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(*E*)-5-(2-BROMOVINYL)URIDINE REQUIRES PHOSPHORYLATION BY THE HERPES SIMPLEX VIRUS (TYPE 1)-INDUCED THYMIDINE KINASE TO EXPRESS ITS ANTIVIRAL ACTIVITY

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Abstract—(*E*)-5-(2-Bromovinyl)uridine (BVUrd), the riboside counterpart of (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVdUrd), effected a dose-dependent inhibition of viral progeny formation and viral DNA synthesis in herpes simplex virus type 1 (HSV-1, strain KOS)-infected human (E_6SM) diploid fibroblast cells. BVUrd was directly phosphorylated in HSV-1-infected cells, presumably by the virus-encoded thymidine kinase (TK), since (i) BVUrd was not phosphorylated by extracts of cells infected with a HSV-1 strain deficient in TK expression and (ii) the phosphorylation was inhibited by a polyclonal anti-HSV-1 antibody. Within the HSV-1-infected cell, BVUrd was incorporated into the viral DNA as BVdUMP (BVdUrd 5′-monophosphate). This incorporation may account for the antiviral action of BVUrd, and implies that, following its initial phosphorylation by the viral TK, BVUrd is converted to its 2′-deoxy counterpart, most likely at the 5′-diphosphate level (BVUDP → BVdUDP).

A few years ago we described the antiviral activity of BVUrd§ and BVUra [1]. Their antiviral activity was remarkably similar to that of BVdUrd [2], in that they were particularly active against HSV-1, less active against HSV-2 and virtually inactive against a dThd TK^- strain of HSV-1 [3].

Although a large variety of 5-substituted 2′-deoxyuridines have been reported to inhibit HSV-1 replication [4], BVUrd is the only ribouridine ever shown to be effective against HSV. This raises the important question on how BVUrd achieves its antiviral effect. For BVdUrd to exert its potent and selective activity against HSV-1 [2], it must, as is the rule for all 5-substituted 2′-deoxyuridines that are selective anti-HSV-agents, be phosphorylated by the virus-encoded TK [3]. This viral TK is quite tolerant to the nature of the pyrimidine C-5 substituent and also recognizes various sugar-modified derivatives of BVdUrd as substrate [5].

That a ribothymidine analogue such as BVUrd should be able to act as substrate for the viral TK is not obvious, however. Furthermore, if BVUrd were to prove apt as substrate for the viral TK, the question arises on how the resulting product would be further processed before interacting with its target, presumed to be the viral DNA polymerase, if identical to that of BVdUrd [3].

The antiviral activity of BVdUrd, BVUrd and BVUra is reversed upon addition of dThd, which suggests that they interfere with the dThd salvage pathway and need to be phosphorylated to exert their antiviral activity [6]. The similarities in the antiviral effects of BVUrd, BVUra and BVdUrd suggest that BVUrd and BVUra should be converted to BVdUrd (or phosphorylated products thereof) at some stage of their metabolism.

BVUra may be directly converted to BVdUrd through the action of pyrimidine nucleoside phosphorylases, as 6-aminothymine (a well-known inhibitor of these enzymes) counteracts the antiviral activity of BVUra and dUrd reverses this counteraction [1]. It is unlikely, however, that BVUrd may be converted to BVdUrd *via* the intermediary formation of BVUra. Such sequence of events would imply two successive phosphorylase reactions, at least one of which should be sensitive to 6-aminothymine (namely the BVUra → BVdUrd reaction); and 6-aminothymine was found not to counteract the antiviral activity of BVUrd [1].

A direct conversion of BVUrd to BVdUrd through exchange of 2′-deoxyribosyl for ribosyl is highly unlikely, as it would require the help of a hypothetical "pentosyl transferase", which is not known to exist. An interesting possibility is that BVUrd may be converted to BVdUrd at the 5′-diphosphate level by ribonucleotide reductase. This would imply, however, that BVUrd is first phosphorylated and, as

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§ Abbreviations used: BVUrd, (*E*)-5-(2-bromovinyl)uridine; BVUra, (*E*)-5-(2-bromovinyl)uracil; BVdUrd, (*E*)-5-(2-bromovinyl)-2′-deoxyuridine; HSV-1, herpes simplex virus type 1; HSV-2, herpes simplex virus type 2; dThd, thymidine (= 2′-deoxythymidine); TK^-, thymidine kinase deficient; BVdUMP, BVdUDP, BVdUTP, (*E*)-(2-bromovinyl)-2′-deoxyuridine 5′-mono-, -di- and -triphosphate; BVUMP, BVUDP, (*E*)-5-(2-bromovinyl)uridine 5′-mono- and -diphosphate; Urd, uridine; dUrd, 2′-deoxyuridine; ATP, adenosine 5′-triphosphate; E_6SM, embryonic skin-muscle; EMEM, Eagle minimum essential medium; MOI, multiplicity of infection; PFU, plaque forming units; PCA, perchloric acid; U, unit; dTMP, thymidine 5′-monophosphate; DEAE, diethylaminoethyl; Thy, thymine.

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BVdUrd owes its selective anti-HSV-1 activity to a specific phosphorylation by the viral dThd kinase, and BVUrd is, like BVdUrd, a selective anti-HSV-1 agent, one may suspect BVUrd to be phosphorylated by the HSV-1 dThd kinase as well.

MATERIALS AND METHODS

Nucleosides and nucleotides. BVUrd and BVdUrd were kindly provided by Dr R. T. Walker (Department of Chemistry, University of Birmingham, U.K.). BVUra was supplied by Dr P. Herdewijn (Rega Institute, Leuven) and BVdUMP was synthesized in our laboratory according to a method published by Hoard and Ott [7]. [*Methyl*-^{3}H]dThd (52 Ci/mmol), Urd (30 Ci/mmol), dUrd (15 Ci/mmol), [^{32}P]orthophosphate (carrier-free) and ATP (3000 Ci/mmol) were purchased from Amersham International Ltd (Buckinghamshire, U.K.).

Cells and viruses. Human diploid (E_6SM) fibroblasts were kindly supplied by Dr A. Billiau (Rega Institute, Leuven) and maintained in EMEM supplemented with 10% (v/v) fetal calf serum and 2 mM L-glutamine (Gibco, Paisley, U.K.). The origin of HSV-1(KOS) and TK$^-$ HSV-1(B2006) has been described elsewhere [8].

Antisera and enzymes. Polyclonal rabbit antiserum raised against HSV-1 infected cells was kindly provided by Dr G. Darby (Wellcome Research Laboratories, Beckenham, U.K.). For identification of the phosphorylating activity, cell-free extracts were preincubated with the antiserum for 30 min at 37° and subsequently overnight at 0° before measuring kinase activity. DNase I from bovine pancreas, phosphodiesterase I from *Crotalus durissus* and calf intestinal alkaline phosphatase were purchased from Boehringer Mannheim GmbH (Mannheim, F.R.G.).

dThd kinase assay. E_6SM cells were grown in polystyrene 75 cm^2 flasks and either mock infected or infected with HSV-1(KOS) or TK$^-$ HSV-1(B2006) at a multiplicity of infection (MOI) of 10 plaque forming units (PFU) per cell. After a 1-hr virus adsorption period, residual virus was removed and cells were further incubated in EMEM supplemented with 3% (v/v) fetal calf serum and 2 mM L-glutamine for an additional 15 hr. Cells were washed with ice-cold buffer (100 mM Tris–HCl pH 8.0, 20 mM β-mercaptoethanol) and stored at −20°. Cells were thawed on ice, scraped off and sonicated in buffer (100 mM Tris-HCl pH 8.0, 20 mM β-mercaptoethanol, 0.9% NaCl). The samples were cleared by ultracentrifugation at 100,000 *g* for 30 min. $(NH_4)_2SO_4$ was added to the supernatants to a final saturation level of 65%. After centrifugation at 25,000 *g* during 30 min, the pellet was suspended in buffer (100 mM Tris–HCl pH 8.0, 20 mM β-mercaptoethanol, 0.9% NaCl, 15% glycerol) and stored in aliquots at −70°.

The dThd kinase activity was measured in 50 mM Tris–HCl pH 8.0, containing 125 μg/ml pyruvate kinase (Sigma Chemical Co., St Louis, Missouri), 5 mM 2-phosphoenol pyruvate (Sigma), 5 mM $MgCl_2$, 6 mM KF and 0.1 mM β-mercaptoethanol. The reaction was followed by using radioactive substrates at 200 μM (i.e. [*methyl*-^{3}H]dThd, [5-^{3}H]Urd or [6-^{3}H]dUrd) and adding ATP to a final concentration of 5 mM. Alternatively, when a radiolabeled substrate was not available, [γ-^{32}P]ATP was used at 5 mM. The γ-phosphate group should be transferred by the kinase onto the substrate, thus allowing to obtain the phosphorylated nucleoside analogue in radiolabeled form [9].

Cell free extract was added and after incubation at 37°, 100-μl samples were withdrawn and poured into 100 μl 2 N perchloric acid. After 10 min on ice, the acid-precipitable material was spun down. Part of the supernatant was neutralized with K_2CO_3 and analysed by thin layer chromatography in either FINK 8 [10] for separation of nucleoside and nucleoside-5′-monophosphate or in propanol/water (70/30) for separation of nucleoside-5′-monophosphate and [γ-^{32}P]ATP. After chromatography radioactivity was measured either by cutting the plates into 0.5 cm fractions or by scanning with a Berthold II LB 2723 scanner. One unit (U) is defined as the amount of extract phosphorylating 1 pmole dThd per min.

Metabolism of BVUrd. E_6SM cells in polystyrene 75 cm^2 flasks were either mock-infected or infected with HSV-1(KOS) at a MOI of 0.03 PFU/cell. After 18 hr of incubation, cells were trypsinized and centrifuged. The cell pellet was homogenized by sonication in 50 mM Tris–HCl (pH 8), 5 mM β-mercaptoethanol and 50% (v/v) glycerol.

Kinase activity was determined in the buffer described above. BVUrd or BVdUrd were added at 25 μM and ATP at 5 mM. After addition of cell extract, the sample was incubated at 37°. Proteins were removed as described above and, after neutralization, the sample was analysed by HPLC.

BVUra and its corresponding nucleosides and nucleotides were separated and quantitated by HPLC on a Radial pak C18 reverse column (Waters). A triphasic elution system was used as follows: (i) a step of 15 min in 10 mM potassium phosphate pH 5.5 (buffer A), (ii) a gradient from 100% to 40% of buffer A within 15 min, with methanol–potassium phosphate buffer (80:20) as diluting buffer, and (iii) a 5 min step in the end conditions of the previous gradient. Under these conditions retention times for BVUra, BVdUrd, BVUrd and BVdUDP were 31.30, 33.20, 32.20 and 27.10 min, respectively. As a control, phosphorylation of dThd was followed by adding 2 μM of [*methyl*-^{3}H]dThd to the reaction mixture in absence of BVdUrd. The tritiated dTMP formed was captured onto a DEAE cellulose filter and radioactivity was determined.

Virus yield reduction. Confluent E_6SM cells in polystyrene 60-mm petri dishes were infected with HSV-1(KOS) at a MOI of 1 PFU/cell in 0.5 ml EMEM supplemented with 3% (v/v) fetal calf serum, 2 mM L-glutamine and 0.075% (v/v) $NaHCO_3$. After a 1-hr virus adsorption period, residual virus was replaced by 2 ml of medium containing BVUrd at 0, 0.5, 1, 5, 10 and 50 μM. Cells were incubated for an additional 24 hr, freeze-thawed once and scraped off. Cell debris was removed by low speed centrifugation (800 *g*) at 4°. The supernatant was stored at −70°, and the virus yield of each sample was determined by plaque formation in Vero cells as previously described [11].

CsCl gradient analysis. In parallel with the virus

Table 1. Initial velocities of the dThd kinase reaction in cell-free extracts of E_6SM cells, as evaluated with various substrates

Substrate	Cell-free dThd kinase extracts from E_6SM cells		
	HSV-1(KOS)-infected	TK⁻HSV-1(B2006)-infected	Mock-infected
	Initial velocity (U/mg protein)*		
dThd	3104 ± 640	35 ± 8	26 ± 4
dUrd	4880 ± 760	20 ± 6	29 ± 9
Urd	25 ± 4	26 ± 2	20 ± 9
BVdUrd	2153 ± 350	<20	<20
BVUrd	2340 ± 180	<20	<20
None	<20	<20	<20

* Average values ± standard deviation for 3–5 separate experiments.

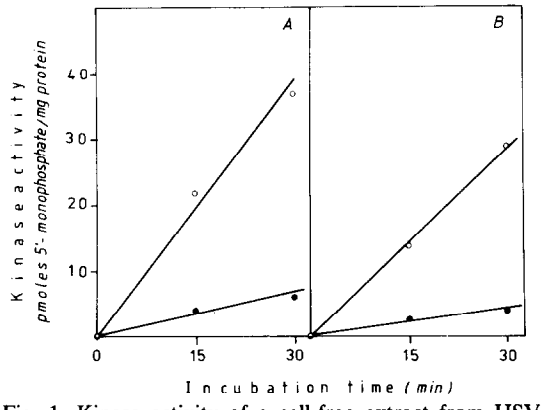

Fig. 1. Kinase activity of a cell-free extract from HSV-1(KOS)-infected E_6SM cells, incubated in the presence of 5 mM [γ-^{32}P]ATP and either BVdUrd (A) or BVUrd (B) at 200 μM. The extract was preincubated with non-immune serum (○) or with polyclonal antiserum raised against HSV-1(KOS)-infected cells (●).

yield reduction assay the effect on viral DNA synthesis was determined. Confluent E_6SM cells in polystyrene 60 mm petri dishes were infected as described above, except that phosphate-free medium (Gibco) was used and that [^{32}P]orthophosphate was added at 25 μCi/petri dish to radiolabel newly synthesized DNA. After a 24-hr incubation period, the cells were lysed and further processed as described elsewhere [11].

Identification of BVdUMP in the viral DNA. The procedure described by Chen *et al.* [12] was followed. 10^7 E_6SM cells were infected with HSV-1(KOS) at a MOI of 1 PFU/cell. After 1-hr virus adsorption at 37°, residual virus was removed, cells were washed with phosphate-free medium and [^{32}P]orthophosphate was added together with BVUrd to a final concentration of 20 μCi/ml and 5 μM, respectively. After 24 hours incubation viral and cellular DNA were separated on CsCl gradient, viral DNA was pooled and desalted on a 1.5 ml Sephadex G50 column. Carrier DNA (from calf thymus, Sigma), isopropanol and Na-acetate buffer pH 7.5 were added to final concentrations of 2 μg/ml, 50% (v/v) and 100 mM, respectively. The sample was stored overnight at −20°, the DNA was pelleted by centrifugation in an Eppendorf 5412 centrifuge and redissolved in DNase buffer (50 mM Tris–HCl pH 7.4, 1 mM $MgCl_2$). The DNase I and phosphodiesterase I reactions were carried out as described [12]. Remaining enzyme was precipitated with PCA, samples were neutralized with K_2CO_3 and concentrated by centrifugation under vacuum. To identify the released [^{32}P]labeled 5′-monophosphates, the sample was chromatographed on thin layer in FINK 8, plates were cut into 0.5 cm fractions and radioactivity was determined. This solvent allowed separation of the common 2′-deoxynucleoside-5′-monophosphates from BVdUMP, as well as BVdUMP from BVUMP (obtained as product of the thymidine kinase reaction with BVUrd and [γ-^{32}P]ATP as substrates).

Protein determination. Protein content was measured by the Biorad Protein Assay with bovine serum albumin as a standard (Richmond, California, U.S.A.).

RESULTS

The possibility that BVUrd may be phosphorylated by the HSV-1 dThd kinase was examined by incubating it with [γ-^{32}P]ATP and cell-free extracts from mock-infected, TK⁻ HSV-1(B2006)-infected or HSV-1(KOS)-infected E_6SM cells (MOI: 10 PFU/cell). BVdUrd, dThd and dUrd, which are well-known substrates for the viral dThd kinase, were included in the experiment. As shown in Table 1, dThd and dUrd are phosphorylated by all three cell-free extracts, albeit at a 100-fold lower rate by the TK⁻HSV-1- and mock-infected cell extracts than by the HSV-1(KOS)-infected cell extracts. The 100-fold higher velocity obtained with the latter cell extracts is obviously due to the presence of the virus-encoded dThd kinase [13]. From the values obtained for the initial velocity it appeared that BVdUrd and BVUrd acted equally well as substrates for the HSV-1 dThd kinase. These values were of the same order of magnitude as those recorded for dThd and dUrd; Urd, in marked contrast with BVUrd, did not seem to act as substrate for HSV-1 dThd kinase (Table 1). The reaction products obtained for BVdUrd and BVUrd were identified as their phosphorylated forms since they were converted again to the nucleo-

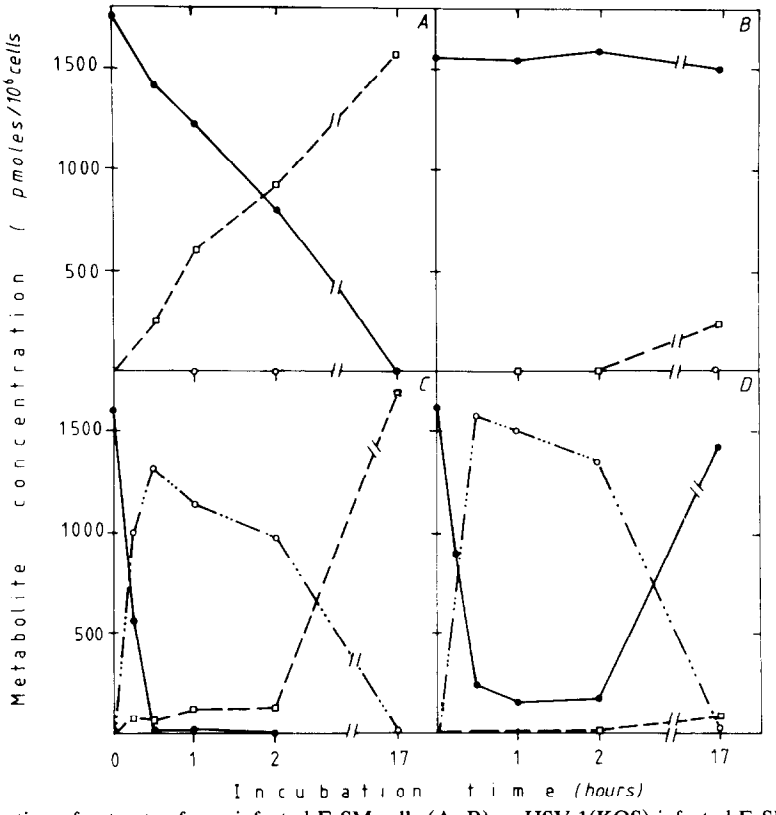

Fig. 2. Incubation of extracts of non-infected E_6SM cells (A, B) or HSV-1(KOS)-infected E_6SM cells (C, D) with 5 mM ATP and either 25 μM BVdUrd (A, C) or BVUrd (B, D). Formation of BVUra (□): A, B, C and D. Formation of BVdUMP (○): A and C. Formation of BVUMP (○): B and D. Fate of BVdUrd (●): A and C. Fate of BVUrd (●): B and D.

side form following alkaline phosphatase treatment.

To ascertain that the phosphorylation of BVdUrd and BVUrd by the HSV-1(KOS)-infected cell extract was achieved by an enzyme of viral origin, the cell extract was preincubated with polyclonal rabbit antiserum at a ratio of 1 μl antiserum to 1 U dThd kinase activity. This ratio was sufficient to block the dThd phosphorylating activity of the cell-free extract of HSV-1(KOS)-infected cell extract by more than 90% (data not shown). This treatment also caused a significant reduction in the phosphorylation rate of both BVdUrd and BVUrd (Fig. 1). In a control experiment where mock-infected cell extract was preincubated with polyclonal rabbit antiserum no such inhibition of dThd kinase activity was observed, even at a ratio of 3 μl antiserum to 1 U dThd kinase activity (data not shown).

When the metabolic fate of BVdUrd and BVUrd was monitored by HPLC analysis, no phosphorylation of either BVdUrd or BVUrd could be detected after they had been incubated with extracts of mock-infected cells (Fig. 2A and B, respectively). When an extract of HSV-1(KOS)-infected cells (MOI: 0.03 PFU/cell) was used, both BVdUrd and BVUrd were phosphorylated to a significant extent (Fig. 2C and D, respectively). The reaction products eluted at 27.10 min in the case of BVdUrd and at 24 min in the case of BVUrd. Following treatment with alkaline phosphatase, the peaks of the reaction products shifted to peaks with the same retention times as BVdUrd and BVUrd, respectively. Moreover, the retention time of the reference compound BVdUMP was the same as the retention time of the reaction product formed after incubation of BVdUrd with the extract of HSV-1(KOS)-infected cells.

Upon a prolonged incubation time, the amounts of nucleoside, nucleoside 5′-monophosphate and free base diminished, regardless of whether BVdUrd or BVUrd were used as the substrate (Fig. 2C and D, respectively). This may be due to the formation of other phosphorylated derivatives (5′-di- or 5′-triphosphates).

It is noteworthy that BVdUrd was converted to BVUra when incubated with extracts of both mock-infected and HSV-1(KOS)-infected cells (Fig. 2A and C). On the contrary, no such conversion was detected with BVUrd as the substrate (Fig. 2B and D). The phosphorylation of BVdUrd by HSV-1-infected cell extract occurred with a higher initial velocity than its phosphorolysis, so that the degradation of BVdUrd by the virus-infected cell extract was retarded as compared with its degradation by the mock-infected cell extract. After prolonged (17 hr) incubation, the only reaction product found if BVdUrd served as a substrate was BVUra and the main product detected, if BVUrd served as substrate, was BVUrd itself. This means that cellullar phosphatases may convert BVUMP and BVdUMP back

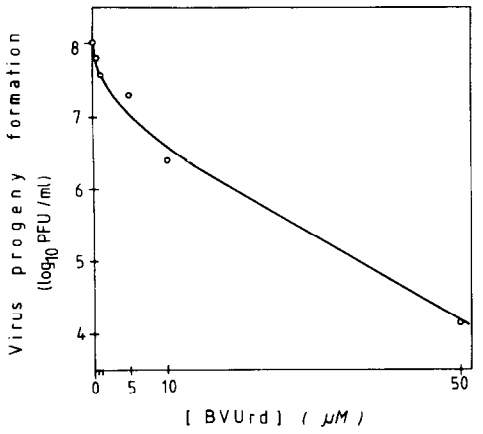

Fig. 3. Antiviral activity of BVUrd against HSV-1(KOS) in E_6SM cells. Virus yield was determined after the virus-infected cells had been incubated for 24 hours in the presence of BVUrd at the indicated concentrations.

to BVUrd and BVdUrd, respectively. While BVdUrd would be further degraded to BVUra by dThd phosphorylase, BVUrd, that is not a substrate of this enzyme [6], would resist degradation.

When BVUrd was added at various concentrations immediately after E_6SM cells had been infected with HSV-1(KOS) (MOI: 1 PFU/cell), a dose-dependent reduction of viral progeny formation was observed (Fig. 3). Virus yield was reduced by 50% at a BVUrd concentration of 1 μM and this reduction increased to 4 log if BVUrd was added at 50 μM.

BVUrd also effected a dose-dependent inhibition of viral DNA synthesis (Fig. 4). About 50% inhibition of viral DNA synthesis was achieved at a BVUrd concentration of 1 μM (Fig. 4B), and at 50 μM BVUrd, viral DNA synthesis was completely abolished (Fig. 4E). The viral DNA formed in the presence of 1 μM and 5 μM BVUrd was more heterodisperse (Fig. 4B and C) than viral DNA formed in the absence of BVUrd (Fig. 4A).

When the viral DNA, formed in HSV-1(KOS)-infected E_6SM cells which had been exposed to 5 μM BVUrd in the presence of [^{32}P]orthophosphate, was isolated by CsCl gradient analysis and subjected to enzymatic hydrolysis (see Materials and Methods), [^{32}P]BVdUMP could be detected in the hydrolysate (data not shown). This indicates that following exposure of the HSV-1-infected cells to BVUrd, the latter was incorporated as its BVdUMP metabolite into the viral DNA.

DISCUSSION

Based on our findings, the metabolism of BVUrd in HSV-1(KOS)-infected cells can be rationalized as shown in Fig. 5. BVUrd is not degraded to BVUra in either mock-infected or HSV-1-infected E_6SM

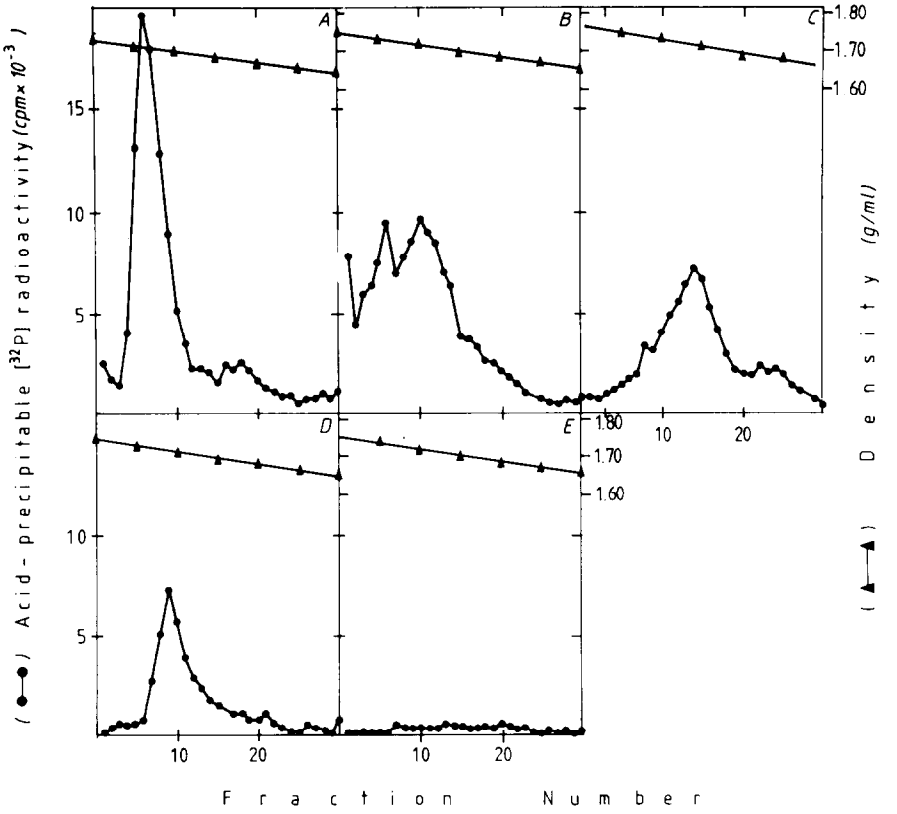

Fig. 4. CsCl gradient analysis of HSV-1(KOS)-infected E_6SM cells, incubated for 24 hr in the presence of [^{32}P]orthophosphate (25 μCi/petri dish) and BVUrd at 0 μM (A), 1 μM (B), 5 μM (C), 10 μM (D) or 50 μM (E).

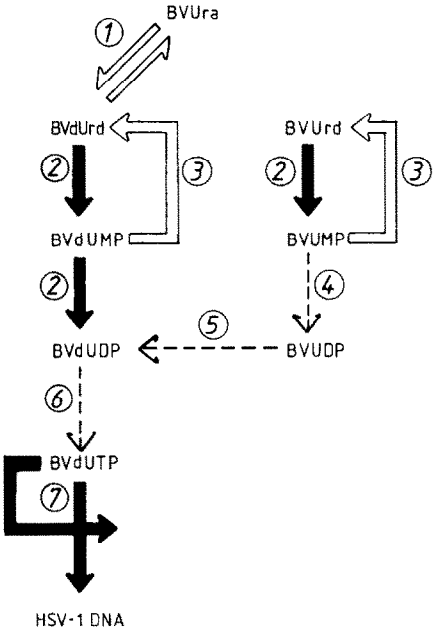

Fig. 5. Model for the metabolism of BVdUrd and BVUrd in the HSV-1-infected cell. Reactions catalysed by cellular or viral enzymes are depicted by open or full arrows, respectively. Putative enzymatic conversions are shown by dotted lines. BVdUrd can be metabolized by dThd phosphorylase to BVUra (1). BVdUrd and BVUrd can both be phosphorylated by HSV-1 dThd kinase (2) to BVdUMP and BVUMP, respectively. The nucleoside 5′-monophosphate is substrate for cellular phosphatases (3). BVdUMP can be further phosphorylated to its 5′-diphosphate and 5′-triphosphate by the HSV-1 dThd kinase (2) and a cellular nucleoside 5′-diphosphate kinase (6), respectively. BVdUTP can both be incorporated into viral DNA and act as an inhibitor of this reaction (7). BVUMP may be further phosphorylated by either HSV-1 dThd kinase or cellular dTMP kinase to its 5′-diphosphate form (4). Reduction of BVUDP to BVdUDP (5) may then be catalysed by ribonucleotide reductase of either cellular or viral origin or both.

cells. BVUrd is not a substrate for the pyrimidine deoxyribonucleoside phosphorylase found in E_6SM, and pyrimidine ribonucleoside phosphorylase, the only enzyme apt to degrade the ribonucleoside, is absent in these cells. In contrast, BVdUrd which is a substrate for both types of pyrimidine phosphorylases [14, 15], is readily degraded to BVUra in both mock-infected and HSV-1-infected E_6SM cells. Thus, the hypothetical conversion of BVUrd to BVdUrd *via* the intermediary formation of BVUra can be excluded. A similar conclusion was reached previously [1] based on the use of inhibitors of pyrimidine nucleoside phosphorylases.

The present study demonstrates that BVUrd is phosphorylated to BVUMP in HSV-1-infected cells but not in TK⁻HSV-1- or mock-infected cells. The phosphorylation of BVUrd must be carried out by a viral enzyme, since this phosphorylation was suppressed by a polyclonal antibody to HSV-1. In all likelihood, the HSV-1-encoded dThd kinase could be held responsible for the phosphorylation of BVUrd as it was absent from TK⁻HSV-1(B2006)-infected cells. Urd kinase activity does not seem to be induced following infection with HSV-1(KOS). Phosphorylation of BVdUrd by the HSV-1-encoded dThd kinase has been demonstrated in previous studies. We found that the ratio of BVdUMP to dTMP formed by this enzyme was 0.7. This is in agreement with the values reported in the literature: 0.9 [16] and 1.1 [17].

The phosphorylation of BVdUMP to its 5′-diphosphate (BVdUDP) is catalysed by the HSV-1-encoded dThd kinase and its subsequent phosphorylation to the 5′-triphosphate (BVdUTP) is assumed to be catalysed by a cellular kinase [18, 19]. BVdUTP can act as substrate of cellular and viral DNA polymerases and thus be incorporated (as BVdUMP) into (viral and cellular) DNA. The incorporation of BVdUMP into viral DNA renders the latter unstable [20]. In addition, BVdUTP can also act as an inhibitor of the HSV DNA polymerase reaction [21].

How is BVUMP further processed after it has been formed from BVUrd in the HSV-1-infected cells? HPLC analysis has indicated that after prolonged incubation the 5′-diphosphate of BVUrd (BVUDP) may be formed. Phosphorylation of BVUMP to BVUDP could be catalysed by either the HSV-1-encoded dThd kinase, or a cellular nucleoside-5′-monophosphate kinase such as dTMP kinase, or both. When the HSV-1(KOS)-infected cells are exposed to BVUrd, a clear dose-dependent inhibitory effect is seen both on viral progeny formation and viral DNA synthesis. Moreover, we have demonstrated the presence of BVdUMP in viral DNA extracted from HSV-1(KOS)-infected cells which had been exposed to BVUrd.

In Fig. 5 we propose a scheme for the metabolic fate of BVUrd in the HSV-1-infected cell. The first step is the phosphorylation of BVUrd by the HSV-1 dThd kinase to BVUMP, which in turn is converted to BVUDP. The latter may then be transformed to BVdUDP by a cellular or viral ribonucleotide reductase [22]. From this point onwards BVdUDP would join the "BVdUrd" pathway and, hence, the eventual antiviral action of BVUrd would be based on the same mechanism as that elucidated previously for BVdUrd [18, 19]. The selectivity of BVUrd as an antiherpetic agent would reside in the preferential phosphorylation of the compound by the virus-encoded dThd kinase, which would thus restrict its further action to the virus-infected cell. That BVUrd follows the direct phosphorylation route in E_6SM cells (infected with HSV-1) is aided by the fact that these cells do not contain a pyrimidine ribonucleoside phosphorylase able to degrade BVUrd to BVUra (see above).

The metabolic pathway deciphered for BVUrd in HSV-1-infected cells (Fig. 5) has been deduced from studies with cell culture systems. The kinetics of interaction of BVUrd (and its metabolites) with the specific enzymes involved in this pathway, i.e. HSV-1 dThd kinase, dTMP kinase, ribonucleotide reductase, remains the subject of further study.

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