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Phosphorylation of 5-Bromodeoxycytidine in Cells Infected with Herpes Simplex Virus

(deoxycytidine kinase/thymidine kinase/halogenated pyrimidine nucleosides/antiviral chemotherapy)

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ABSTRACT **5-Bromodeoxycytidine is phosphorylated to 5-bromodeoxycytidine 5′-monophosphate in extracts of cells infected with herpes simplex virus but not in extracts of uninfected cells. The conversion of 5-bromodeoxycytidine to nucleotides and its utilization for DNA synthesis in uninfected cells occurs by deamination of 5-bromodeoxycytidine to 5-bromodeoxyuridine followed by phosphorylation of 5-bromodeoxyuridine to 5-bromodeoxyuridine 5′-monophosphate. In contrast, in cells infected with herpes simplex virus, 5-bromodeoxycytidine is phosphorylated directly to 5-bromodeoxycytidine 5′-monophosphate, which can then be deaminated to 5-bromodeoxyuridine 5′-monophosphate and incorporated into DNA. These results indicate a difference in the substrate specificity of nucleoside kinases induced by herpes simplex virus and the enzymes present in uninfected cells. It is suggested that this difference in substrate specificity between virus-induced and host-cell enzymes may allow selective chemotherapy of herpes simplex infections with 5-bromo- or 5-iododeoxycytidine.**

Several enzymes related to DNA synthesis, including thymidine kinase (1–3), deoxycytidine kinase (4–6), deoxycytidylate deaminase (7), ribonucleotide reductase (8), and DNA polymerase (7), are induced in cells infected with herpes simplex virus (HSV). The synthesis of virus-induced enzymes in HSV-infected cells suggests the possibility of selective antiviral chemotherapy based on biochemical differences between HSV-induced and host-cell enzymes. Evidence presented in this paper indicates that 5-bromodeoxycytidine (BrdC) is readily phosphorylated by extracts of HSV-infected cells, whereas BrdC is a poor substrate for phosphorylation by extracts of uninfected cells. This difference in substrate specificity between HSV-induced and host-cell nucleoside kinases may contribute to selective chemotherapy of HSV infections.

BrdC is incorporated into the DNA of uninfected mouse cells as BrdUMP (9). The conversion of BrdC to BrdUMP could occur by two pathways: (*i*) deamination of BrdC to BrdU followed by phosphorylation to BrdUMP, and/or (*ii*) phosphorylation of BrdC to BrdCMP followed by deamination to BrdUMP. These reactions could be catalyzed by the sequential actions of (*i*) cytidine deaminase (EC 3.5.4.5) and thymidine kinase (EC 2.7.1.21), and (*ii*) deoxycytidine kinase and deoxycytidylate deaminase. Mutant lines of mouse cells lacking thymidine kinase, and therefore resistant to BrdU, are also resistant to BrdC (9). In addition, Cooper and Greer (10) showed that inhibition of cytidine deaminase *in vivo* reduces the incorporation of BrdC into the DNA of mouse tissues. These results suggest that the conversion of BrdC to cytotoxic nucleotides, and the utilization of BrdC for DNA synthesis, occur mainly by deamination of BrdC to BrdU followed by phosphorylation of BrdU by thymidine kinase. Cooper and Greer (11) found that BrdC and IdC are poor substrates for deoxycytidine kinase in extracts of mouse and human lymphoid cells. The K_m of mouse deoxycytidine kinase for BrdC is approximately 2.0 mM, as opposed to a K_m of 10 μM for deoxycytidine (dC). Similarly, the K_m of the human enzyme is 400 μM for BrdC and 2.0 μM for dC. Since BrdCMP is a good substrate for deoxycytidylate deaminase (12), these results suggest that the restricted substrate specificity of deoxycytidine kinase results in the lack of utilization of BrdC by the deoxycytidine kinase-deoxycytidylate deaminase pathway in uninfected mouse and human cells.

In contrast to the cross-resistance of BrdU- and IdU-resistant mouse cells to BrdC and IdC (9, 13), an IdU-resistant strain of HSV appears to retain the sensitivity of wild-type HSV to IdC (14). Although the biochemical basis of IdU-resistance in this HSV mutant has not been established, this observation suggested the possibility that IdC might be phosphorylated in HSV-infected cells without prior deamination to IdU. The results of the present investigation demonstrate that HSV-infected cells phosphorylate BrdC directly to BrdCMP and utilize BrdC for DNA synthesis without prior deamination of the nucleoside to BrdU.

METHODS

Virus Stocks. A stock of HSV subtype 1 (HSV-1) strain 2BB, which had been prepared and titered in primary rabbit-kidney cells, was obtained from Dr. Harold G. Haines, Department of Dermatology. Stocks of HSV-1 strains F and MP, and HSV subtype 2 (HSV-2) strain G, were purchased from the American Type Culture Collection. These virus stocks had been prepared in HEp-2 cells in the laboratory of Dr. B. Roizman, University of Chicago. Plaque assays were performed as described by Roizman and Roane (15) except that BHK cells were used.

Cell Lines. Baby hamster kidney cells BHK 21/C13 (BHK). and human laryngeal epidermoid carcinoma cells (HEp-2) were grown in Eagle's minimal essential medium supplemented with 10% fetal-calf serum and antibiotics (50 units/ml

Abbreviations: HSV, herpes simplex virus; PFU, plaque-forming units.

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TABLE 1. *Deamination of [³H]dC and [³H]BrdC in extracts of BHK and HEp-2 cells*

Cell line	Tetrahydrouridine (μg/ml)	Incubation time (min)	nmoles deaminated [³H]BrdC	nmoles deaminated [³H]dC
BHK	0	120	<0.04	<0.04
HEp-2	0	15	2.0	1.7
HEp-2	100	15	<0.1	<0.1

Cell extracts were prepared in buffer B. Reaction mixtures (0.1 ml) contained the following amounts of protein: BHK, 0.15 mg; HEp-2, 0.05 mg.

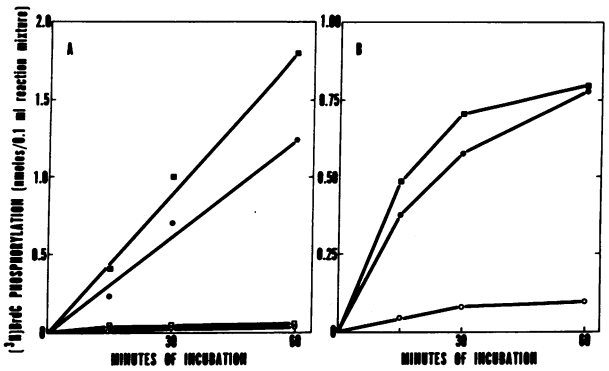

FIG. 1. Phosphorylation of [³H]BrdC by extracts of uninfected and HSV-infected cells. (*A*) Extracts of BHK cells infected with HSV-1 strain 2BB (5 PFU/cell) were prepared in buffer A 13 hr after infection (●). Extracts of BHK cells infected with HSV-2 strain G (3 PFU/cell) were prepared in buffer B 9 hr after infection (■). Uninfected BHK cell extracts were prepared in buffer A (○) and in buffer B (□). Reaction mixtures (0.1 ml) contained the following amounts of protein: uninfected cells in buffer A, 500 μg; uninfected cells in buffer B, 120 μg; 2BB-infected cells, 80 μg; G-infected cells, 21 μg. (*B*) Extracts of uninfected HEp-2 cells (○) and of HEp-2 cells infected with HSV-2 strain G (3 PFU/cell) were prepared in buffer B 15 (●) and 24 (■) hours after infection. Reaction mixtures (0.1 ml) contained 100 μg/ml of tetrahydrouridine and the following amounts of protein: uninfected cells, 150 μg; infected cells 15 hr after infection, 70 μg; infected cells 24 hr after infection, 50 μg.

of penicillin plus 50 μg/ml of streptomycin). A transformed hamster cell line, 333-8-9 T1, which was isolated after infection of hamster-embryo fibroblasts with UV-irradiated HSV-2 (16), was generously provided by Dr. Ronald Duff. These cells were grown in medium 199 supplemented with 10% fetal-calf serum, 10% tryptose phosphate broth, and antibiotics. Human lymphoid cells were grown in RPMI 1640 medium supplemented with 10% fetal-calf serum and antibiotics. The RPMI 4098 and RPMI 6237 cell lines were established from the peripheral blood of normal human donors and were purchased from Associated Biomedic Systems. The RAJI and Jijoye cell lines were established from donors with Burkitt's lymphoma and were purchased from the American Type Culture Collection. All cells were grown at 37° in a humidified 5% CO_2 atmosphere. Media, sera, and antibiotics were purchased from Grand Island Biological Company.

Cervical Carcinoma Specimens. Two cervical carcinoma specimens (stage 1B) were removed from women, 27 and 59 years of age, who had not received previous radiation therapy. The tumors were diagnosed as invasive squamous cell carcinoma by the Department of Pathology, Jackson Memorial Hospital, Miami. Tissues were used for enzyme extraction immediately after they were obtained from surgery.

HSV Infection. Monolayers of BHK or HEp-2 cells were infected with HSV diluted in growth medium. An inoculum of 1.0 ml was used to infect cells in 100-mm dishes and an inoculum of 0.25 ml for cells in 60-mm dishes. After two hours at 37° the inoculum was aspirated, the cells were washed with growth medium, fresh medium was added, and the cells were incubated at 37°.

Preparation of Cell Extracts and Enzyme Assays. Cells were washed twice with 0.9% NaCl; suspended in preparative buffer (1–5 × 10^7 cells/ml), and disrupted by sonication. Cervical carcinoma tissue (1 g) was homogenized in 5 ml of buffer in a Sorvall omnimixer. Extracts were centrifuged at 100,000 × *g* for 1 hr and the supernatant fluid was used immediately for enzyme assays. Protein was determined by the method of Lowry *et al.* (17).

Maximal deoxycytidine kinase activity from cells infected with HSV-1 strains F and 2BB was obtained when extracts were prepared in buffer consisting of 25 mM Tris-HCl (pH 8.0), 10 mM 2-mercaptoethanol, 10% glycerol, 1.0 mM $MgCl_2$, and 0.2 mM dTTP (buffer A). However, deoxycytidine kinase from cells infected with HSV-2 strain G was optimally prepared in buffer containing 25 mM Tris-HCl (pH 8.0), 25 mM 2-mercaptoethanol, and 10% glycerol (buffer B). dTTP (0.1 mM) inhibited the phosphorylation of [³H]dC by 85% in extracts of HSV-2-infected cells. However, dTTP concentrations of less than 0.2 mM did not significantly inhibit [³H]dC phosphorylation in extracts of uninfected or HSV-1-infected cells. The activity of deoxycytidine kinase from uninfected BHK or HEp-2 cells was similar in extracts prepared in either buffer A or B. Nucleoside kinase activities could not be reproducibly demonstrated in extracts of BHK cells infected with HSV-1 strain MP, apparently due to instability of the enzyme induced by this strain in both buffers A and B.

Nucleoside kinase activities were determined in reaction mixtures which contained 50 mM Tris·HCl (pH 8.0), 10 mM NaF, 5.0 mM $MgCl_2$, 10 mM ATP, 20 μM [³H]dC or [³H]-BrdC (New England Nuclear, 25 μCi/μmole), and 0.05 ml of cell extract in a total volume of 0.1 ml. After incubation at 37°, [³H]nucleotide formation was determined by adsorption to discs of DEAE-chromatography paper (11). Specific activities were determined from initial rates of [³H]nucleotide formation, which were proportional to the concentration of cell extract.

Reaction mixtures for determination of cytidine deaminase were as described above except that ATP was omitted. After incubation at 37°, reactions were terminated by boiling. Aliquots (20 μl) of the reaction mixtures were applied to Whatman DE81 chromatography paper along with carrier unlabeled nucleosides (either dC and dU or BrdC and BrdU). Chromatograms were developed with 0.03 N formic acid. The positions of carrier nucleosides were identified under UV light, and the UV-absorbing areas were cut out and counted. The R_F values of dC, BrdC, dU, and BrdU were 0.8, 0.7, 0.5, and 0.1, respectively.

TABLE 2. *Nucleoside kinase activities in extracts of uninfected and HSV-infected cells with [^{3}H]dC and [^{3}H]BrdC as substrates*

Cell line	Infecting HSV strain	Preparative buffer	[^{3}H]Nucleotide formation nmoles/hr per mg of protein	
			[^{3}H]dC	[^{3}H]BrdC
BHK	none	A	2.2	<0.1
	none	B	2.8	0.3
	HSV-1 2BB	A	11	13
	HSV-1 F	A	15	14
	HSV-2 G	B	59	100
HEp-2	none	B	8.0	1.1
	HSV-2 G	B	8.0	30

Extracts of HSV-infected BHK cells were prepared 13 hr after infection with 2BB (5 PFU/cell) (PFU, plaque-forming units) and 9 hr after infection with F (20 PFU/cell) and G (3 PFU/cell). Extracts of G-infected HEp-2 cells (3 PFU/cell) were prepared 24 hr after infection. Extracts were prepared in either buffer A or buffer B as indicated. For extracts of uninfected and HSV-infected HEp-2 cells, 100 μg/ml of tetrahydrouridine was added to reaction mixtures.

Incorporation of [^{3}H]BrdU and [^{3}H]BrdC into DNA. Cells were incubated in growth medium containing 0.05 μCi/ml (210 μCi/μmole) of [^{3}H]BrdU or [^{3}H]BrdC. The cells were washed twice with 0.9% NaCl and cold 5% trichloroacetic acid was added. Precipitates were collected on glass-fiber filters, washed three times with cold 5% trichloroacetic acid, once with 95% ethanol, dried, and counted.

DNA hydrolysates were prepared after 10^7 HSV-infected BHK cells were labeled as described above. Cells were washed twice with 0.9% NaCl and three times with cold 5% perchloric acid. Precipitates were collected in 5% perchloric acid, washed with 95% ethanol, and dried in a vacuum desiccator. Nucleic acids were hydrolyzed in 0.1 ml of 7.5 N perchloric acid at 100° for 1 hr. NaOH (0.1 ml of 7.5 N) was added and insoluble material was removed by centrifugation. An aliquot (20 μl) of the supernatant fluid was applied to Whatman 1 or 3MM chromatography paper along with carrier 5-bromouracil and 5-bromocytosine. Chromatograms were developed with water-saturated *n*-butanol. The positions of 5-bromouracil (R_F = 0.6) and 5-bromocytosine (R_F = 0.4) were identified under UV light, and the UV-absorbing areas were cut out and counted. No deamination (less than 1%) of [^{3}H]BrdC was detected under these hydrolysis conditions.

TABLE 3. *Identification of the [^{3}H]nucleotide product formed by phosphorylation of [^{3}H]BrdC in extracts of HSV-infected cells*

Cell line	Infecting HSV strain	cpm recovered	
		BrdC	BrdU
BHK	HSV-1 2BB	120	<10
BHK	HSV-2 G	200	<10
HEp-2	HSV-2 G	90	<10

^{3}[H]Nucleotides formed by incubation of [^{3}H]BrdC with extracts of HSV-infected cells, as described in Fig. 1, were isolated by adsorption to DEAE-cellulose discs. Discs containing 900 to 2000 cpm (0.45 to 1.0 nmole) of [^{3}H]nucleotide product were removed from scintillation vials, dried, and eluted with 0.5 ml of 1.0 M KCl-0.5 M Tris·HCl (pH 8.0). Alkaline phosphatase (2 units, Sigma, type III) was added to the eluant and the reaction mixture was incubated for 1 hr at 37°. A 50-μl aliquot was chromatographed along with carrier BrdC and BrdU on DEAE-cellulose chromatography paper with 0.03 N formic acid. The positions of BrdC and BrdU were identified under UV light; these areas were cut out and counted.

TABLE 4. *Utilization of [^{3}H]BrdC and [^{3}H]BrdU for DNA synthesis in uninfected and HSV-infected cells*

Cell line	Infecting HSV strain	[^{3}H]Nucleoside incorporation (cpm)	
		[^{3}H]BrdU	[^{3}H]BrdC
BHK	none	2400	50
	HSV-1 F	2600	1800
	HSV-1 MP	4400	2200
	HSV-2 G	2500	2300
HEp-2	none	250	30
	HSV-2 G	460	240

Cells (2×10^6) were incubated for 2 hr at 37° with [^{3}H]BrdU or with [^{3}H]BrdC (0.05 μCi/ml, 210 μCi/μmole). Cells infected with F (20 PFU/cell), MP (20 PFU/cell), or G (3 PFU/cell) were labeled from 5 to 7 hr after infection. For infected and uninfected HEp-2 cells, 100 μg/ml of tetrahydrouridine was added during the labeling period. The incorporation of [^{3}H]nucleosides into trichloroacetic acid-precipitable material was determined.

RESULTS

Phosphorylation of [^{3}H]BrdC by Extracts of HSV-Infected Cells. Since deamination of [^{3}H]nucleoside substrates would interfere with the determination of nucleoside kinase activity, it was necessary to inhibit cytidine deaminase with tetrahydrouridine (18) in extracts of cells that contained significant cytidine deaminase activity. In most experiments, BHK cells were used since extracts of these cells did not catalyze the deamination of either [^{3}H]dC or [^{3}H]BrdC (Table 1).

As reported for mouse and human cell extracts (11), extracts of uninfected BHK cells did not catalyze the phosphorylation of [^{3}H]BrdC at an appreciable rate compared to the rate of [^{3}H]dC phosphorylation (Table 2 and Fig. 1*A*). The specific activity of deoxycytidine kinase, determined with [^{3}H]dC as substrate, increased 5- to 7-fold after infection of BHK cells with HSV-1 strains F and 2BB, and 20- to 30-fold after infection with HSV-2 strain G (Table 2). In contrast to the results obtained with uninfected cell extracts, [^{3}H]BrdC was readily phosphorylated by extracts of HSV-infected BHK cells (Fig. 1*A*). The rate of [^{3}H]BrdC phosphorylation was approximately equal to that of [^{3}H]dC phosphorylation in extracts of HSV-1-infected cells, and approximately twice the rate of [^{3}H]dC phosphorylation in extracts of HSV-2-infected cells (Table 2).

The formation of [^{3}H]nucleotides from [^{3}H]BrdC in extracts of HSV-infected BHK cells could occur either by phosphorylation of [^{3}H]BrdC to [^{3}H]BrdCMP or by deamination of [^{3}H]BrdC to [^{3}H]BrdU followed by phosphorylation of [^{3}H]BrdU to [^{3}H]BrdUMP. Although no deamination of either [^{3}H]dC or [^{3}H]BrdC was detectable in extracts of

BHK cells, it was necessary to further establish that [^{3}H]-BrdC was phosphorylated directly to [^{3}H]BrdCMP in extracts of HSV-infected cells. [^{3}H]Nucleotides formed from [^{3}H]BrdC by incubation with extracts of HSV-infected cells were digested to [^{3}H]nucleosides by treatment with alkaline phosphatase. The [^{3}H]nucleoside product was then chromatographed in the presence of unlabeled carrier BrdC and BrdU. All of the [^{3}H]nucleotide product (greater than 95%) formed from [^{3}H]BrdC by incubation with extracts of BHK cells infected with either HSV-1 strain 2BB or HSV-2 strain G chromatographed with BrdC after alkaline phosphatase treatment (Table 3). These results demonstrate the direct phosphorylation of [^{3}H]BrdC to [^{3}H]BrdCMP. No significant deamination of [^{3}H]BrdCMP was observed, suggesting that deoxycytidylate deaminase was not active in these extracts. This is probably due to the instability of this enzyme in the absence of dCTP or high concentrations of dCMP (12).

Because of the possible therapeutic significance of these results, the HEp-2 cell line was chosen to investigate the phosphorylation of [^{3}H]BrdC by extracts of HSV-infected cells of human origin. Unlike BHK cells, HEp-2 cells had high levels of cytidine deaminase activity (Table 1) when assayed with either [^{3}H]dC or [^{3}H]BrdC as substrate. It was therefore necessary to add tetrahydrouridine to the reaction mixture, which resulted in greater than 95% inhibition of the deamination of both [^{3}H]dC and [^{3}H]BrdC (Table 1). In the presence of tetrahydrouridine, [^{3}H]BrdC was readily phosphorylated by extracts of HSV-2-infected HEp-2 cells, whereas it was a poor substrate for phosphorylation by extracts of uninfected HEp-2 cells (Fig. 1*B*). The rate of phosphorylation of [^{3}H]BrdC by extracts of uninfected HEp-2 cells was less than 15% the rate of [^{3}H]dC phosphorylation. However, [^{3}H]BrdC was phosphorylated approximately three-fold more rapidly than [^{3}H]dC by extracts of HSV-2-infected HEp-2 cells (Table 2). As for HSV-infected BHK cells, the product of [^{3}H]BrdC phosphorylation by extracts of HSV-infected HEp-2 cells was [^{3}H]BrdCMP (Table 3). It may be noted that no increase in the specific activity of deoxycytidine kinase, determined with [^{3}H]dC as substrate, was observed 10, 15, or 24 hr after infection of HEp-2 cells with HSV-2 strain G.

Utilization of [^{3}H]BrdC for DNA Synthesis in HSV-Infected Cells. In order to demonstrate the phosphorylation of [^{3}H]-BrdC by HSV-infected cells *in vivo*, the extent of incorporation of [^{3}H]BrdC and [^{3}H]BrdU into DNA was determined. Since BHK cells lack cytidine deaminase activity, the utilization of [^{3}H]BrdC for DNA synthesis in this cell line must occur by way of phosphorylation to [^{3}H]BrdCMP, rather than by way of deamination to [^{3}H]BrdU followed by phosphorylation to [^{3}H]BrdUMP. Therefore, uninfected BHK cells did not incorporate [^{3}H]BrdC into DNA. HSV-infected BHK cells, however, utilized [^{3}H]BrdC for DNA synthesis as readily as [^{3}H]BrdU (Table 4). Due to their high cytidine deaminase activity, uninfected HEp-2 cells utilized [^{3}H]BrdC and [^{3}H]BrdU equally well for DNA synthesis. However, when tetrahydrouridine was used to inhibit cytidine deaminase, the incorporation of [^{3}H]BrdC into the DNA of uninfected HEp-2 cells was reduced to approximately 10% the extent of [^{3}H]BrdU incorporation. In contrast, the incorporation of [^{3}H]BrdC into DNA of HSV-2-infected HEp-2 cells, in the presence of tetrahydrouridine, was 50 to 60% the extent of [^{3}H]BrdU incorporation (Table 4). These results suggest that [^{3}H]BrdC can be phosphorylated without deamination to [^{3}H]BrdU in HSV-infected, but not in uninfected, BHK and HEp-2 cells.

It was of interest to determine if [^{3}H]BrdCMP was incorporated into the DNA of HSV-infected cells without deamination, or if the utilization of [^{3}H]BrdC for DNA synthesis occurred by way of deamination of [^{3}H]BrdCMP to [^{3}H]BrdUMP. DNA hydrolysates of BHK cells infected with HSV-1 strain F or HSV-2 strain G were prepared after infected cells were labeled with [^{3}H]BrdU or [^{3}H]BrdC as described in Table 4. The DNA hydrolysates were chromatographed with carrier 5-bromouracil and 5-bromocytosine as described in *Methods*. For both F- and G-infected cells labeled with [^{3}H]BrdC, more than 90% of the radioactivity in hydrolyzed DNA chromatographed with 5-bromouracil. This result indicates that [^{3}H]BrdC is incorporated into the DNA of HSV-infected cells mainly as [^{3}H]-BrdUMP, presumably after deamination of [^{3}H]BrdCMP to [^{3}H]BrdUMP by deoxycytidylate deaminase.

Nucleoside Kinase in Extracts of HSV-Transformed Cells, Human Lymphoid Cells, and Cervical Carcinoma Tissue. Because of the possible oncogenicity of HSV-2 in cervical carcinoma (19), and of the herpes-like Epstein–Barr virus in Burkitt's lymphoma (20), it was of interest to investigate the possibility that [^{3}H]BrdC might be phosphorylated in these cells and in the 333-8-9 T1 hamster-embryo fibroblasts which were transformed by infection with UV-irradiated HSV-2 (16). Extracts of two cervical carcinomas, 333-8-9 T1 cells, and human lymphoid cell lines established either from normal donors (RPMI 4098 and RPMI 6237) or from donors with Burkitt's lymphoma (RAJI and Jijoye) did not catalyze the phosphorylation of 20 μM [^{3}H]BrdC at a significant rate (less than 10% the rate of [^{3}H]dC phosphorylation). Tetrahydrouridine (100 μg/ml) was used to inhibit cytidine deaminase in extracts of cervical carcinoma tissue and of 333-8-9 T1 cells. Extracts of both cervical carcinomas and 333-8-9 T1 cells were prepared in buffer B. Extracts of lymphoid cells were prepared in both buffers A and B. In addition to the lack of [^{3}H]BrdC phosphorylation in cell extracts, Jijoye and 333-8-9 T1 cells did not utilize [^{3}H]BrdC for DNA synthesis (less than 10% of [^{3}H]BrdU incorporation).

DISCUSSION

The results of this study suggest that cells infected with HSV convert BrdC to nucleotides by a pathway that is not present in uninfected cells. In uninfected cells, BrdC is a poor substrate for phosphorylation to BrdCMP; instead, the conversion of BrdC to nucleotides proceeds by deamination of BrdC to BrdU which is then phosphorylated to BrdUMP by thymidine kinase. In HSV-infected cells, however, BrdC is phosphorylated directly to BrdCMP without prior deamination to BrdU. BrdCMP is probably then deaminated to BrdUMP, which can be phosphorylated to BrdUTP and incorporated into DNA. 5-Methyldeoxycytidine and IdC may also be good substrates for the HSV-induced nucleoside kinase.

The phosphorylation of BrdC in HSV-infected cells may be related to the hypothesis that HSV-induced thymidine kinase and deoxycytidine kinase represent enzymatic activities of the same protein (5). Hay *et al.* (5) proposed that HSV induces a

single pyrimidine deoxyribonucleoside kinase with two catalytic sites; one for the phosphorylation of dT and one for the phosphorylation of dC. Alternatively, HSV-induced thymidine kinase and deoxycytidine kinase could be activities of a single catalytic site. In contrast to the HSV-induced nucleoside kinase, deoxycytidine kinase and thymidine kinase appear to be genetically (21) and biochemically (22, 23) distinct enzymes in uninfected mammalian cells, and mammalian deoxycytidine kinase has a low affinity for dC analogs substituted with methyl, bromo, or iodo groups in the 5-position (11). If a common catalytic site is involved in the phosphorylation of both dT and dC by the HSV-induced nucleoside kinase, then this enzyme might have a higher affinity for 5-substituted dC analogs than the deoxycytidine kinase of uninfected cells.

The difference in substrate specificity between HSV-induced and host-cell nucleoside kinases, reported in this study, might contribute to enhanced antiviral selectivity in the chemotherapy of HSV infections. The work of Kaufman and others (24, 25) has demonstrated the activity of IdU in the therapy of HSV infections of the cornea. IdC (14), BrdU (26, 27), and BrdC (27) appear to be as effective as IdU as inhibitors of HSV replication in tissue culture. Furthermore, BrdU (24) and IdC (28) are as active as IdU in the therapy of herpetic keratitis of the rabbit cornea. BrdC or IdC may be more selective antiviral agents than BrdU or IdU, since BrdC, and possibly IdC, would be phosphorylated by HSV-infected cells but not by uninfected cells. 5-Trifluoromethyldeoxyuridine has been reported to be a more potent antiviral agent than BrdU or IdU in the therapy of corneal HSV infections (29, 30). If 5-trifluoromethyldeoxycytidine is phosphorylated by HSV-induced nucleoside kinase, then similar considerations would apply to the therapy of HSV infections with this nucleoside analog.

Human tissues contain high cytidine deaminase activities (31). Therefore, inhibition of this enzyme with tetrahydrouridine would be required to prevent the deamination of BrdC to BrdU, which is toxic to normal as well as to HSV-infected cells. Tetrahydrouridine is an effective inhibitor *in vivo* of cytidine deaminase in mice (18, 32), and previous work in this laboratory has shown that tetrahydrouridine reduces the utilization of BrdC for DNA synthesis by normal mouse tissues *in vivo* (10). Therefore, administration of tetrahydrouridine together with BrdC might result in the selective conversion of BrdC to nucleotides in HSV-infected cells, which can phosphorylate BrdC directly to BrdCMP without prior deamination of BrdC to BrdU.

In addition to decreasing the toxicity of BrdC to uninfected cells, tetrahydrouridine might increase the antiviral activity of BrdC *in vivo* by inhibiting its catabolism. Cooper and Greer (10) have shown that inhibition of cytidine deaminase by administration of tetrahydrouridine to mice increases the utilization of exogenous dC for DNA synthesis and increases the toxicity of 5-fluorodeoxycytidine, which is a good substrate for mouse deoxycytidine kinase (11). The antineoplastic activity of cytosine arabinoside in mice is also increased by tetrahydrouridine administration (32). These considerations suggest that more effective and selective chemotherapy of HSV infections may be achieved with BrdC, in combination with tetrahydrouridine, than is currently obtained with BrdU or IdU. It is possible that combination chemotherapy with tetrahydrouridine and BrdC or IdC would be effective for systemic HSV infections as well as for topical treatment of ocular or cutaneous infections.

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