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Influence of Acyclovir and Bucyclovir on Nucleotide Pools in Cells Infected with Herpes Simplex Virus Type 1

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The effects of the acyclic guanosine analogs acyclovir (ACV) and (*R*)-9-(3,4-dihydroxybutyl)guanine (bucyclovir, BCV) on the deoxyribonucleoside triphosphate (dNTP) pools of herpes simplex type 1 (HSV-1)-infected African green monkey kidney (GMK) and human embryonic lung fibroblast (HL) cells were investigated. HSV-1 infection increased the dNTP pools in both cell types compared with those in uninfected cells. Mock-infected GMK cells showed a 10-fold-higher dTTP concentration than comparable HL cells. ACV or BCV treatment of HSV-1-infected cells yielded further increases of the dNTP pools. ACV- or BCV-treated, HSV-1-infected HL cells showed 20- to 50-fold-higher concentrations of ACV triphosphate and BCV triphosphate, respectively, than similarly treated GMK cells. This is in accord with previous results, which showed that ACV and BCV are less active in GMK cells than in HL cells. This difference in activity is attributed to the substantial deoxythymidine pools previously found in GMK cells. The results are discussed in relation to known metabolic and kinetic parameters.

The antiviral nucleoside analogs 9-[(2-hydroxyethoxy)methyl]guanine (acyclovir, ACV) and (*R*)-9-(3,4-dihydroxybutyl)guanine (bucyclovir, BCV) are both selectively phosphorylated by herpes simplex virus (HSV)-coded deoxythymidine kinase and, after further cellular phosphorylation to triphosphate, selectively interfere with viral DNA synthesis (5, 17).

It has been shown that the activity of multiple antiviral substances is dependent on the host cell used (3, 7, 12, 13). Previous investigation has shown that ACV is 10 to 100 times more effective in human lung fibroblasts (HL) than in African green monkey kidney (GMK) cells. One reason for this is that GMK cells contain substantially higher levels of deoxythymidine than HL cells (10, 11). Intracellular deoxythymidine pools may decrease the phosphorylation of antiviral nucleoside analogs, which explains part of the decreased ACV and BCV activity to HSV-1 seen in GMK cells. The present investigation was performed to analyze the nucleoside triphosphate (NTP) levels in the two cell types during various states of infection and antiviral treatment.

MATERIALS AND METHODS

Cells. African green monkey kidney cell line (GMK) and a human fetal lung fibroblast cell line (HL) were grown in Eagle medium with 2% fetal calf serum and antibiotics (penicillin, 100 IU/ml; streptomycin, 100 μg/ml).

Viruses. F9004 is an HSV-1 laboratory strain propagated in our laboratory.

Drugs. ACV, ACV-triphosphate (ACV-TP), BCV, and BCV-triphosphate (BCV-TP) were kindly given to us by Alf Larsson, Astra läkemedel AB, Södertälje, Sweden.

Preparation of samples. GMK and HL cells grown to confluence in glass bottles were infected at a multiplicity of infection of 4 to 5. After adsorption for 1 h at 37°C, the virus-containing medium was removed, and medium containing ACV or BCV was added. The cells were harvested at different times in the presence of 6% trichloroacetic acid. The nucleotides were extracted by a Freon-amine method developed by Khym (14) with modifications by Chen et al. (1). The samples were lyophilized and dissolved in water to a 200-μl volume. Ribonucleotides were degraded by a periodate oxidation procedure developed by Garrett and Santi (9).

Chromatographic equipment. High-pressure liquid chromatographic analyses were performed on a chromatograph purchased from Waters Associates, Inc. (Milford, Mass.).

Chromatographic procedure. Deoxyribonucleoside triphosphates (dNTPs) were separated on a strong anion-exchange column (Partisil 10-SAX, 4.6 mm by 25 cm; Whatman, Clifton, N.J.), using an isocratic procedure. The mobile phase consisted of 0.4 M H_3PO_4 in water (pH 3.25) and 2.5% acetonitrile with a flow rate of 2 ml/min. The peak identification procedure included standard addition, absorbance ratio at two wavelengths (254 and 280 nm), and retention time. BCV-TP and dGTP peaks were not separated from each other.

Ribonucleoside triphosphates (rNTPs) were separated by using an ion-pair, reversed-phase, isocratic procedure developed in our laboratory. Radially compressed C-18 columns (Resolve, 10 μm, 8 mm by 10 cm; Waters Associates) were used. The mobile phase consisted of 10 mM tetra-*n*-butylammonium hydrogen sulfate, 25 mM K_2HPO_4, and 150 mM ammonium sulfate in water. The pH was adjusted to 7.50. The flow rate was 2.5 ml/min.

RESULTS

Influence of ACV and BCV on NTP pools in HSV-1-infected cells. The dNTP levels in mock- and HSV-1-infected HL and GMK cells were analyzed by high-pressure liquid chromatography after infection (Table 1). The dNTP pool levels peaked 8 to 12 h after infection. Noninfected GMK cells showed a 10-fold-higher intracellular concentration of dTTP than HL cells. After HSV-1 infection without antiviral substances present, the dNTP pools increased. Addition of 10 to 100 μM ACV or BCV to HSV-1-infected cells caused an increase in all four dNTP pool levels in both cell types. The increases appeared to be similar in the two cell types, except that the dTTP increase was more pronounced in HSV-1-infected, ACV-treated HL cells than in similarly

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TABLE 1. Concentration of dNTP 8 h after HSV-1 or mock infection of HL and GMK cells

Cells and treatment	dNTP concn (pmol/10^6 cells)			
	dCTP	dTTP	dATP	dGTP
HL cells				
Uninfected	<5	<5	<5	<5
Uninfected + 100 μM ACV	<5	10	<5	<5
Uninfected + 100 μM BCV	<5	<5	<5	—[a]
HSV-1 infected	12	84	13	16
HSV-1 infected + 10 μM ACV	65	652	94	43
HSV-1 infected + 100 μM ACV	76	602	98	55
HSV-1 infected + 10 μM BCV	15	310	66	—
HSV-1 infected + 100 μM BCV	39	543	205	—
GMK cells				
Uninfected	8	98	6	<5
Uninfected + 100 μM ACV	21	76	9	<5
Uninfected + 100 μM BCV	8	65	<5	—
HSV-1 infected	49	220	5	12
HSV-1 infected + 10 μM ACV	60	400	80	34
HSV-1 infected + 100 μM ACV	31	880	8	35
HSV-1 infected + 10 μM BCV	60	412	7	—
HSV-1 infected + 100 μM BCV	6	104	7	—

[a] —, The dGTP peak could not be separated from the BCV-TP peak.

treated GMK cells. The rNTP levels were analyzed by high-pressure liquid chromatography in uninfected and HSV-1-infected HL and GMK cells (Table 2). HSV-1 infection and ACV or BCV treatment yielded minor variations with respect to rNTP pool levels.

ACV-treated, HSV-1-infected HL cells exhibited 20-fold-higher levels of ACV-TP than comparable GMK cells (Table 3). No ACV-TP was detected in uninfected cells incubated with ACV.

BCV was phosphorylated to BCV-TP in large amounts in 10 μM BCV-treated, HSV-1-infected cells. No BCV-TP was detected in uninfected cells incubated with BCV.

dTTP levels at different times after infection. The dTTP pools were measured in HL and GMK cells by high-pressure liquid chromatography at different times after HSV-1 infection and ACV or BCV addition (Fig. 1). The dTTP pool levels increased after HSV-1 infection. Addition of ACV to HSV-1-infected cells accentuated the dTTP levels further.

Inhibition of HSV-1 by ACV and BCV in HL and GMK cells. The concentrations of ACV and BCV for a 50% inhibition of HSV-1 replication were determined. The 50% inhibiting concentration values were considerably lower for both ACV and BCV in HSV-1-infected HL cells compared with those in HSV-1-infected GMK cells (Table 3). This corresponds to the higher amounts of ACV-TP and BCV-TP in HSV-1-infected HL cells compared with HSV-1-infected GMK cells.

TABLE 2. Concentration of rNTPs 8 h after HSV-1 or mock infection of HL and GMK cells[a]

Cells and treatment	rNTP concn (nmol/10^6 cells)			
	CTP	UTP	GTP	ATP
HL	1.2	6.9	3.4	14
HL, HSV-1	3.4	11	4.2	22
HL, HSV-1, 100 μM BCV	2.5	10	2.5	9.1
HL, 100 μM BCV	2.4	3.5	1.4	5.8
GMK	1.0	3.9	0.8	5.3
GMK, HSV-1	1.6	6.4	1.7	8.7
GMK, HSV-1, 100 μM BCV	0.8	3.4	0.5	2.5
GMK, 100 μM BCV	1.0	4.0	1.0	7.4

[a] Experiments were performed in the presence and absence of 100 μM BCV as described in Materials and Methods.

DISCUSSION

The levels of naturally occurring dNTPs appeared to be similar in mock-infected GMK and HL cells with one exception: dTTP concentrations were 10-fold higher in GMK cells than in HL cells. This appears to be in concordance with the higher deoxythymidine pools found in GMK as compared with HL cells (10, 11). Infection with HSV-1 yielded increases of the dNTP pool levels of both cell types tested. This result is similar to those of earlier reports (8). Treatment of HSV-1-infected cells with ACV and BCV yielded further increases of dNTP pool levels. Similar results have been obtained for ACV by Furman and co-workers (8). Both cell types tested exhibited similar patterns in this respect. Our results are consistent with the studies of Cheng et al. (2), who analyzed dNTP pools of HSV-1-infected cells in the presence of dihydroxypropoxymethylguanine.

Higher concentrations of the active metabolites ACV-TP and BCV-TP were found in HSV-1-infected HL cells than in comparable GMK cells (Table 3). It was further shown that considerably more BCV-TP was accumulated in BCV-treated, HSV-1-infected cells than ACV-TP ACV-treated, HSV-1-infeced cells. The ACV-TP and BCV-TP concentrations in HSV-1-infected HL cells were of the same magnitude as found by others (6, 19). BCV is more readily phosphorylated by viral deoxythymidine kinase and also by cellular GMP kinase than ACV. BCV shows a 100-fold-lower K_i value for HSV-1 deoxythymidine kinase than ACV (1.5 μM compared with 173 μM; 15, 17). ACV is therefore more sensitive to competition from intracellular deoxythymidine than BCV (16). BCV-monophosphate also has a lower K_m value for hog brain GMP kinase than ACV-monophosphate (18, 19). These differences may account for the higher rate of formation of BCV-TP compared with that of ACV-TP. These kinetic parameters for formation and utilization of ACV-TP and BCV-TP may account for part or all of the observed difference in triphosphate pool levels of these antiviral compounds. BCV-TP exhibits lower HSV-1 polymerase-inhibiting activity than ACV-TP (K_i, 0.74 μM compared with 0.003 μM [4, 19]). This difference may explain part or all of the lower antiviral activity shown by BCV in cell culture.

The higher levels of the active metabolites ACV-TP and BCV-TP found in HSV-1-infected HL cells compared with those in HSV-1-infected GMK cells was paralleled by increased antiviral activity in this cell type. Careful estimation

TABLE 3. Concentrations of ACV and BCV giving a 50% inhibition of virus replication (IC_{50}) and concentrations of ACV-TP and BCV-TP

Cells	IC_{50} (μM)		pmol/10^6 cells			
			ACV-TP[a]		BCV-TP + dGTP[b]	
	ACV	BCV	10 μm	100 μM	10 μm	100 μM
HL	0.7	6.1	15	283	6,380	ND[c]
GMK	4.9	94	<10	11	196	ND

[a] HSV-1-infected cells (multiplicity of infection = 4), 10 or 100 μM ACV addition, at 15 h after infection.

[b] HSV-1-infected cells (multiplicity of infection = 4), 10 or 100 μM BCV addition, at 15 h after infection.

[c] ND, Not done.

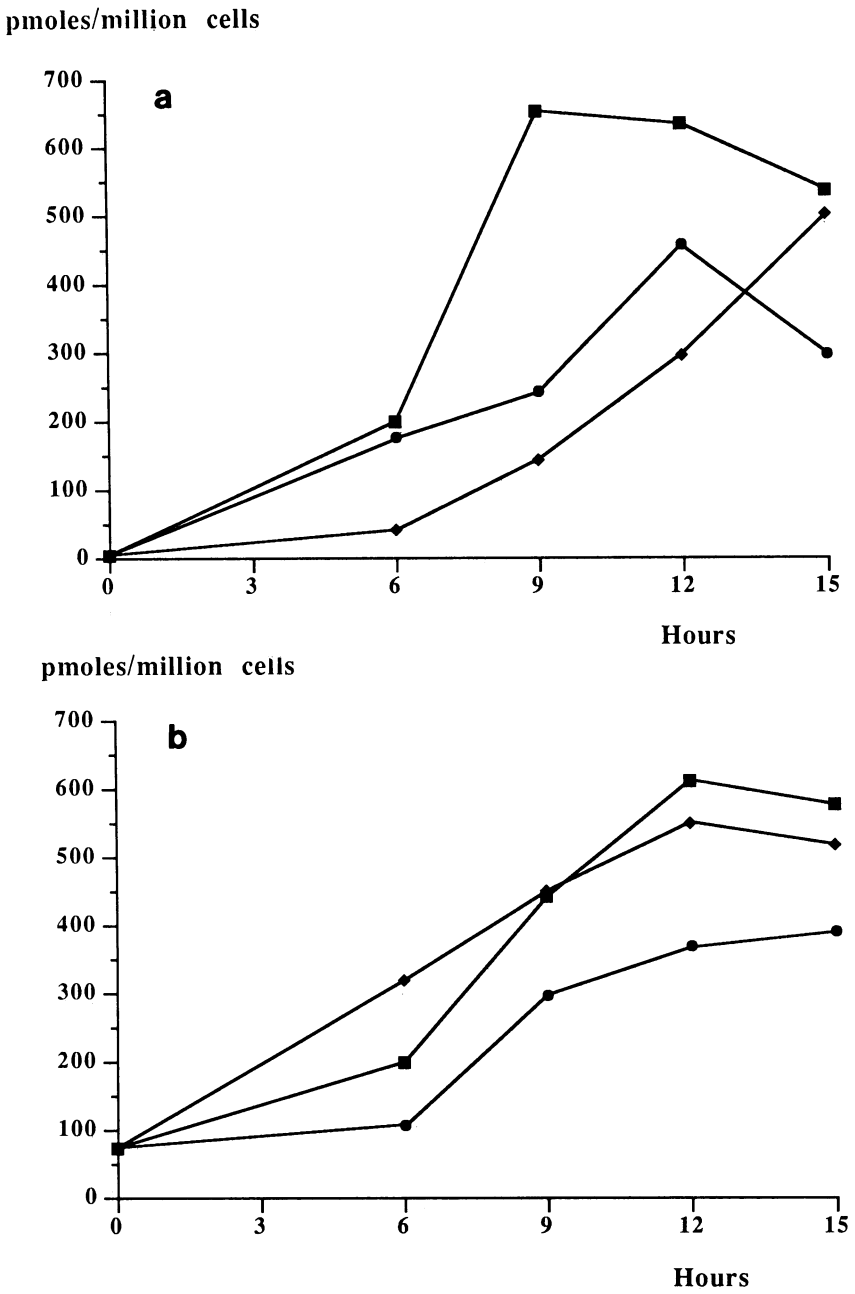

FIG. 1. Concentration of dTTP at different times after HSV-1 infection in HL (a) and GMK (b) cells. Symbols: ●, no BCV or ACV; ■, 10 μM ACV; ◆, 10 μM BCV. Experimental procedures were as described in Materials and Methods.

of cell culture pool sizes and enzyme kinetic parameters for different antiviral substances may be of value for the prediction of antiviral efficiency in different cell cultures. Knowledge of these parameters may be helpful when attempting to simulate the in vivo environment as well as when predicting the in vivo efficiency of new antiviral substances.

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