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INCORPORATION INTO NUCLEIC ACIDS OF THE ANTIHERPES GUANOSINE ANALOG BUCICLOVIR, AND EFFECTS ON DNA AND PROTEIN SYNTHESIS

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Abstract—Using cells expressing herpes simplex virus (HSV) thymidine kinase, we investigated the metabolism of the acyclic antiherpes guanosine analog buciclovir, in relation to the effects of the drug on viral DNA and protein synthesis. In these cells the predominant metabolite of buciclovir was its triphosphate, as in the HSV-1 infected Vero cells investigated in parallel. Further metabolism of buciclovir led to incorporation into RNA and DNA. Buciclovir inhibited DNA synthesis, not RNA synthesis, and prevented an increase in the size of newly synthesized DNA.

To study the relative effects of BCV on cellular and viral DNA synthesis, human TK^- cells transformed to a TK^+ phenotype with HSV-2 DNA, were infected with HSV-1. In these HSV-1 infected cells buciclovir-triphosphate caused a preferential inhibition of viral DNA synthesis. Despite incorporation of buciclovir into RNA, and the presence of buciclovir-triphosphate from the time of infection onwards, no effect was observed on the synthesis of the β proteins ICP-6 and ICP-8. Presumably as a consequence of inhibition of viral DNA synthesis, the synthesis of a $\beta\gamma$ protein (gD) and a γ protein (gC) were inhibited, and synthesis of the β proteins (ICP-6 and ICP-8) was not shut-off. Glycosylation of gC that was still synthesized, was not inhibited. Thus, the biological effects of buciclovir can be explained by its inhibition of DNA synthesis.

A number of acyclic guanosine analogs are potent and selective inhibitors of herpes simplex virus (HSV)§ multiplication. Some of these, acyclovir (ACV: 9-[(2-hydroxyethoxy)methyl]guanine), gancyclovir (GCV: 9-[(1,3-dihydroxy-2-propoxy)-methyl]guanine), buciclovir (BCV: (*R*)-9-(3,4-dihydroxybutyl)guanine) and 9-(4-hydroxybutyl)-guanine (HBG) have a similar mechanism of antiviral action. First, these guanosine analogs are selectively phosphorylated to monophosphates by HSV thymidine kinase. After further phosphorylation by cellular enzymes, triphosphates of the guanosine-analogs are formed in infected cells. These triphosphates are potent and specific inhibitors of HSV-DNA polymerase (see Refs 1–4 for reviews).

In infected cells and in non-infected cells expressing the viral thymidine kinase, ACV-TP terminates DNA synthesis [5]. In experiments using purified HSV-DNA polymerase, it was shown that ACV-TP, BCV-TP and HBG-TP inactivate the polymerase [6, 7]. It is possible, therefore, that incorporation of BCV into the 3′-terminal end of the growing DNA chains can lead to irreversible termination of HSV-DNA synthesis. However, incorporation of this guanosine analog into DNA has not been directly demonstrated. GCV has been shown to be incorporated internally into DNA in HSV-1 infected cells [8]. This is possible since this compound, like BCV, has two hydroxyl groups in the acyclic side-chain, which may mimic the 5′ and the 3′ hydroxyl groups in normal nucleosides.

Using cells expressing viral thymidine kinase we investigated the metabolism of BCV, its phosphorylation and incorporation into nucleic acids. In addition, we studied the consequences of the metabolism of BCV on cellular DNA and RNA synthesis and herpes virus DNA and protein synthesis.

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§ The abbreviations used are: AC4 cells, human cells expressing the HSV-2 thymidine kinase; ACV, acyclovir: 9-[(2-hydroxyethoxy)methyl]guanine; BCV, buciclovir: (*R*)-9-(3,4-dihydroxybutyl)guanine; HBG, 9-(4-hydroxybutyl)guanine; GCV, gancyclovir: 9-[(1,3-dihydroxy-2-propoxymethyl]guanine; HSV, herpes simplex virus; HPLC, high-pressure liquid chromatography; LMTK-1 cells, murine cell-line expressing the HSV-1 thymidine kinase; MP, DP and TP: mono-, di- and triphosphate, respectively; PBS, phosphate-buffered saline; PFU, plaque-forming unit; ICP, intracellular protein of HSV-infected cells.

EXPERIMENTAL PROCEDURES

Chemicals and enzymes. BCV was synthesized at Astra Alab AB [9]. [8-^{3}H]BCV, specific activity 11.2 Ci/mmol, and deoxy[1′,2′,2,8-^{3}H]adenosine, specific activity 72 Ci/mmol, were purchased from Amersham International, U.K. [Methyl-^{3}H]thymidine, specific activity 43 Ci/mmol, [2-^{14}C]thymidine, specific activity 55.3 mCi/mmol, [5,6-^{3}H]uridine, specific activity 38.4 Ci/mmol, [$^{32}PO_4$]$^{3-}$, carrier-free, L-[^{35}S]methionine, 235 Ci/mmol, were purchased from New England Nuclear (Boston, MA).

Snake venom phosphodiesterase I (type IV), alkaline phosphatase (type III-S), RNAse A, proteinase K (type XI, fungal) were from Sigma Chemical Co. (St Louis, MO). Reagents for SDS-gel-electrophoresis and blotting were bought from Bio-Rad (Richmond, CA). All other reagents were of analytical grade whenever possible.

Cells and viruses. Vero cells (CCL 81) were from Flow Laboratories (Irvine, Scotland); LMTK-1 cells were provided by Dr S. Kit (Houston, TX). These cells have been constructed by biochemically transforming mouse LM(TK$^-$) cells with a DNA plasmid coding for HSV-1 TK [10]. AC4 cells, provided by Dr S. Bacchetti, Ontario, Canada are the result of transforming human TK$^-$ cells with HSV-2 DNA and selecting for the TK$^+$ phenotype [11]. Cells expressing HSV-TK were grown in minimal essential medium including HAT-mixture (0.4 μM aminopterin, 100 μM hypoxanthine and 16 μM thymidine) but were transferred into HAT-free medium at least two passages before being used in experiments. The HSV-1 strain used, C42 has been described earlier [12].

Purification of [8-^{3}H]-labeled-BCV. The radiolabeled nucleoside analog was purified prior to use on a reverse phase column (C_{18} column from Waters, Radial-Pak μBondapak, 5 μm particle size) eluted with 10 mM KH_2PO_4, pH 6.0, containing 5% methanol. The purified nucleoside eluted as a single peak when reinjected into the same system.

Extraction and HPLC analysis of metabolites of BCV in cell culture. The intracellular low-molecular weight metabolites were extracted by the PBS-ethanol method described earlier [13]. The extracts were analysed for the presence of mono-, di- and triphosphates by HPLC on a weak anion exchange column (Waters, Radial-Pak, μBondapak NH_2) by a stepwise elution procedure using 0.1 M KH_2PO_4, pH 3.0 for 6 min and then changing to 1 M KH_2PO_4, pH 3.0. Metabolites were identified as described [13].

Digestion of nucleic acids labeled with radioactive acyclic nucleoside analogs. Rapidly growing LMTK-1 cells were incubated for 4 hr with 1 or 10 μM [8-^{3}H]BCV, washed, treated with protease, extracted with phenol/chloroform, and precipitated with ethanol as described for preparing nucleic acids for Cs_2SO_4 centrifugation. The nucleic acids obtained after the second ethanol precipitation were treated with a mixture of nucleases to obtain nucleosides, as described [14]. In short, nucleic acids obtained after ethanol-precipitation and originating from 8×10^5 cells were dissolved in 1 ml buffer (10 mM $MgCl_2$, 30 mM Tris–HCl, pH 8.0) containing 0.2 mg snake venom phosphodiesterase I, 0.16 mg alkaline phosphatase, type III-S and 0.2 mg RNAse A and incubated 6 hr at 37°. After incubation, the digested material was centrifuged 5 min at 10,000 *g* and the supernatant was analyzed by HPLC [12]. When using this procedure 80% or more of the radioactivity was recovered in the supernatant.

Separation of DNA and RNA by isopycnic centrifugation in Cs_2SO_4. The method used is a modification of the procedure used by Kufe *et al.* [15]. The cell monolayers were washed and digested by incubation with proteinase K as described below for HSV-DNA isolation. Digested samples were extracted once with phenol, once with phenol/chloroform (1:1) and once with chloroform, all extraction steps were performed at 60° and with one 10 min centrifugation at 10,000 rpm in a HB-4 rotor between extractions. The nucleic acids were precipitated from the solution by the addition of 0.1 vol. of 4 M NaCl and 2 vol. of absolute ethanol. After standing overnight at −20°, samples were centrifuged at 10,000 *g* for 30 min and the resulting pellets were resuspended in 1 ml 0.005 M EDTA. To the solution 0.1 vol. of 4 M NaCl and 2 vol. of ice-cold ethanol were again added and the material was reprecipitated at −70° for 4 hr. The pellets were suspended in 0.5 ml 0.005 M EDTA, and deionized formamide was added to a final concentration of 50% (v/v). The nucleic acid extracts were then heated to 80° for 5 min. Samples were made up to 2.5 ml in 0.005 M EDTA and mixed with an equal volume of saturated Cs_2SO_4 to yield a starting density of 1.52 g/ml. Centrifugation was carried out in a vertical rotor (TV-865) at 50,000 rpm for 24 hr at 20°. Fractions (0.1 ml) were collected from the bottom and assayed for acid-precipitable radioactivity as described below. Cells labeled with [^{3}H]thymidine showed a peak in the density region between 1.42 and 1.48 g/ml whereas the main peak after incubation with [^{3}H]uridine banded between 1.62 and 1.68 g/ml. To determine the effects of BCV (1 and 10 μM) on incorporation of $[^{32}PO_4]^{3-}$ into nucleic acids, rapidly growing LMTK-1 cells were treated for 4 hr with 100 μCi of $[^{32}PO_4]^{3-}$ in 2 ml phosphate-free medium at 37°, and nucleic acids were isolated and analyzed.

Analysis of DNA synthesis in LMTK-1 cells treated with BCV by centrifugation in alkaline sucrose. The method used is a modification of the method used by Furman *et al.* [5]. Exponentially growing LMTK-1 cells were incubated for 40 hr with [^{14}C]thymidine (0.1 μCi/ml) to label DNA. Each cell culture dish was then washed three times with 3 ml of PBS, new growth medium (2 ml) was added and the cultures were incubated for 2 hr at 37°. To the growth medium of some dishes 0.5 μM BCV was added, and after 30 min 50 μCi of [^{3}H]thymidine was added to all dishes. After 4 hr all dishes were washed three times with 3 ml PBS, and the first half of the series of the dishes was harvested. The other half was supplied with fresh growth medium and the incubation was continued for an additional 16 hr in the absence of drug (chase experiment). The cells were digested with 1 ml each of proteinase K for 30 min at 55° to remove proteins [13]. An aliquot containing the equivalent of 2×10^5 cells was gently added to a solution of 1% sarcosyl, 10 mM EDTA, and 1 M NaOH on top of an alkaline sucrose gradient. Linear gradients from 5 to 20% (w/w) sucrose solutions were prepared that contained 0.7 M NaCl, 0.3 M NaOH and 0.01 M EDTA.

The gradients were centrifuged at 40,000 rpm for 100 min at 20° in a Beckman SW 40 rotor. Fractions (0.125 ml) were collected from the bottom and 50 μl of each fraction was spotted on Munktell filter paper, washed in 5% TCA to precipitated DNA before liquid scintillation spectrometry.

Separation of HSV DNA and cellular DNA by

isopycnic centrifugation in CsCl. Confluent AC4 cells were pretreated for 2 hr with different concentrations of BCV (0.01, 0.1, 0.5, 1.0 μM). The cell monolayers were then washed twice with 5 ml of PBS to remove extracellular drug. The cultures were infected with HSV-1, strain C42 (multiplicity of infection = 1) and 5 ml growth medium including [^{3}H]-deoxyadenosine (10 μCi/ml) was added to label DNA during a 16 hr incubation period. Cells were harvested after three washes with 5 ml PBS, transferred into Eppendorf vials and pelleted by low speed centrifugation. One ml proteinase K (0.1 mg/ml in 0.01 M Tris–HCl (pH 7.4), 0.1 M NaCl, 0.001 M EDTA, 0.2% SDS and 0.5% sarcosyl) was added to each vial and proteins were digested for 3 hr at 37° [16]. To each sample CsCl was added in 0.01 M Tris–HCl (pH 7.4), 0.1 M NaCl and 0.001 M EDTA to a final density of 1.700 g/cm^3. The samples were centrifuged for 24 hr at 50,000 rpm in a vertical rotor, TV-865. Samples were collected from the bottom of the tubes and 50 μl of each fraction wure spotted on 25 mm filter paper dishes (Munktell, Sweden) which were washed in TCA to precipitate nucleic acid and remove unincorporated precursors, and subsequently treated with alkali to digest RNA and washed again [16] before analysis by scintillation spectroscopy.

Radioimmunoprecipitation and SDS-polyacrylamide electrophoresis (SDS-PAGE). Detergent extracts from [^{35}S] methionine-labeled cells, infected with HSV-1, strain C42, at a multiplicity of five, were immunoprecipitated with gC-1 specific monoclonal antibody B1C1 [17] as previously described [18]. The precipitates were solubilized and electrophoresed in 9.25% polyacrylamide gels according to the method by Morse *et al.* [19]. Gels were treated with "Amplify" (Amersham), and subjected to fluorography on Kodak X-omat AR film. During labeling, methionine-free medium was used. To assay for protein synthesis, cells labeled with [^{35}S]methionine as indicated below, were harvested and subjected SDS-PAGE [19]. Blotting of proteins was on nitrocellulose paper (Bio Rad) for 2 hr, at 60 V, constant voltage, in a buffer containing 25 mM Tris–Cl, 192 mM glycine, pH 8.3, and 20% methanol [20]. Blotting papers were subjected to autoradiography using Kodak X-omat AR films.

Table 1. Incorporation of BCV into nucleotides and nucleic acids

Compound	pmol/10^6 cells		
	HSV-1 infected Vero cells	LMTK-1 cells	
		2 hr	4 hr
BCVMP	6.3	<5.0	27.6
BCVDP	23.8	<5.0	56.0
BCVTP	250	172	286
DNA	—	<0.01	0.05
RNA	—	<0.01	0.73

Confluent Vero cells were infected with HSV-1, strain C42 at a multiplicity of infection of 1 PFU/cell, and 10 μM of BCV was added to the growth medium. After 16 hr, cells were extracted and analysed as described under Experimental Procedures. Rapidly growing LMTK-1 cells were treated for 2 or 4 hr with 10 μM of labeled BCV and analysed as described under Experimental Procedures.

RESULTS

Metabolism of BCV in HSV-1-infected Vero cells and in LMTK-1 cells

In HSV-1 infected Vero cells and in LMTK-1 cells, the only metabolites of BCV observed were the mono-, di- and triphosphates. After an incubation time of 16 hr for HSV-1 infected Vero cells (i.e. one infection cycle) the extent of phosphorylation of BCV was the same as after incubation time of 4 hr for LMTK-1 cells (Table 1). Using these experimental conditions, LMTK-1 cells were exposed to radiolabeled BCV and the nucleic acids were extracted. A part of the extract was digested by nucleases and the resulting material was analyzed by HPLC (Fig. 1A). This showed that 85–93% of the total nucleic acid digest migrated as BCV. The extracts showed an additional peak of radioactivity that migrated as [^{3}H]–H_2O that originated from the exchange of [^{3}H] from BCV to H_2O and amounted to 2% of the radioactivity in BCV (Fig. 1A).

Another part of the nucleic acid labeled with BCV was subjected to Cs_2SO_4-centrifugation to analyze the incorporation into DNA and RNA (Fig. 1B). The incorporation into RNA after labeling with 10 μM of BCV amounted to 0.73 pmol of nucleoside/10^6 cells, and the incorporation into DNA to 0.05 pmol/10^6 cells (Table 1).

Analysis of cellular DNA synthesis in LMTK-1 cells after treatment with BCV

The effects of BCV on incorporation of $[^{32}PO_4]^{3-}$ into nucleic acids was studied in growing LMTK-1 cells. A comparison of Fig. 2A and Fig. 2B shows that BCV inhibited the incorporation into DNA. On the other hand the incorporation of RNA by $[^{32}PO_4]^{3-}$ was not inhibited by the guanosine analog (1 or 10 μM). These results suggest that BCV preferentially inhibits DNA synthesis.

Rapidly growing LMTK-1 cells were incubated with [^{14}C-]thymidine for 40 hr. Subsequently, as described under Experimental Procedures, cells were incubated for 4 hr with [^{3}H]thymidine in the absence or presence of BCV (0.5 μM). The DNA was isolated and subjected to alkaline-sucrose density gradient centrifugation (Fig. 3). The sedimentation profiles show that BCV (Fig. 3B, filled symbols) prevented an increase in DNA size observed in untreated cells (Fig. 3A). When after 4 hr incubation BCV (Fig. 3B, open symbols) was removed, and the cells incubated for an additional 16 hr, only a very small increase in DNA size could be observed. The DNA from cultures submitted to a similar pulse-chase protocol, but not treated with BCV, banded around fraction 20 (not shown), which indicated that in the absence of the drug an increase in DNA-size could be observed. These results suggest that BCV can prevent DNA-chain elongation.

Effects of BCV on viral DNA and protein synthesis

Because BCV could be incorporated into DNA and prevent DNA-chain elongation, its effects on DNA synthesis in HSV-infected cells has been stud-

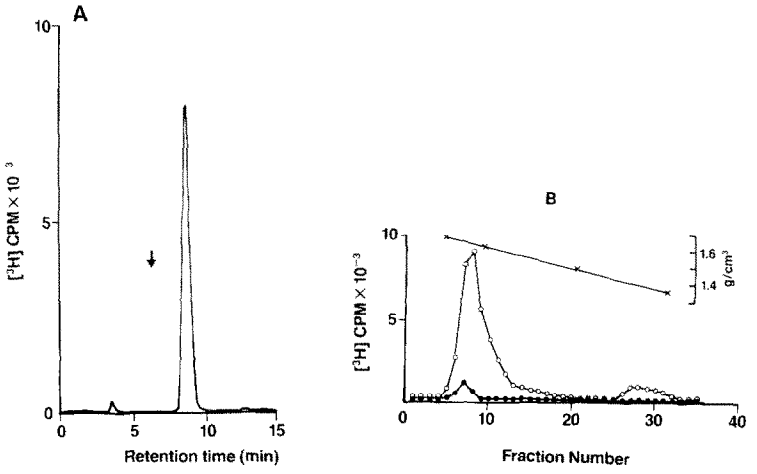

Fig. 1. (A) HPLC analysis of the digested nucleic acid extract of LMTK-1 cells after labeling for 4 hr with [8-^{3}H]BCV. The extraction procedure, digestions and HPLC systems used are described in the Experimental Procedures. Arrow indicates elution position of guanosine and deoxyguanosine; the peak eluting at 3 min is [^{3}H]H_2O; the peak eluting between 8 and 9 min is BCV. (B) Incorporation of BCV into nucleic acids of LMTK-1 cells. Exponentially growing LMTK-1 cells were incubated for 4 hr with 1 μM (●—●) or 10 μM (○—○) of radioactive BCV. The nucleic acids were extracted, purified and subjected to isopycnic centrifugation in Cs_2SO_4. Material in frqctions 6–10 is RNA; DNA is present in fractions 26–31.

ied. For these experiments AC-4 cells were used, which are transformed with the HSV-2 thymidine kinase. These cells phosphorylate BCV to a similar extent as LMTK-1 cells, but, in contrast to LMTK-1 cells, also support a productive HSV-1 multiplication (data not shown). Pretreating AC-4 cells with various concentrations of BCV before infection enabled the study of the effects of BCV-TP from the time of infection onwards, as BCV-TP is metabolically stable in these cells [data not shown; cf. ref. 13].

DNA was labelled with [^{3}H]deoxyadenosine, added immediately after infection, and was extracted 16 hr later. At the highest concentration tested (0.5 μM) treatment with BCV before infection decreased HSV-DNA synthesis (high density material) to about 35% of untreated cultures, whereas cellular DNA synthesis was unaffected (Fig. 4). The results also show that more than 25% of the radioactive DNA had a density different from HSV- or cellular DNA. The nature of this material is unknown.

Because BCV can be incorporated into RNA (see Fig. 1B) we investigated whether the drug added to AC4 cells before and during infection inhibits the synthesis of the viral β proteins ICP-6 and ICP-8. Cells were pulse-labeled with [^{35}S]-methionine from 9 to 10, 14 to 15 and 17 to 18 hr post infection. It is shown in Fig. 5, that ICP-6 and ICP-8 are still synthesized early post infection (9–10 hr), despite the presence of BCV-TP from the time of infection. In addition, the figure shows that the synthesis of ICP-6 and ICP-8 is not shut-off late post infection (14 hr–15 hr and 17 hr–18 hr). This is in-line with the drug inhibiting DNA synthesis [21], and hence late (γ) protein synthesis (see also below).

Effects of BCV on viral glycoprotein synthesis

Nucleoside analogs can inhibit glycosylation [22, 23]. Here we investigated the effect of BCV on the synthesis of gC and its precursor pgC, which are sensitive probes for effects on glycosylation [22]. As shown in Fig. 6, BCV decreased the total amount of

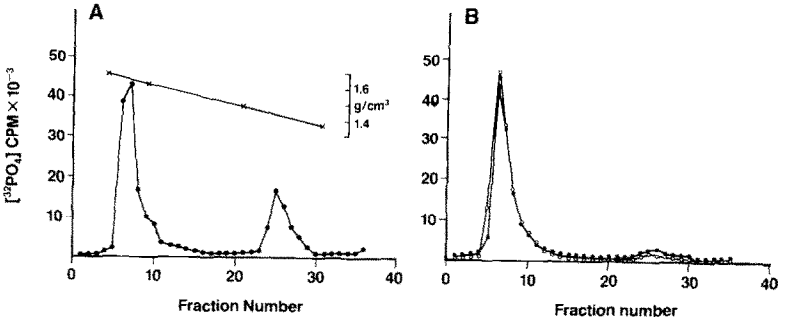

Fig. 2. Incorporation of [^{32}P] into RNA and DNA of LMTK-1 cells. Profiles from Cs_2SO_4 gradients from untreated cells (panel A) and cells treated with 1 (●—●) or 10 μM (○—○) BCV (panel B).

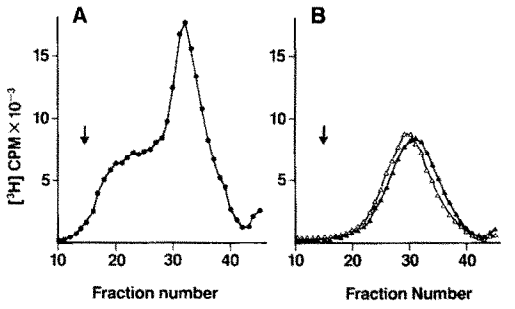

Fig. 3. Analysis of DNA size by velocity centrifugation in alkaline sucrose density gradients. Centrifugation profiles of [^{3}H]thymidine-labeled DNA obtained after 4 hr incubation without drug treatment (●—●, panel A), or in the presence of 0.5 μM BCV (▲—▲, panel B). The profiles obtained after incubation for 4 hr with 0.5 μM BCV followed by 16 hr incubation without drug are shown in panel B (△—△). The arrows indicate the position of [^{14}C]thymidine-labeled DNA ("background-labeling"; see Experimental Procedures).

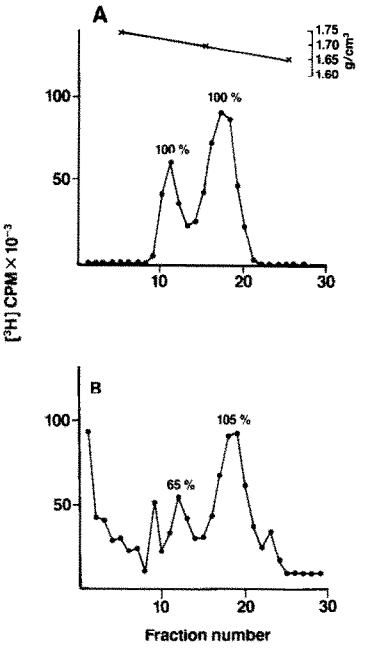

Fig. 4. DNA synthesis in HSV-1 infected AC4 cells pretreated with BCV. Confluent AC4 cells were pretreated for 4 hr without (panel A), or with 0.5 μM BCV (panel B). Medium with BCV was then removed and the cell monolayers were infected with 1 PFU/cell of HSV-1, C42, for 60 min at room temperature, new medium without BCV was added and cells were incubated for 16 hr in the presence of radioactive deoxyadenosine to label DNA. Cells were extracted and HSV-DNA (high density) and cellular DNA (low density) were separated by CsCl isopycnic density centrifugation. The percentage figures indicate incorporation into viral or cellular DNA relative to untreated controls.

[^{35}S]methionine-labeled gC, which is a γ-protein. However, the ratio of the labelling of pgC and gC is less strongly affected by BCV than by tunicamycin or (E)-5-(2-bromovinyl)-2′-deoxyuridine [22]. Furthermore, as shown in the figure, the electrophoretic mobilities of pgC and gC are the same as in untreated cells. Using a Western-blotting technique, the same results were obtained with another glycoprotein, gD (data not shown), which suggested that protein glycosylation is not affected by BCV.

DISCUSSION

The major metabolite of the acyclic guanosine analog BCV in herpes virus infected cells and in cells transformed with the herpes virus thymidine kinase gene (LMTK-1 cells) is BCV-TP. A small fraction of the triphosphate in the LMTK-1 cells is incorporated into nucleic acids, preferentially into RNA. However, RNA synthesis is not inhibited by BCV, but DNA synthesis is inhibited. It should be noted that, at least in infected cells, the formation of up to 6000 pmol of BCV-TP per 10^6 cells does not decrease the pool sizes of deoxynucleoside triphosphates [24]. Rather, in the presence of BCV-TP, DNA synthesis in intact cells is terminated, resulting in a higher proportion of low-molecular weight DNA than in cells devoid of BCV-TP. It is not known whether incorporation of BCV into DNA is the cause of termination of DNA synthesis shown in pulse-chase experiments (Fig. 3), as BCV-TP is also an inhibitor of DNA polymerase [7, 13] and high levels of BCV-TP persist in the cell when the drug is removed from the medium [13]. Nevertheless, the finding of termination of DNA synthesis here is in line with the results that high (cytotoxic) concentrations of BCV can cause chromosomal breaks and induce sister-chromatid exchange [25, 26]. The antiviral effects of compounds such as BCV, having a long intracellular half-life as a triphosphate and the triphosphate irreversibly terminating DNA synthesis, may be long-lasting allowing an unfrequent administration *in vivo* [12, 27]. However, specific phosphorylation in HSV-infected cells is of paramount importance to reduce the risk of unwanted side-effects to uninfected cells.

We have not been able to show incorporation of BCV into nucleic acids of HSV infected cells treated with the drug during the whole viral replication phase [13]. This may be explained by the fact that BCV-TP is a better substrate for cellular than for viral DNA polymerase, as cellular DNA synthesis is shut-off in infected cells. It is therefore likely that inhibition of viral DNA synthesis in transformed, infected cells treated with BCV before infection is caused by the direct inhibition of the viral polymerase by BCV-TP in competition with dGTP. Here, and elsewhere [28], we have shown that BCV preferentially inhibits viral DNA synthesis over cellular DNA synthesis, presumably because the K_i of BCV-TP for the viral DNA polymerase is approximately 200 times lower than the K_i for the cellular DNA polymerase [7, 13]. Indeed, BCV-resistance of HSV can be mapped into the viral polymerase (*pol*) locus [29].

Although BCV can be incorporated into RNA, inhibition of early viral protein synthesis in thymidine kinase-transformed, infected cells could not be demonstrated despite presence of BCV-TP from the

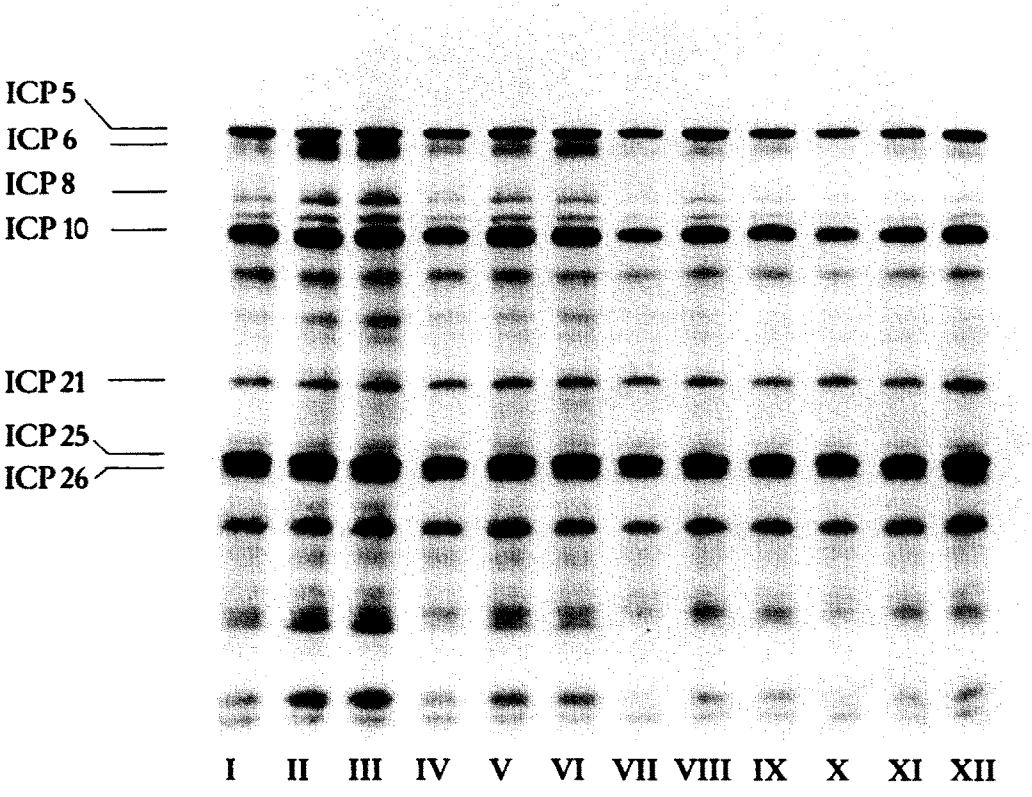

Fig. 5. Autoradiogram of a polyacrylamide slab gel containing electrophoretically separated proteins from HSV-1 (C42) infected AC4 cells, labeled with [^{35}S]-Methionine. I, untreated, labeled from 0 to 18 hr p.i.; II, 1.0 μM BCV added at 0 hr p.i.; labeled from 0 to 18 hr p.i.; III, 0.1 μM BCV before and 1.0 μM BCV after infection; labeled from 0 to 18 hr p.i.; IV, untreated, labeled from 9 to 10 hr p.i.; V, 1.0 μM BCV added 0 hr p.i.; labeled from 9 to 10 hr p.i.; VI, 0.1 μM BCV before and 1.0 μM BCV after infection, labeled from 9 to 10 hr p.i.; VII, untreated, labeled from 14 to 15 hr p.i.; VIII, 1.0 μM BCV added 0 hr p.i., labeled from 14 to 15 hr p.i.; IX, 0.1 μM BCV before and 1.0 μM BCV after infection, labeled from 14 to 15 hr p.i.; X, untreated labeled from 17 to 18 hr p.i.; XI, 1.0 μM BCV added 0 hr p.i., labeled from 17 to 18 hr p.i.; XII, 0.1 μM BCV before and 1.0 μM BCV after infection, labeled from 17 to 18 hr p.i.

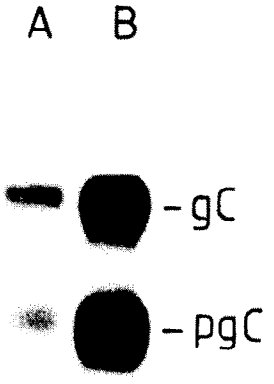

Fig. 6. Electrophoretic characterization of gC from BCV-treated and untreated cells. Radioimmunoprecipitation with monoclonal antibody B1C1 and subsequent SDS-polyacrylamide gel electrophoresis of [^{35}S]-methionine-labeled extract from HSV-1-infected GMK-cells was performed as described in Methods. Both samples were run on the same gel and the gel was exposed for 72 hr to X-ray film. BCV-treated (100 μM) cells (lane A) and untreated control (lane B).

time of infection. In fact, the effects of BCV on protein synthesis in these cells, that is the lack of shut-off on synthesis the viral β-proteins, the inhibition of synthesis of the viral γ-proteins and the lack of effect on glycosylation can be explained by BCV inhibiting only viral DNA synthesis (cf. Refs 31 and 21).

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