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Metabolic activation of the nucleoside analog 9-{[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl}guanine in human diploid fibroblasts infected with human cytomegalovirus

(antiviral chemotherapy/acyclovir/acyclovir analog/mode of drug action)

KAREN K. BIRON*, SYLVIA C. STANAT*, JANN B. SORRELL*, JAMES A. FYFE†, PAUL M. KELLER†, CATHERINE U. LAMBE†, AND DONALD J. NELSON†

Departments of *Virology and †Experimental Therapy, The Wellcome Research Laboratories, Research Triangle Park, NC 27709

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ABSTRACT **9-{[2-Hydroxy-1-(hydroxymethyl)ethoxy]-methyl}guanine (BW B759U) is a more potent inhibitor of human cytomegalovirus (HCMV) *in vitro* than is the related nucleoside analog acyclovir (ACV). BW B759U was selectively activated to the 5′-triphosphate (BW B759U-triphosphate) in cells infected with HCMV to levels at least 10-fold higher than those measured for ACV-triphosphate and up to as much as 100-fold higher than the levels found in uninfected cells. BW B759U-triphosphate accumulated in HCMV-infected cells with time; the rate of this increase was dependent upon the drug dose and virus multiplicity of infection. Enzyme activities that catalyzed the phosphorylation of thymidine and 2′-deoxycytidine increased 3- to 7-fold in extracts of cells early after HCMV infection but thereafter declined. No concomitant increase in the rate of BW B759U phosphorylation was detected under these assay conditions. Maximal rate of accumulation of both BW B759U-triphosphate and ACV-triphosphate after a short exposure to drug occurred in the late phase of the infective cycle, as the titer of extracellular virus reached a peak in untreated cultures, but after the decline of stimulated host deoxypyrimidine kinase activities. Once formed, the BW B759U-triphosphate pool decreased very slowly and thus it persisted for several days in both HCMV-infected and uninfected cells.**

The therapy of herpesvirus infections has recently advanced with the development of nucleoside analogs that are active only in the herpesvirus-infected cells. This selectivity appears to depend upon several factors: the level of nucleoside activation in the infected cell, the competing intracellular deoxynucleoside triphosphate pools, and the relative sensitivity of the target enzyme, viral DNA polymerase. With herpes simplex virus (HSV) or varicella zoster virus (VZV), specific phosphorylation of most of the antiviral nucleoside analogs is initiated by the virus-encoded deoxypyrimidine kinases. The anti-herpes nucleoside analog acyclovir (ACV) and its congener 9-{[2-hydroxy-1-(hydroxymethyl)ethoxy]-methyl}guanine (BW B759U) exhibit comparable antiviral activity against HSV and VZV *in vitro* (1–5). However, BW B759U is a more potent inhibitor of both human CMV and Epstein–Barr virus (EBV) *in vitro* than is ACV (1–3, 5). As is the case with ACV, BW B759U is activated to the triphosphate form in HSV- and VZV-infected cells and the initial step of this phosphorylation is associated with the activity of the virus-specified thymidine kinase (TK) (refs. 4, 6–8; unpublished data). TK-deficient mutants of HSV and VZV are resistant *in vitro* to both ACV and BW B759U (refs. 1–3; unpublished data).

Human cytomegalovirus (HCMV), on the other hand, reportedly does not induce such a novel TK activity in infected cells (9, 10), yet HCMV is susceptible to inhibition by at least two nucleoside analogs, 2′-fluoro-5-iodoarabinosylcytosine (FIaC) and BW B759U, whose anti-HSV activities depend on the expression of HSV TK (1–3, 11, 12). Recently, we reported that the triphosphate of BW B759U, like ACV-triphosphate, serves as a competitive inhibitor of the HCMV DNA polymerase, although the K_i values for BW B759U-triphosphate were consistently 5-fold higher than those measured for ACV-triphosphate (ref. 5; see Table 1). In the present study, we report that BW B759U, in contrast to ACV, is preferentially activated in human diploid fibroblasts infected with HCMV.

MATERIALS AND METHODS

Cells and Virus. Human foreskin fibroblast (HFF) cells were derived in this laboratory from tissues obtained from Duke University Hospital Pediatrics Department (Durham, NC) and were used up to passage 11. Human diploid embryonic lung fibroblast (MRC-5) cells were obtained from American Type Culture Collection and were used between passage 22 and passage 26. Monolayer cultures were grown in Eagle's minimal essential medium (ME medium) containing 50 units of penicillin per ml and 50 μg of streptomycin per ml supplemented with 10% fetal bovine serum (Sterile Systems, Logan, Utah) and 1% L-glutamine. HCMV strain AD169 was also obtained from American Type Culture Collection. Kerr strain of HCMV was obtained from E. S. Huang (Cancer Research Center, University of North Carolina, Chapel Hill). The clinical strain Wade was isolated from a congenitally infected infant by J. Zeller (Duke University Hospital Infectious Diseases Laboratory).

Virus stocks of AD169 were stored in liquid nitrogen and were amplified before use by low multiplicity of infection (moi) passage in MRC-5 cells [<0.05 plaque-forming units (pfu) per cell]. Supernatant virus was used at time of peak titer (days 8–11) and was back titrated at the time of use to determine approximate moi.

Cells and virus stocks were routinely checked for mycoplasma contamination by electron microscopy and [^{125}I]iodoxydeoxycytidine autoradiography methods.

Cell Cytotoxicity Assay. The cytotoxicity of the nucleoside analogs was determined by Naomi Cohn (The Wellcome Research Laboratories) by using a cell growth assay that allows

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Abbreviations: EBV, Epstein–Barr virus; HCMV, human cytomegalovirus; HSV, herpes simplex virus; VZV, varicella zoster virus; ACV, acyclovir or 9-[(2-hydroxyethoxy)methyl]guanine; FIaC, 2′-fluoro-5-iodoarabinosylcytosine; BW B759U, 9-{[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl}guanine, also abbreviated 2′ NDG, BIOLF-62, and DHPG by other investigators; HFF, human foreskin fibroblast; TK, thymidine kinase; pfu, plaque-forming unit(s); moi, multiplicity of infection.

two or three doublings of control cells. Cells were seeded at low densities and incubated with varying concentrations of drug incorporated into the medium. Cells were then detached by trypsin treatment (0.25%, GIBCO) and resuspended in medium. Cell numbers were determined with a model Z_b Coulter Counter.

Plaque Reduction Assay. Confluent monolayers of diploid fibroblasts in Falcon 12-well plates were infected with 50–100 pfu of HCMV. Unadsorbed virus was removed after $1\frac{1}{2}$ hr and the monolayers were overlaid with ME medium containing 0.6% SeaPlaque agarose (Marine Colloids, Rockland, ME), 2% fetal bovine serum, and the appropriate concentration of drug. Assay plates were incubated at 37°C in a humidified atmosphere containing 5% CO_2 for 8 days. Monolayers were then formalin fixed and stained with crystal violet, and plaques were counted at 30× magnification by using a Bausch and Lomb dissecting microscope. Data were analyzed with a computer probability analysis (version 79.3 of procedure Probit, Statistical Analysis Systems, Raleigh, NC).

DNA Polymerase Purification and Assay. The virus-specific DNA polymerase was partially purified from extracts of AD169-infected MRC-5 cells by sequential ion-exchange chromatography on DEAE-cellulose (Whatman DE52) and cellulose phosphate (Whatman P11) as described (5). Polymerase activity was measured by using the optimal synthetic template primer poly(dC)·oligo(dG) in the presence of 60 mM ammonium sulfate (5). Kinetic constants were determined by the use of the computer program of Cleland (13) and enzyme inhibition was analyzed according to the method of Spector and Hajian (14).

Enzyme Assays. Cytosol extracts of cells were prepared as described (15) and desalted before use. Reaction mixtures to measure phosphorylation of 2′-deoxycytidine (16) contained the following: 6 mM ATP, 6 mM $MgCl_2$, 0.2% (wt/vol) bovine serum albumin, 10 units of creatine kinase per ml, 6 mM creatine phosphate, 100 mM Tris·HCl (pH 7.5), 2 mM dithiothreitol, and 0.3 mM 2′-deoxy[2-^{14}C]cytidine (14 cpm/pmol, Moravek Biochemicals, City of Industry, CA). The reaction mixtures with thymidine (2′-deoxyribosylthymine) as phosphate acceptor were the same, except 2 mM ATP, 2 mM $MgCl_2$, 1.0% bovine serum albumin, and 0.1 mM [2-^{14}C]thymidine (53 cpm/pmol, ICN) were used. Identical sets of reaction mixtures for measuring the phosphorylation of BW B759U contained 2 mM [8-^{14}C]BW B759U (74 cpm/pmol) in place of deoxycytidine or thymidine. The deoxycytidine and thymidine reaction mixtures were spotted onto DEAE paper, washed, dried, and assayed for radioactivity (15). Reactions containing BW B759U were stopped by mixing 10-μl samples into 0.5 ml of 0.1 M HCl and analyzing with cation-exchange columns as described (17). All rates of phosphorylation were constant during the 60-min assay time. Protein concentrations were estimated by the Coomassie brilliant blue dye method (18).

ACV was chemically synthesized at The Wellcome Research Laboratories by published procedures (19) and was radiolabeled at the 8 position with ^{14}C. BW B759U (synthesized by procedures to be published elsewhere) was similarly radiolabeled with ^{14}C.

HPLC Analysis. Neutralized perchloric acid extracts of cells (20) were evaporated to dryness and redissolved in water. Both ribonucleotides and deoxyribonucleotides were separated by anion-exchange HPLC on a Whatman Partisil PXS10/25 SAX or Whatman Partisil 10 SAX column. Ribonucleotides were separated by elution with a linear gradient between 0.3 M and 0.8 M KH_2PO_4 (pH 3.5) developed over 30 min and at a flow rate of 1.0 ml/min.

Cell extracts were treated with sodium periodate to remove ribonucleotides, by the method of Garrett and Santi (21) modified by final acidification to pH 4–4.5 to prevent dGTP degradation. Deoxyribonucleoside 5′-triphosphates were separated with a linear gradient elution between 0.3 M and 0.8 M KH_2PO_4 at pH 3.5 or pH 3.78.

RESULTS

Antiviral and Cytotoxic Activity of BW B759U. As reported by other investigators (1–3, 5), BW B759U exerts stronger inhibitory activity on HCMV *in vitro* than does ACV (Table 1). The range of ED_{50} values reported to date for BW B759U has been similar for laboratory strains and clinical isolates (refs. 1–3; Table 1). The compound is highly effective at concentrations below the cytotoxic level for human diploid fibroblasts.

Inhibition of HCMV DNA Polymerase by the Triphosphates of BW B759U and ACV. The kinetics of inhibition of the partially purified HCMV DNA polymerase were studied (5). Analysis of Lineweaver–Burk plots of the data indicated competitive inhibition by ACV-triphosphate or BW B759U-triphosphate for the incorporation of the normal substrate, dGTP, catalyzed by this enzyme. The kinetic values for competitive inhibition of the HCMV-induced enzyme by BW B759U-triphosphate were ≈5-fold higher than the K_i values obtained for ACV-triphosphate. ACV-triphosphate is also a more effective inhibitor of the DNA polymerases of HSV (4, 23) and VZV (5) or cell DNA polymerase α (4, 23) than is BW B759U-triphosphate.

HPLC Analysis of BW B759U Phosphate Derivatives in HCMV-Infected and Uninfected Cells. The ability of HCMV infection to induce the intracellular formation of BW B759U-triphosphate was first examined in HFF cells. The HPLC analysis of perchloric acid extracts of the cells indicated that BW B759U-triphosphate levels increase in HCMV-infected cells with time (Table 2). Under these experimental conditions, appreciable levels of BW B759U-triphosphate were not apparent before 48 hr postinfection. The low levels of BW B759U-triphosphate initially measured in uninfected cells declined despite continued incubation of uninfected HFF cells in the presence of BW B759U.

In a similar experiment with ACV, the formation of ACV-triphosphate after HCMV infection was compared to that of BW B759U-triphosphate in MRC-5 cells. Cells were either mock-infected or infected with AD169, radiolabeled compounds were added at 26 hr postinfection, and samples were then harvested at the indicated times (Fig. 1). These results confirm that HCMV infection of human diploid fibroblasts results in the selective formation of BW B759U-triphosphate. The amounts of triphosphates that were detected depended upon the stage of infection and the length of exposure time of the cells to 50 μM [^{14}C]BW B759U. Furthermore, the levels of BW B759U-triphosphate measured in HCMV-infected cells in this experiment were almost 10-fold

Table 1. Inhibition of plaque formation and apparent kinetic constants

Virus strain or cell type	ID_{50}, μM*		K_i, μM†	
	BW B759U	ACV	BW B759U-TP	ACV-TP
AD169	1.7	108	1.4 ± 0.27	0.33 ± 0.05
Wade	0.8	54	ND	ND
MRC-5	350	3000	ND	ND
HeLa S-3‡	ND	ND	2.5 ± 0.7	0.37 ± 0.07

ND, not done; TP, triphosphate.
*Determined by plaque reduction in human diploid fibroblasts (5).
†DNA polymerase inhibition kinetic constants ± standard errors were determined by directly fitting the data to a hyperbola by the method of Wilkinson (22) by the use of the computer program of Cleland (13).
‡St. Clair *et al.* (4).

Table 2. Formation of BW B759U-triphosphate in HFF cells with and without HCMV infection

HCMV infection	Time, hr	[^{14}C]BW B759U-TP, pmol per 10^6 cells
Present	12	1.8
	24	3.4
	36	3.7
	48	26
	60	36
Absent	12	1.6
	24	7.3
	36	3.9
	48	4.3
	60	0.3

HFF cells were mock-infected or infected with HCMV at an moi of 0.1 pfu per cell. One and one-half hours after infection, 50 μM [^{14}C]BW B759U (53.5 mCi/mmol; 1 Ci = 37 GBq) was added and incubation was continued until the indicated times of cell harvest. Neutralized perchloric acid extracts were analyzed by HPLC. TP, triphosphate.

higher than those measured for ACV-triphosphate after HCMV infection and treatment with 100 μM [^{14}C]ACV.

The formation of BW B759U-triphosphate in HCMV-infected MRC-5 cells was measured as a function of drug concentration and time (Fig. 2). The MRC-5 cells were infected with strain AD169 at an moi of 0.5 pfu per cell, and infected cultures exhibited advanced cytopathic effect by day 5. The level of BW B759U-triphosphate increased in infected cells as a function of both concentration of drug and length of exposure time to the drug during this phase of the infection cycle.

Since the above experiments were performed with a laboratory strain of HCMV, it was of interest to confirm this preferential phosphorylation of BW B759U in cells infected with a low-passage clinical isolate. The Wade isolate, containing a mixture of cell-associated and cell-free virus, was used as the inoculum for MRC-5 cells. Radiolabeled BW B759U or ACV was added when 60% of the cell monolayer showed cytopathic effect. Again, a marked selectivity in the formation of BW B759U-triphosphate was observed in HCMV-infected cells (Fig. 3).

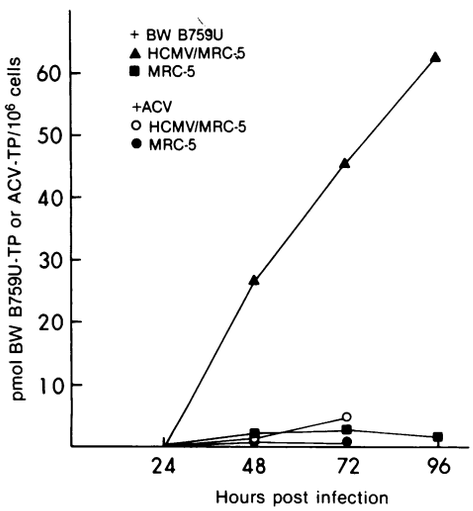

FIG. 1. Formation of BW B759U-triphosphate (BW B759U-TP) or ACV-triphosphate (ACV-TP) in cells infected with HCMV. Human diploid lung fibroblasts (MRC-5) were mock-infected or infected with HCMV strain AD169 at an moi of 0.1 pfu per cell. After 26 hr, 50 μM [^{14}C]BW B759U (53.5 mCi/mmol) or 100 μM [^{14}C]ACV (53.8 mCi/mmol) was added and samples were incubated until the indicated time of harvest. Cells were then washed, perchloric acid extracted, and analyzed by HPLC.

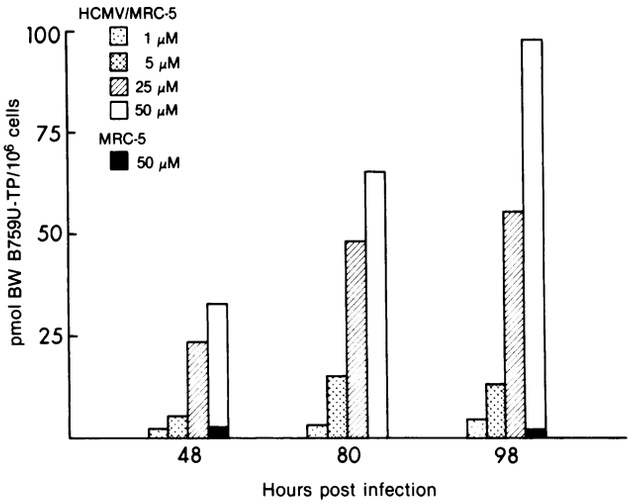

FIG. 2. Correlation of BW B759U-triphosphate (BW B759U-TP) levels with drug concentration and treatment time. MRC-5 cells were mock-infected or infected with HCMV strain AD169 at an moi of 0.5 pfu per cell. At 24 hr postinfection, [^{14}C]BW B759U was added to the cultures and incubation was continued until the indicated times of harvest. Cells were processed for HPLC analysis. HCMV-infected MRC-5 cells were treated with [^{14}C]BW B759U at a concentration of 1, 5, 25, or 50 μM. MRC-5 cells were incubated with 50 μM [^{14}C]BW B759U.

Relative Rate of BW B759U Phosphorylation in Uninfected and HCMV-Infected Cells. The relative rate of triphosphate formation was assessed throughout the virus replication cycle by pulse-labeling experiments, and this activity was related to several known parameters of the infectious cycle. These parameters included the generation of extracellular infectious virus as well as the induction of host deoxycytidine and thymidine phosphorylation activities. HCMV infection of cells has been shown to stimulate the levels of cytosol TK and deoxycytidine kinase (9, 10). In these experiments, infected cells were pulse-labeled with [^{14}C]BW B759U (50 μM) or [^{14}C]ACV (100 μM) for 24-hr intervals throughout the replication cycle. Uninfected and HCMV-infected MRC-5 cells maintained without drug treatment were harvested daily for measurement of phosphorylating activities. The amounts of BW B759U-triphosphate and ACV-triphosphate accumulated per 24 hr of treatment over the 7-day infection cycle in

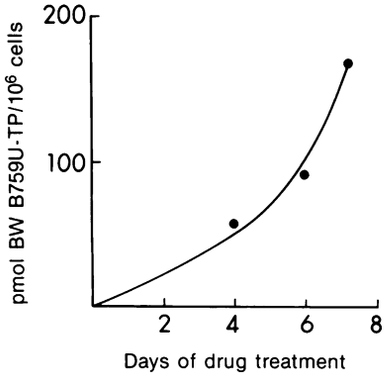

FIG. 3. Phosphorylation of BW B759U in cells infected with a clinical isolate of HCMV. MRC-5 cells were infected with both cell-associated and cell-free virus of the Wade clinical isolate. Radiolabeled BW B759U was added when infected cultures showed cytopathic involvement of 60% of the monolayers. Samples were harvested three times throughout the slow progression of the infection and were analyzed for HPLC. TP, triphosphate.

one such experiment are indicated in Fig. 4*a*. Maximal rate of accumulation of the triphosphate of both compounds occurred late in the infective cycle, at a time that coincided with the peak titer of extracellular virus production. When the levels of phosphorylating enzymes in control and infected cells were measured, a stimulation of both thymidine and deoxycytidine phosphorylating activities was apparent by 36–48 hr postinfection (Fig. 4*b*). These activities peaked on day 3 postinfection, well before the phosphorylation of BW B759U and ACV reached a maximum under the conditions of this experiment (Fig. 4*c*). A subsequent experiment of identical design but with an moi lower by a factor 5, confirmed the time differential between the induced host deoxycytidine kinase and TK activities and maximal rate of phosphorylation of BW B759U (data not shown). This difference is clearly demonstrated by a plot of the ratio of host TK activity to levels of BW B759U-triphosphate in the two separate experiments (Fig. 4*c*).

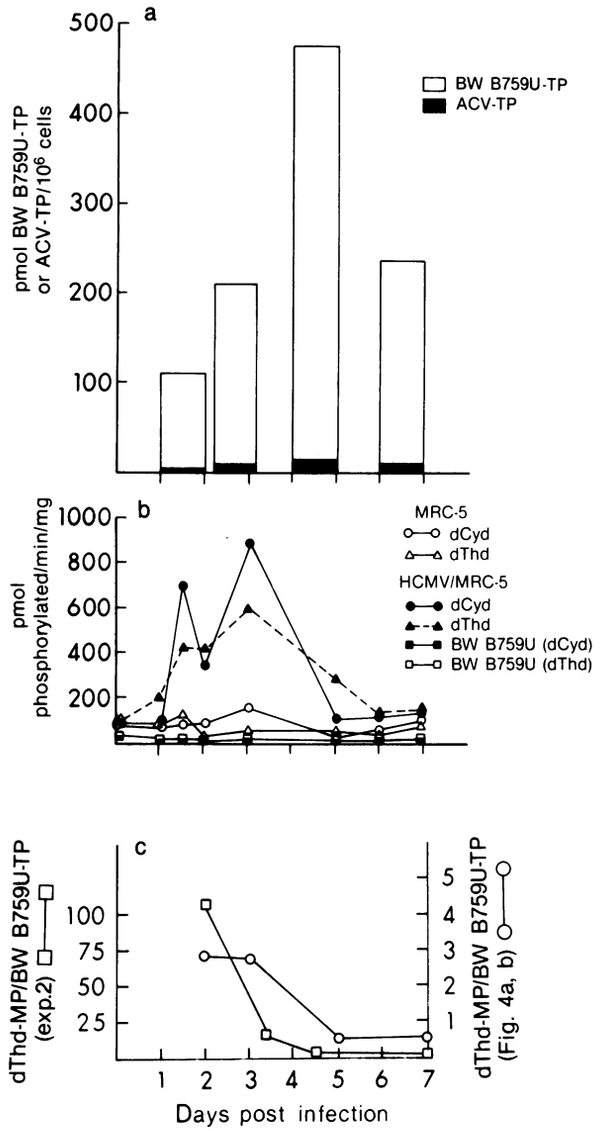

FIG. 4. (*a*) The relative rates of accumulation of BW B759U-triphosphate (BW B759U-TP) and ACV-triphosphate (ACV-TP) in HCMV-infected MRC-5 cells were measured throughout the replicative cycle. Cells were infected at an moi of 0.5 pfu per cell and then were treated for 24-hr periods with either 50 μM [^{14}C]BW B759U or 100 μM [^{14}C]ACV before harvesting for HPLC analysis. (*b*) Cytosol extracts of infected (▲ and ●) and uninfected MRC-5 (△ and ○) cells were assayed for TK (dThd) and deoxycytidine (dCyd) kinase activities throughout the infection cycle. Phosphorylation of BW B759U was also measured under conditions optimal for either host TK [□, BW B759U (dThd)] or deoxycytidine kinase [■, BW B759U (dCyd)]. The units of activity are expressed as pmol of product formed per min per mg of extracted protein. (*c*) The ratio of TK activity (measured as pmol of product formed per min per mg of protein extract) to levels of BW B759U-triphosphate as plotted using the data in *a* and *b* (○) or data from a similar experiment with an moi lower by a factor of 5 as described in the text (□).

Table 3. Intracellular stability of BW B759U-triphosphate

Time after drug removal, hr	Unlabeled BW B759U	[^{14}C]BW B759U-TP, pmol per 10^6 cells	% of initial [^{14}C]BW B759U-TP
0	−	209	100
1	−	274	131
	+	184	88
6	−	127	61
	+	133	64
24	−	78	37
	+	84	40

MRC-5 cells were infected with a mixture of cell-free and cell-associated HCMV strain AD169 and incubated until the infection was advanced as evidenced by cytopathic effect. Fifty micromolar [^{14}C]BW B759U was added for 24 hr. One sample was harvested for determination of BW B759U-triphosphate (BW B759U-TP) levels. The remaining samples were washed to remove residual radiolabeled drug, and one half of the samples were refed medium containing 50 μM unlabeled BW B759U. The other half received medium with no addition. At various times after drug removal, samples were harvested and BW B759U-triphosphate levels were measured by HPLC analysis.

Phosphorylation of BW B759U was also examined in these same extracts of uninfected and HCMV-infected MRC-5 cells. Under conditions optimal for assay of either host deoxycytidine kinase or TK, no significant differences were seen between infected and uninfected extracts, regardless of time after infection. The thymidine phosphorylating activity was partially purified from cytosol extracts of rapidly growing MRC-5 cells. As was true with purified TK from Vero cells (unpublished data), no phosphorylation of BW B759U was detected with this partially purified host cell TK (<3% of the rate of thymidine phosphorylation).

Persistence of the Triphosphate Form of BW B759U in HCMV-Infected Cells. The stability of BW B759U-triphosphate was examined by replacement studies in cultures of MRC-5 cells infected with AD169 (Table 3). Radiolabeled BW B759U was removed and replaced with fresh medium, with or without unlabeled drug. The radioactivity in the BW B759U-triphosphate was determined by HPLC. These data indicate that the intracellular half-life (or time to 50% of initial level of the radiolabeled BW B759U-triphosphate) was >6 hr, with ≈40% of the triphosphate levels still present by 24 hr after removal. A half-life of ≈12 hr was measured in a subsequent experiment in which 17% of the BW B759U-triphosphate persisted 54 hr after removal (data not shown). The addition of 50 μM unlabeled BW B759U to the replacement medium after radiolabeled drug removal did not significantly change the levels of the radioactive triphosphate recovered when compared with the samples in which no drug was added. Total levels of BW B759U-triphosphate, however, continued to rise. Thus, the triphosphate of BW B759U, once formed, seemed quite stable and appeared to be conserved under these experimental conditions.

DISCUSSION

BW B759U, a congener of ACV, is a more effective antiviral agent against HCMV *in vitro* than is ACV (Table 1; refs. 1–3). The molecular basis for this difference, however, has not yet been elucidated. We have ruled out differences in the

sensitivity of the HCMV DNA polymerase to competitive inhibition by the triphosphates of these two nucleoside analogs (ref. 5; Table 1). Data presented in this communication suggest that one major difference in the sensitivity of HCMV to BW B759U and ACV resides in the ability of the infected cell to preferentially generate high levels of the triphosphate of BW B759U. The triphosphate of BW B759U, once formed, appears quite stable and persists for days in the HCMV-infected cell. This is in contrast to the shorter half-life measured for ACV-triphosphate in the HSV-infected cell (24). The addition of an unlabeled extracellular pool of BW B759U to the cells had no significant effect on the slow rate of loss of [^{14}C]BW B759U-triphosphate from the cells. In the case in which the replacement medium contained unlabeled BW B759U, the triphosphate continued to accumulate, although the ^{14}C-labeled component decreased at a rate that was insensitive to the presence of an unlabeled pool of BW B759U.

The uninfected cell is also capable of producing low levels of BW B759U-triphosphate, but at low levels detectable only with radiolabeled drug. The triphosphate form, however, does not accumulate in the uninfected cell, and the transient increase detected after initial exposure of newly seeded cultures to the compound disappeared upon continued incubation. ACV-triphosphate levels, on the other hand, were not detectable in these uninfected cells even with radiolabeled compound.

The identification of the enzyme(s) responsible for the phosphorylation of BW B759U remains to be determined. The uninfected cell contains an activity (or activities) capable of phosphorylating BW B759U, although the efficiency is low. Infection of these cells with HCMV results in the appearance of elevated levels of BW B759U-triphosphate. The kinetics of induction of the BW B759U phosphorylating activity (or activities) are clearly quite different from those measured for the induction of host cytosol deoxycytidine kinase and TK activities under the experimental conditions described here (Fig. 4). The induced BW B759U phosphorylating activity reached a maximum in the untreated infected cells later in the infection cycle, after the induced host activities had declined and coincident with high titers of extracellular virus (Fig. 4). Furthermore, the amounts of BW B759U-triphosphate formed during a short drug pulse late in infection were higher than those measured when drug was added early after virus infection and maintained continuously until time of harvest late in the infection cycle. (Figs. 2 and 4). This may be a result of unrestricted viral DNA synthesis prior to drug treatment, causing amplification of the infection within either the individually infected cells or within the total cell population.

It has recently been demonstrated that BW B759U is a more potent inhibitor of HCMV DNA synthesis in the infected, drug-treated cell than is ACV (25, 26). The data presented here indicate that although BW B759U-triphosphate is somewhat less effective an inhibitor than is ACV-triphosphate for the HCMV DNA polymerase, this compound is more efficiently activated in the HCMV-infected cell to provide levels of BW B759U-triphosphate up to 30-fold higher than those measured for ACV-triphosphate. These experiments do not distinguish between the direct expression of a virus-coded phosphorylating activity or, alternatively, virus-dependent induction or modulation of a host BW B759U phosphorylating function. However, the ability of the HCMV-infected cell to specifically generate stable BW B759U-triphosphate provides an *in vitro* rationale for the superior antiviral activity of this nucleoside analog when compared to ACV.

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