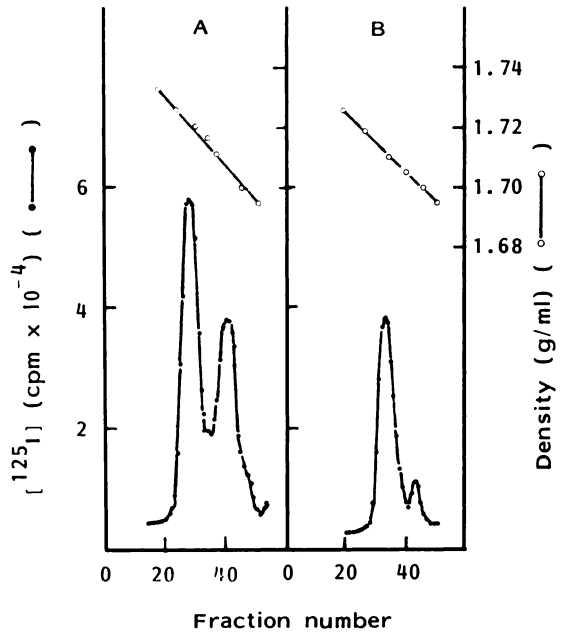

Fig. 5. Isopycnic CsCl centrifugation of DNA isolated from TK⁺ VZV-infected HEF cells. Twenty-four hr after infection, [^{125}I]IVDU was added at a final concentration of 0.3 μM (A) and 0.03 μM (B). After a 24-hr labeling period, the cells were processed and their DNA was analyzed as described in Materials and Methods.

IVDU-labeled VZV DNA purified from the VZV nucleocapsids. As illustrated in Fig. 6, approximately 10% substitution of dThd was achieved upon the addition of 0.3 μM [^{125}I]IVDU. The extent of incorporation of IVDU into DNA could also be quantitated by measuring the resulting shift in CsCl density, using isopycnic centrifugation (19, 29, 30). VZV DNA has a density of 1.708 g/ml and a G + C content of 47%. The molecular weight of the VZV DNA should increase 1.11177-fold and the density should increase 1.8989-fold, if 100% of the dTMP residues are substituted by IVDUMP. The VZV DNA density increased from 1.708 g/ml to 1.727 g/ml following incubation in the presence of 0.3 μM IVDU (Fig. 5). From this result, the percentage of substitution of dTMP by IVDUMP can be estimated at approximately 8%. At an IVDU concentration of 0.003 or 0.03 μM, respectively, 0.1–1.0% of the dThd moieties were replaced by IVDU (Fig. 6).

Discussion

Several reports point to the relationship between the incorporation of 5-(2-halovinyl)-2′-deoxyuridines (BVDU and IVDU) into HSV-1 DNA, and a reduction of virus infectivity and DNA integrity (12, 14, 19). Although BVDU and IVDU are more inhibitory to VZV than HSV-1 (2, 8, 19), no evidence has so far been presented that these drugs are incorporated into VZV DNA and/or change the physical properties of VZV DNA.

In this study we have demonstrated that IVDU is selectively taken up by TK⁺ VZV-infected cells. IVDU is not taken up to an appreciable extent by mock- or TK⁻ VZV-infected cells even if incubated with IVDU at a concentration 100 times higher than the ID_{50} for VZV replication. A selective uptake of IVDU and its carbocyclic analogue (C-IVDU) also occurs by TK⁺ HSV-1-infected but not mock- or TK⁻ HSV-1-infected cells (14, 31). From these findings one may thus infer that the cellular uptake of IVDU depends on the presence of viral TK, be it encoded by VZV or HSV-1.

[^{125}I]IVDU was incorporated effectively into DNA of VZV-infected cells. Similarly, the incorporations of IVDU, BVDU, or C-IVDU into HSV-1 DNA has been reported previously (12, 14, 19). In the present study, however, the incorporation of IVDU into VZV DNA was ascertained by purifying VZV DNA from the nucleocapsids isolated from the cells which had been infected with TK⁺ VZV and incubated in the presence of [^{125}I]IVDU. The radioactivity associated with the nucleocapsids was about 10–25% of the corresponding cell-associated radioactivity; 80–95% of the nucleocapsid-associated radioactivity appeared to reside in the DNA. The specific activity (cpm of [^{125}I]IVDU per μg of VZV DNA) of purified VZV DNA increased proportionally with the concentration of [^{125}I]IVDU in the culture medium (Table 2). These DNAs showed the same electrophoretic mobility in agarose gel as standard VZV DNA. The molecular weight of these DNAs was estimated at approximately 50 × 10^6. These results suggest that high molecular weight VZV DNA was synthesized in VZV-infected cells even when incubated in the presence of 0.3 μM IVDU. Thus, IVDU does not appear to inhibit DNA elongation at this concentration.

For HSV-1-infected cells, Mancini *et al.* (19) showed that the extent of virus yield reduction correlated with the amount of BVDU substituted for dThd in HSV-1 DNA. BVDU-substituted DNA was more labile, as determined by a dose-dependent increase in single strand breaks when examined by centrifugation in alkaline sucrose gradients. We have now calculated the extent of substitution of dTMP by IVDUMP from the specific activity of [^{125}I]IVDU-labeled VZV DNA purified from the VZV nucleocapsids. The substitution of IVDUMP for dTMP in VZV

TABLE 1

Incorporation of [^{125}I]IVDU in VZV-infected HEF cells, VZV nucleocapsids, and VZV DNA

HEF cells infected with	[^{125}I]IVDU[a]	Incorporation of [^{125}I]IVDU					Specific activity
		Cell (A)	Nucleocapsid (B)[b]	(B/A)	DNA (C)[c]	(C/B)	
	μM			cpm			cpm/μg VZV DNA
TK⁺ VZV	3 × 10^{-4}	12,080	2,628	(22%)	2,502	(95%)	1.9 × 10^3
	3 × 10^{-3}	120,240	23,986	(20%)	21,528	(90%)	2.5 × 10^4
	3 × 10^{-2}	795,040	93,996	(12%)	83,025	(88%)	1.7 × 10^5
	3 × 10^{-1}	8,079,760	910,368	(11%)	750,225	(82%)	1.9 × 10^6
TK⁻ VZV	3 × 10^{-1}	7,603	543		461		
Mock	3 × 10^{-1}	6,814					

[a] [^{125}I]IVDU was added to VZV- or mock-infected cells (4 × 10^7 cells) at the indicated (final) concentrations.
[b] Nucleocapsids (B) were purified from VZV-infected cells (A) which had taken up [^{125}I]IVDU.
[c] DNA was purified from nucleocapsids (B).

TABLE 2

Hybridization of DNA isolated from VZV-infected cells exposed to [^{125}I]IVDU, with different DNA probes

Probe	[^{125}I]IVDU-labeled DNA annealed to the DNA filter[b]			
Cold DNA fixed onto filter[a]	VZV DNA	VZV-infected cellular DNA	VZV-infected cell DNA[c]	VZV-infected cell DNA[d]
VZV DNA	2360[e]	830	940	290
HEF DNA	320	2630	170	1100
Calf thymus DNA	210	1280	90	480
λ phage DNA	50	330	40	170

[a] Five μg of DNA each.
[b] A total of 20,000 cpm of each DNA was carried onto the filters.
[c] CsCl density, 1.727 g/ml.
[d] CsCl density, 1.712 g/ml.
[e] cpm hybridized.

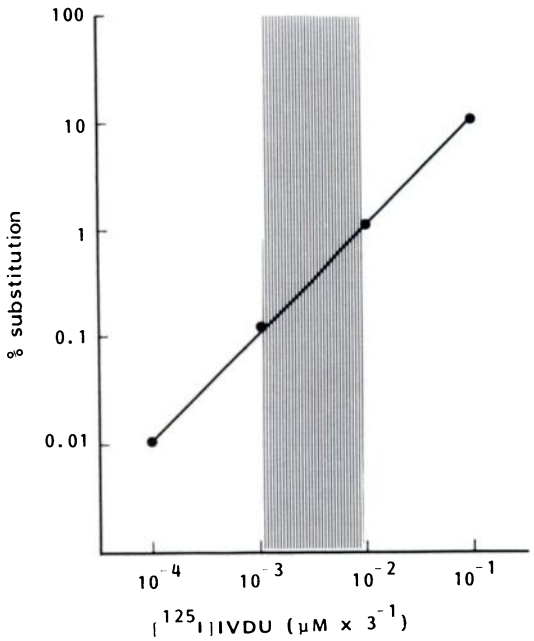

Fig. 6. Percentage substitution of dTMP by IVDUMP in VZV DNA. The extent of substitution was calculated from the specific radioactivity of [^{125}I]IVDU-labeled VZV DNA purified from the VZV nucleocapsids. ■ corresponds to the range of ID_{50} of IVDU for different VZV strains.

DNA increased proportionally with the concentration of IVDU added. At the IVDU concentrations of 0.003 μM, 0.03 μM, and 0.3 μM, the percentage substitution of dThd by IVDUMP was about 0.1%, 1.0%, and 10%, respectively. Since the ID_{50} of IVDU for VZV replication ranges from 0.003 μM to 0.03 μM (8), one may infer that 0.1–1.0% substitution of IVDU for dThd in the viral DNA may suffice for inhibition of VZV replication. In the study with HSV-1 (19), the ID_{50} of BVDU for HSV-1 replication was 0.12 μM, and 5–10% substitution of BVDU for dThd in HSV-1 DNA was attained at this concentration. Thus, for IVDU or BVDU to inhibit VZV replication, a much lower substitution rate of IVDU (or BVDU) for dThd into viral DNA is required than for inhibition of HSV-1 replication (0.1–1.0%, as compared to 5–10%).

At high concentrations, IVDU was also incorporated into cellular DNA of TK$^+$ VZV-infected cells. A similar incorporation into cellular DNA has been observed with BVDU, IVDU, and C-IVDU in HSV-1-infected cells (12, 14, 19). Incorporation of IVDU into cellular DNA was demonstrated by DNA·DNA hybridization techniques. This incorporation occurred only in the cells infected with TK$^+$ VZV. No incorporation whatsoever was noted into cellular DNA of mock- or TK$^-$ VZV-infected cells.

In conclusion, the selectivity of IVDU as an anti-VZV agent primarily depends on a specific phosphorylation by the viral TK, whereas its antiviral action seems to be related to its incorporation into viral DNA. Antiviral activity is noted if as few as 0.1% (or more) of the dThd residues of VZV DNA are replaced by IVDU.

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