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Metabolism of Carbovir, a Potent Inhibitor of Human Immunodeficiency Virus Type 1, and Its Effects on Cellular Metabolism

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Carbovir (CBV) [the (−)-enantiomer of the carbocyclic analog of 2′,3′-dideoxy-2′,3′-didehydroguanosine] is a potent inhibitor of human immunodeficiency virus type 1 (HIV) replication in vitro. We have characterized the metabolism of CBV and its effect on cellular metabolism in an effort to better understand its mechanism of action. CBV was primarily metabolized to the 5′-triphosphate of CBV (CBV-TP) to concentrations sufficient to inhibit HIV reverse transcriptase. Infection of CEM cells with HIV did not affect the metabolism of CBV. In CEM cells, there was no evidence of the degradation of CBV by purine nucleoside phosphorylase. The half-life of CBV-TP in CEM cells was 2.5 h, similar to that of the 5′-triphosphate of zidovudine (AZT). However, unlike the levels of the 5′-triphosphate of AZT, CBV-TP levels declined without evidence of a plateau. CBV did not affect the metabolism of AZT, and AZT did not affect the metabolism of CBV. A small amount of CBV was incorporated into DNA in intact CEM cells, and this incorporation was increased by incubation with mycophenolic acid, an inhibitor of IMP dehydrogenase. CBV specifically inhibited the incorporation of nucleic acid precursors into DNA but had no effect on the incorporation of radiolabeled precursors into RNA or protein. CBV did not decrease the level of TTP, dGTP, dCTP, or dATP. These results suggested that the cytotoxicity of CBV was due to the inhibition of DNA synthesis. Further studies are necessary to identify the target(s) responsible for growth inhibition.

Carbovir (CBV) is a carbocyclic nucleoside analog with potent activity against human immunodeficiency virus type 1 (HIV) replication in cell cultures (5, 6, 18, 19). It is phosphorylated by the cellular 5′-nucleotidase (3, 4, 8) in cells to the 5′-monophosphate, which is then phosphorylated to the 5′-triphosphate by the cellular mono- and diphosphate kinases. Its anti-HIV activity is due to the inhibition of HIV reverse transcriptase by the 5′-triphosphate (12, 20). The 5′-triphosphate of CBV (CBV-TP) is a substrate for DNA synthesis by HIV reverse transcriptase with either a natural RNA template or a natural DNA template, and the incorporation of the 5′-monophosphate of CBV into the DNA chain by HIV reverse transcriptase results in chain termination (12). An important distinguishing characteristic of CBV-TP is its lack of activity against human DNA polymerases α, β, and γ (12, 20), which are likely targets for the development of toxicity for any nucleoside analog. In addition, combinations of CBV and zidovudine (AZT) are synergistic in their ability to inhibit HIV replication in cell cultures (13). CBV may be sufficiently different from existing anti-HIV nucleoside analogs in terms of toxicity (7, 9, 12) and chemical stability to warrant its evaluation as a potential anti-AIDS drug.

An understanding of the metabolism and effects on cellular processes of any drug is important to its rational use in the treatment of disease. This is particularly true for nucleoside analogs, because metabolism to phosphorylated anabolites is usually required for activity. In this work, we have characterized the metabolism of CBV and studied its effects on biochemical processes in an attempt to determine the mechanism of growth inhibition and the mechanism of the synergy that is observed with CBV and AZT.

(A preliminary report of this work has been presented [10].)

MATERIALS AND METHODS

Materials. The (−)-enantiomer of CBV was synthesized as previously described (18). dATP, dGTP, dCTP, TTP, phosphodiesterase I, and *Escherichia coli* DNA polymerase I were purchased from Pharmacia LKB Biotechnology (Piscataway, N.J.). [*methyl*-^{3}H]thymidine (7 Ci/mmol), [5-^{3}H]uridine (20 Ci/mmol), [5-^{3}H]cytidine (26 Ci/mmol), [8-^{3}H]CBV (5 Ci/mmol), [*methyl*-^{3}H]AZT, [8-^{14}C]adenine, [8-^{14}C]inosine, [8-^{3}H]deoxyguanosine, and [8-^{14}C]guanine were obtained from Moravek Radiochemicals (Brea, Calif.). [8-^{3}H]dATP (25 Ci/mmol), [*methyl*-^{3}H]TTP (30 Ci/mmol), [8-^{3}H]dGTP (16.9 Ci/mmol), [5-^{3}H]dCTP (26.4 Ci/mmol), and L-[4,5-^{3}H]leucine (69 Ci/mmol) were obtained from Amersham Corp. (Arlington Heights, Ill.). Mycophenolic acid, CsCl, DNase I, and alkaline phosphatase were obtained from Sigma Chemical Co. (St. Louis, Mo.). Proteinase K was obtained from Boehringer Mannheim Biochemicals (Indianapolis, Ind.).

Cell cultures and HIV infection. CEM cells, obtained from the American Type Culture Collection (Rockville, Md.), were grown in RPMI 1640 medium (Sigma) containing 10% fetal bovine serum (Hyclone, Logan, Utah) that had been heat inactivated at 56°C for 30 min, 100 μg of penicillin per ml, 100 U of streptomycin per ml, 20 μg of gentamicin per ml, and 25 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) buffer. Cells were routinely tested for the presence of mycoplasmas and were discarded at passage

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20; new cultures were established from cryopreserved, mycoplasma-free cell stocks. Cells were maintained in a humidified atmosphere of 5% CO_2 in air at 37°C. All experiments were conducted with cells that were proliferating at maximal rates. The HIV-infected cells used in these studies (see Table 1) were CEM cells infected with the HTLV-III_{RF} strain of the virus (Electronucleonics, Silver Spring, Md.) by suspension of 10^6 cells in 1 ml of cell-free virus stock (0.2 50% tissue culture infective dose) produced by acute infection of CEM cells. Following a 4-h incubation period, the cells were harvested by centrifugation and resuspended in 30 ml of fresh medium. The level of infection produced in this experiment caused substantial cytopathic effects and cell lysis by day 4 postinfection.

Biochemical assays. The effect of CBV on the incorporation of radiolabeled precursors into DNA, RNA, and protein was determined as described previously (2). The deoxyribonucleoside triphosphate pools were measured by the method of Solter and Handschumacher (14) with slight modifications (11). Purine nucleoside phosphorylase activity was measured in 100-μl volumes containing 50 mM potassium phosphate (pH 7.4), 10 μM labeled nucleoside, and 1 mg of protein from the CEM cell extract per ml. The CEM cell extract was prepared by resuspension of the cell pellet in 3 volumes of 0.01 M potassium phosphate (pH 7.4) and incubation on ice for 15 min. The pellet was homogenized, and the sample was centrifuged at 100,000 × *g* for 60 min. After incubation for the desired time at 25°C, the reaction was stopped by boiling. Purine base and nucleoside standards were added to each sample to a final concentration of 0.5 mg/ml, and the base was separated from the nucleoside by paper chromatography (Whatman 3M strips) with 46.9% butyl alcohol–22% propionic acid in water as the solvent. The base and nucleoside, identified by UV light, were cut out of the paper, and radioactivity was counted.

Measurement of acid-soluble metabolites of CBV and AZT. Cells incubated with [^{3}H]CBV were collected by centrifugation, and the pellet was mixed with 110 μl of ice-cold 0.5 M perchloric acid. The acid-insoluble material was removed by centrifugation at 12,000 × *g* for 20 min, and 100 μl of the supernatant fluid was removed and neutralized with 7.5 μl of 1 M potassium phosphate (pH 7.4)–12.5 μl of 4 M KOH. $KClO_4$ was removed by centrifugation at 12,000 × *g* for 20 min, and a portion of the supernatant fluid was injected onto a Partisil-10 SAX anion-exchange column (Keystone Scientific Inc., State College, Pa.). Elution of the nucleotides was accomplished with a 50-min linear gradient of 5 mM $NH_4H_2PO_4$ buffer (pH 2.8) to 750 mM $NH_4H_2PO_4$ buffer (pH 3.7) at a flow rate of 2 ml/min. One-minute fractions were collected, and radioactivity was counted.

Measurement of the incorporation of [^{3}H]CBV into DNA. CEM cells were incubated with 10 μM [^{3}H]CBV (1 Ci/mmol), with and without 5 μM mycophenolic acid, for 24 h. The cells were collected by centrifugation, and the cell pellet was incubated at 37°C overnight in 0.5 ml of 10 mM Tris (pH 8.0)–40 mM EDTA–0.5% sodium dodecyl sulfate–200 μg of proteinase K per ml. The samples were mixed with 10 ml of a CsCl solution such that the final concentration of the CsCl was exactly 1.58 g/ml. The samples were centrifuged at 66,000 × *g* and 20°C for 72 h. The gradients were fractionated, and a portion of each sample was precipitated onto glass fiber filters with a 5% trichloroacetic acid solution containing 10 mM PP_i. These filters were washed three times with this 5% trichloroacetic acid solution, washed twice with 95% ethanol, and dried, and radioactivity was counted. Fractions containing acid-precipitable counts were pooled, dialyzed against water to remove the CsCl, lyophilized, and resuspended in 50 μl of DNase I in 50 mM glycine (pH 9.0). After incubation for 2 h at 37°C, 50 μl of 100 U each of phosphodiesterase I and alkaline phosphatase per ml in 50 mM glycine (pH 9.0) was added to each sample, and the reaction was continued overnight at 37°C. The reaction was stopped by boiling for 2 min, the precipitated proteins were removed by centrifugation, and the samples were analyzed by reverse-phase high-pressure liquid chromatography (HPLC) for the appearance of radiolabeled nucleosides. Nucleosides were separated on a Spherisorb ODS1 (5-μm) column (Keystone Scientific) with a 20-min linear gradient of 50 mM ammonium dihydrogen phosphate buffer (pH 4.5) to 50 mM ammonium dihydrogen phosphate buffer (pH 4.5) containing 20% acetonitrile (vol/vol) at a flow rate of 1 ml/min. One-minute fractions were collected, and radioactivity was counted.

RESULTS

The 5′-monophosphate of CBV, the 5′-diphosphate of CBV, and CBV-TP were detected in CEM cells incubated with 1 μM [^{3}H]CBV for 4 h (Fig. 1). This concentration of CBV is sufficient to inhibit HIV growth by approximately 50% (19). The intracellular concentration of CBV-TP increased during the first 8 h of incubation with 1.0 μM [^{3}H]CBV. CBV-TP levels were lower after 24 h of incubation with 1.0 μM [^{3}H]CBV than after 8 h, even though the concentration of CBV in the medium did not change during the 24-h incubation. There was a linear relationship between the intracellular concentration of CBV-TP (from 0.005 to 3 pmol/10^6 cells) and the concentration of CBV in the medium (from 0.1 to 100 μM) (data not shown), a result indicating that metabolism was not saturated over this range of concentrations in CEM cells. Infection of CEM cells with HIV did not alter the metabolism of CBV (Table 1). In all of these experiments, the primary CBV nucleotide was the triphosphate, which represented 60% of all phosphorylated metabolites of CBV (Fig. 1).

In agreement with the work of Bondoc et al. (4), significant radioactivity was also observed in natural purine nucleotides, such as GDP and GTP, after treatment with [^{3}H]CBV (Fig. 1). As in the previous work (4), the [^{3}H]CBV was purchased from Moravek and found to be >99% CBV by HPLC. However, when we further purified the [^{3}H]CBV by reverse-phase HPLC, the incorporation of radioactivity from [^{3}H]CBV into the natural purine nucleotide pool was decreased by 95% (Fig. 1). This result indicated that the label in the natural purine nucleotide pool was due to a contaminant in the [^{3}H]CBV. In contrast, Bondoc et al. (4) observed that further purification of the [^{3}H]CBV did not affect the incorporation of radioactive label into GTP. Therefore, they concluded that [^{3}H]CBV was being degraded to [^{3}H]guanine by the cells, possibly by purine nucleoside phosphorylase. Because of this suggestion, we determined the ability of calf thymus purine nucleoside phosphorylase to degrade [^{3}H]CBV and found no detectable [^{3}H]guanine under conditions that totally converted inosine to hypoxanthine (data not shown). Furthermore, we tested the ability of crude extracts of CEM cells to degrade [^{3}H]CBV to [^{3}H]guanine under conditions that were optimal for purine nucleoside phosphorylase activity. No [^{3}H]guanine was liberated from [^{3}H]CBV after 20 h of incubation. Under identical reaction conditions, both inosine and deoxyguanosine were totally degraded to their respective bases within 30 min.

An important characteristic of any drug is the time that the

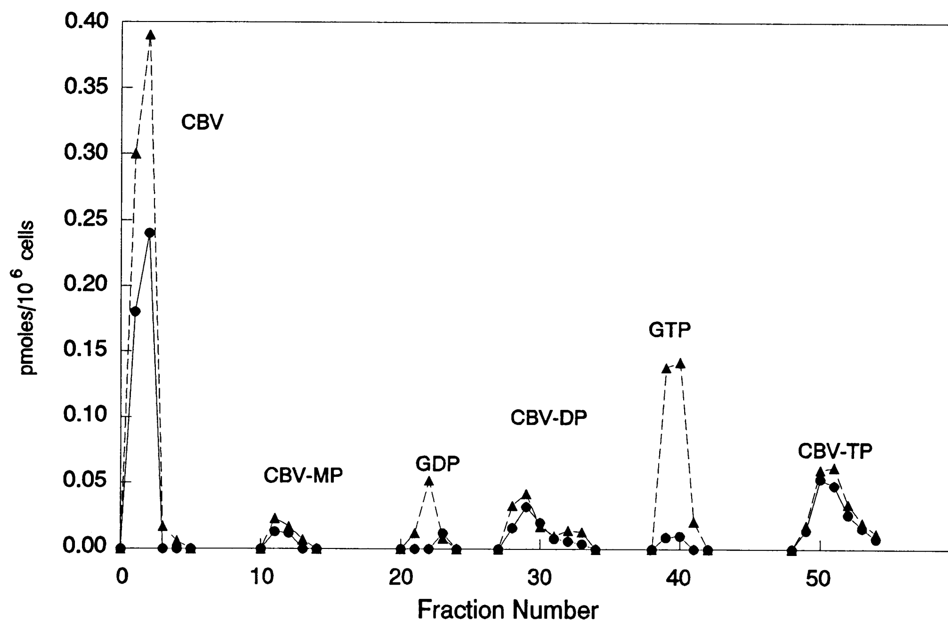

FIG. 1. Phosphorylation of CBV by CEM cells. CEM cells were incubated with either 1 μM [^{3}H]CBV (▲) or 1 μM [^{3}H]CBV that had been further purified by reverse-phase HPLC (●). After 4 h, the acid-soluble metabolites of CBV were separated by HPLC, and the radioactivity in each fraction was determined.

active form of the drug (in this case, CBV-TP) remains in the cell, because such information may suggest the necessary interval over which the drug must be given to maintain sufficient concentrations of the active metabolite for antiviral activity. Therefore, CEM cells were incubated with 1.0 μM [^{3}H]CBV plus 5 μM mycophenolic acid for 8 h, collected by centrifugation, washed, and resuspended in fresh medium containing no drugs, and the amount of CBV-TP was determined at various times (Fig. 2). The half-life for the disappearance of CBV-TP was approximately 2.5 h, similar to the initial half-life for the disappearance of the 5'-triphosphate of AZT (AZT-TP). However, unlike the levels of AZT-TP, the levels of CBV-TP declined in CEM cells without evidence of a plateau. Approximately 10% of the AZT-TP was still present 24 h after the removal of AZT, whereas no CBV-TP was detected 24 h after the removal of CBV. This difference could have been due to the accumulation of large amounts of the 5'-monophosphate of AZT, a continual source for the production of AZT-TP. Mycophenolic acid was included in the experiments with CBV because of the small amount of CBV-TP that accumulated in cells treated with only [^{3}H]CBV and the cost of [^{3}H]CBV required to obtain sufficient counts for this experiment. Mycophenolic acid inhibits IMP dehydrogenase, thereby resulting in increased intracellular IMP levels. IMP is the limiting substrate in the phosphorylation of CBV by 5'-nucleotidase (3). For determination of the effect of mycophenolic acid on the half-life of CBV-TP, a similar experiment was done with cells treated with only CBV (data not shown). In this experiment, the half-life of CBV-TP was 2.0 h, a result indicating that mycophenolic acid did not affect the rate of disappearance of CBV-TP.

TABLE 1. Effect of HIV infection on the metabolism of CBV[a]

h of infection	HIV	No. of cells (10^{6})	pmol of CBV-TP/10^{6} cells	% of control
24	−	0.52	0.041	
	+	0.52	0.045	111
48	−	0.75	0.028	
	+	0.72	0.038	138
72	−	1.35	0.060	
	+	1.10	0.022	38
96	−	3.80	0.038	
	+	0.65	0.032	86

[a] HIV-infected and noninfected CEM cells were incubated with 1 μM [^{3}H]CBV for 8 h at 24, 48, 72, or 96 h after infection with HIV. The acid-soluble metabolites of CBV were separated by HPLC, and the amount of radioactive CBV-TP was determined. The experiment was repeated once, with a similar outcome. In this experiment, the metabolism of CBV in HIV-infected cells at 72 h was 38% of that in control cells. In the repeat experiment, it was 132% of that in control cells. Therefore, we believe that the 72-h result in this experiment was due to an artifact and conclude that HIV infection does not affect CBV metabolism.

The effect of CBV on the incorporation of uridine into RNA, uridine into DNA (after conversion to deoxycytidine nucleotides), thymidine into DNA, and leucine into protein was determined to aid in the identification of the molecular target responsible for the growth inhibition caused by CBV. The concentration of CBV required to inhibit cell growth by 50% was 0.3 mM (data not shown). CBV at 1 mM inhibited the incorporation of [^{3}H]uridine into DNA by 60% but had little effect on the incorporation of [^{3}H]uridine into RNA, [^{3}H]thymidine into DNA, or [^{3}H]leucine into protein (Fig. 3). Treatment of CEM cells with 1 mM CBV also inhibited the incorporation of [^{3}H]cytidine, [^{14}C]guanine, and [^{14}C] adenine into DNA, each by approximately 70%, but did not inhibit their incorporation into RNA (data not shown). Incubation of CEM cells with 1 mM CBV for as much as 48

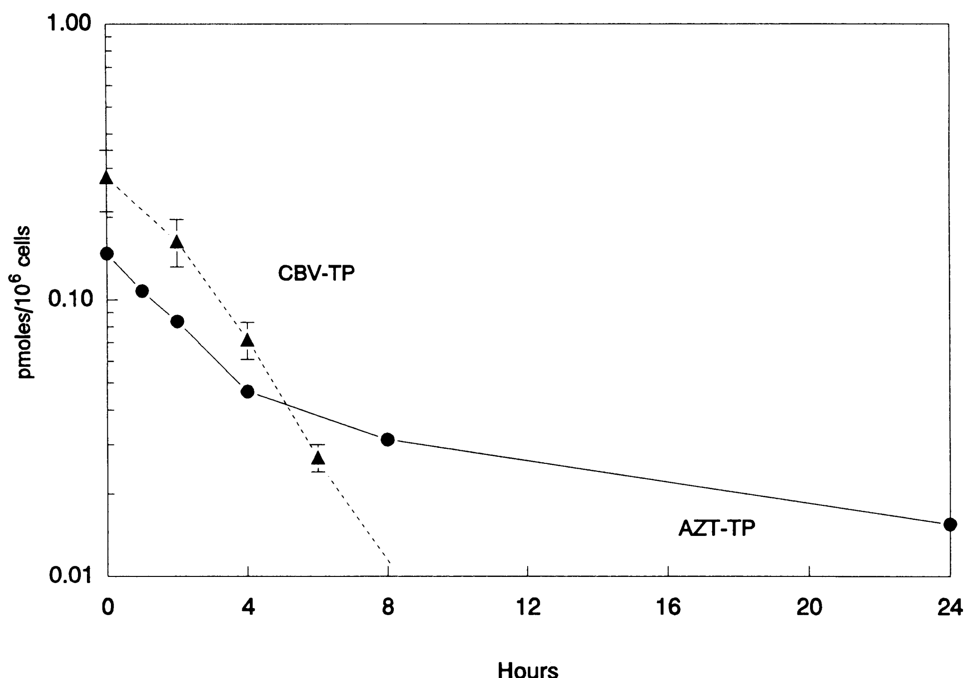

FIG. 2. Rate of disappearance of CBV-TP and AZT-TP from CEM cells. CEM cells were treated with either 5 μM mycophenolic acid plus 1 μM [^{3}H]CBV or 20 nM [^{3}H]AZT for 6 h. The cells were collected by centrifugation, washed free of drug, and resuspended in drug-free medium. At various times after resuspension in drug-free medium, an aliquot of the cell suspension was removed, the acid-soluble metabolites of CBV and AZT were separated by HPLC, and the amount of radioactive CBV-TP or AZT-TP was determined. Each point for CBV-TP is the mean ± the standard deviation for two experiments. Each point for AZT-TP is the value for one experiment only.

h did not result in reduced concentrations of any of the natural deoxynucleoside triphosphates (data not shown).

Very little CBV was detected in the DNA of CEM cells. Incubation with 10 μM [^{3}H]CBV for 24 h resulted in the incorporation of approximately 0.035 pmol of [^{3}H]CBV per 10^6 cells. In cells incubated with 10 μM [^{3}H]CBV plus 5 μM mycophenolic acid for 24 h, approximately 30-fold more [^{3}H]CBV was detected in the DNA (1.06 pmol/10^6 cells). This concentration of mycophenolic acid increased the metabolism of CBV to CBV-TP by approximately 20-fold. Mycophenolic acid (5 μM) decreased the GTP concentration in CEM cells to 30% of control levels, and the combination of 10 μM [^{3}H]CBV plus 5 μM mycophenolic acid decreased the growth of CEM cells by approximately 50%. CEM cell growth was not inhibited when cells were incubated with 10 μM [^{3}H]CBV alone.

In an attempt to explain the synergy seen with CBV and AZT against HIV replication in cell cultures, we measured the effect of both CBV and AZT on deoxynucleoside triphosphate pools. Decreases in the levels of natural substrates for DNA synthesis, if they occurred, could result in increased inhibition of HIV reverse transcriptase by CBV-TP or AZT-TP. At concentrations that are relevant to their antiviral activity, neither CBV nor AZT caused a reduction in the levels of any of the deoxynucleoside triphosphate pools (Table 2). In addition, incubation of CEM cells for 4 or 24 h with 1 μM CBV plus 1 μM AZT did not affect the levels of the natural deoxynucleoside triphosphate pools (data not shown). Therefore, these results indicated that the synergy seen with AZT and CBV was not due to a decrease in TTP pool levels by CBV or a decrease in dGTP pool levels by AZT.

The metabolism of AZT in CEM cells treated with CBV (1, 10, or 100 μM) plus AZT (20 nM) for 4 h was the same as that seen in cells treated with only AZT (20 nM), and the metabolism of CBV in CEM cells treated with AZT (0.01, 0.1, 1, or 10 μM) plus CBV (1 μM) for 4 h was the same as that seen in cells treated with only CBV (1 μM). Furthermore, preincubation of CEM cells with CBV (1, 10, or 100 μM) for 24 h did not affect the metabolism of AZT, and preincubation of CEM cells with AZT (0.01, 0.1, or 1 μM) did not affect the metabolism of CBV. Therefore, an effect on the metabolism of one drug by the other also cannot explain the synergy seen with this combination.

DISCUSSION

In this work, we have characterized the metabolism and biochemical actions of CBV in CEM cells. CBV was metabolized to CBV-TP to concentrations that are sufficient to explain its anti-HIV activity. As for other anti-HIV nucleoside analogs, the selectivity of CBV is due to selective inhibition of HIV DNA synthesis and not to selective phosphorylation in HIV-infected cells. Our studies also indicated that CBV was not a substrate for purine nucleoside phosphorylase. This conclusion is in conflict with the work of Bondoc et al. (4), who showed that CBV was degraded to guanine in CEM cells and suggested that CBV was degraded by purine nucleoside phosphorylase. Because our purification of [^{3}H]CBV did not remove all of the contaminant (as evidenced by a low level of incorporation into the GTP peak even with purified CBV), we believe that the contaminant must have eluted in reverse-phase HPLC close to CBV. This suggestion could explain the conflict between our results and the work of Bondoc et al. (4). It is possible that their

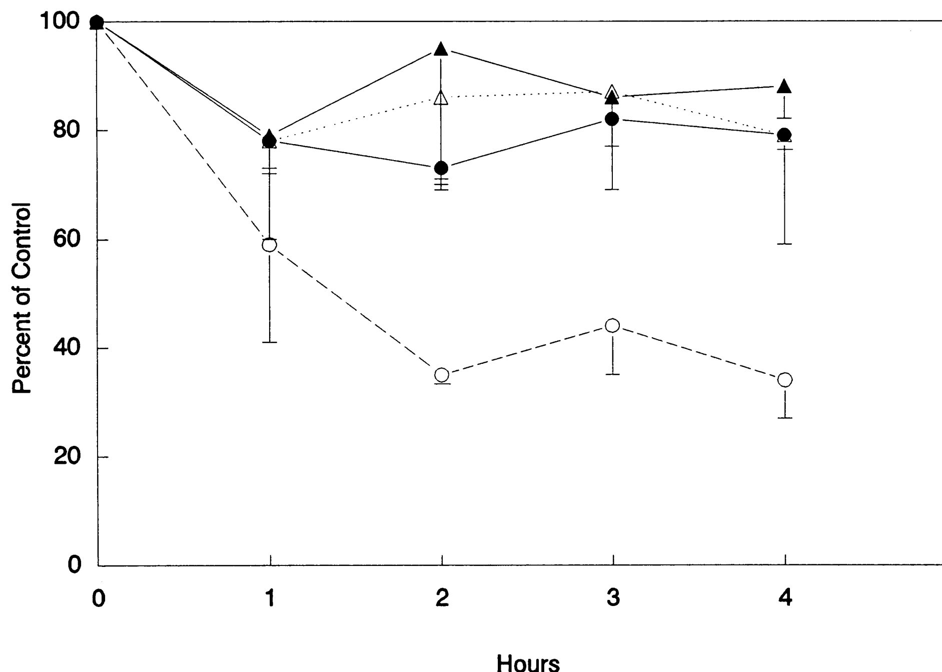

FIG. 3. Effect of CBV on macromolecular synthesis. CEM cells were incubated with and without 1 mM CBV for 4 h in the presence of [^{3}H]thymidine, [^{3}H]uridine, or [^{3}H]leucine. Cells were removed at 1, 2, 3, and 4 h, and the incorporation of [^{3}H]thymidine into DNA (●), [^{3}H]uridine into RNA (▲), [^{3}H]uridine into DNA as deoxycytidine (○), and [^{3}H]leucine into protein (△) was determined. Each point represents the mean ± the standard deviation for two separate experiments.

purification of CBV did not separate the contaminant from CBV.

At high concentrations, CBV was a selective inhibitor of DNA synthesis. It inhibited the incorporation of uridine, cytidine, adenine, and guanine into DNA but did not inhibit their incorporation into RNA. CBV did not inhibit the incorporation of thymidine into DNA. Initially, we concluded from this result that the inhibition of ribonucleotide reductase may be responsible for the cytotoxicity of CBV. However, CBV did not affect deoxynucleoside triphosphate pools at a concentration (1 mM) that resulted in significant inhibition of cell growth. The selective inhibition of DNA synthesis, the lack of effect on deoxynucleoside triphosphate pools, and the incorporation of CBV into DNA suggest that the inhibition of cellular DNA synthesis was primarily responsible for the ability of CBV to inhibit CEM cell growth. At the present time, we have no explanation for the inability of CBV to inhibit the incorporation of thymidine into DNA. Further studies are in progress to clarify this issue.

TABLE 2. Effects of CBV and AZT on deoxynucleoside triphosphate pools[a]

Analog	Concn (μM)	Incubation time (h)	Level (% of control) of:			
			TTP	dATP	dCTP	dGTP
CBV	1	4	124	112	99	110
		24	104	103	119	96
	10	4	109	106	108	115
		24	115	116	132	108
	100	4	111	106	97	104
		24	62	208	99	249
AZT	0.1	4	99	91	74	92
		24	96	94	119	90
	1	4	124	101	47	96
		24	107	115	143	106
	10	4	110	83	84	82
		24	162	134	163	146

[a] CEM cells were incubated with CBV or AZT for either 4 or 24 h. The cells were collected, and the deoxynucleoside triphosphate pool levels were determined. Each value is the average for at least two separate experiments, except for the dCTP levels after 24 h, which were for only one experiment. In these experiments, control cells contained 28 ± 9 pmol of TTP per 10^6 cells, 15 ± 4 pmol of dATP per 10^6 cells, 4.5 ± 2 pmol of dCTP per 10^6 cells, and 14 ± 5 pmol of dGTP per 10^6 cells.

Measurement of the incorporation of the anti-HIV nucleoside analogs into host cell DNA is important in the identification of their mechanisms of toxicity. Very little incorporation of CBV into the DNA of CEM cells treated with [^{3}H]CBV was detected. These results are consistent with our in vitro results for CBV-TP and DNA polymerases α, β, and γ (12, 20). However, combinations of CBV with mycophenolic acid, which optimized conditions for the incorporation of CBV into DNA, did result in significant incorporation of CBV into DNA. Mycophenolic acid potentiates the anti-HIV activity of CBV only at concentrations that also result in increased cytotoxicity (unpublished observation). These results indicate that drug combinations that increase CBV metabolism via the inhibition of IMP dehydrogenase also result in potentiation of the toxicity of CBV.

The DNA polymerase responsible for the incorporation of CBV into DNA in CEM cells has not yet been determined. However, in vitro studies of the interaction of CBV-TP with human DNA polymerases α, β, and γ (12) suggested that DNA polymerase α would be responsible for the majority of the incorporation of CBV into DNA in CEM cells. The ability of DNA polymerases δ and ε to utilize CBV-TP as a substrate has not yet been determined, but these poly-

merases may also contribute to the incorporation of CBV into DNA in CEM cells. The amount of CBV incorporated into DNA was smaller than or equal to the amount of either AZT or 2',3'-dideoxycytidine incorporated into DNA in other studies (1, 15–17). These results suggest that the level of inhibition of host cell DNA polymerases by CBV-TP may be lower than that observed with other anti-HIV nucleoside analogs. However, further experiments comparing the incorporation of these compounds into DNA at equally toxic doses in identical cell culture models are necessary to adequately compare these compounds.

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REFERENCES

1. **Avramis, V. I., W. Markson, R. L. Jackson, and E. Gomperts.** 1989. Biochemical pharmacology of zidovudine in human T-lymphoblastoid cells (CEM). AIDS 3:417–422.
2. **Bennett, L. L., Jr., D. Smithers, D. L. Hill, L. M. Rose, and J. A. Alexander.** 1978. Biochemical properties of the nucleoside of 3-amino-1,5-dihydro-5-methyl-1,4,5,6,8-pentaazaacenaphthylene (NSC-154020). Biochem. Pharmacol. **27**:233–241.
3. **Bondoc, L. L., Jr., G. S. Ahluwalia, N. R. Hartman, D. A. Cooney, H. Mitsuya, D. G. Johns, and A. Fridland.** 1991. Modulation of the antiretroviral activity and anabolism of purine 2',3'-dideoxynucleoside analogs by inhibitors of IMP dehydrogenase. Antiviral Res. **15**(Suppl. 1):80.
4. **Bondoc, L. L., Jr., W. M. Shannon, J. A. Secrist III, R. Vince, and A. Fridland.** 1990. Metabolism of the carbocyclic nucleoside analogue carbovir, an inhibitor of human immunodeficiency virus, in human lymphoid cells. Biochemistry **29**:9839–9843.
5. **Carter, S. G., J. A. Kessler, and C. D. Rankin.** 1990. Activities of (−)-carbovir and 3'-azido-3'-deoxythymidine against human immunodeficiency virus in vitro. Antimicrob. Agents Chemother. **34**:1297–1300.
6. **Coates, J. A. V., H. J. Inggall, B. A. Pearson, C. R. Penn, R. Storer, C. Williamson, and J. M. Cameron.** 1991. Carbovir: the (−) enantiomer is a potent and selective antiviral agent against human immunodeficiency virus in vitro. Antiviral Res. **15**:161–168.
7. **Du, D.-L., D. A. Volpe, C. K. Grieshaber, and M. J. Murphy, Jr.** 1992. In vitro toxicity of 3'-azido-3'-deoxythymidine, carbovir, and 2',3'-didehydro-2',3'-dideoxythymidine to human and murine haematopoietic progenitor cells. Br. J. Haematol. **80**:437–445.
8. **Johnson, M. A., and A. Fridland.** 1989. Phosphorylation of 2',3'-dideoxyinosine by cytosolic 5'-nucleotidase of human lymphoid cells. Mol. Pharmacol. **36**:291–295.
9. **Kurtzberg, J., and S. G. Carter.** 1990. Differential toxicity of carbovir and AZT to human bone marrow hematopoietic progenitor cells in vitro. Exp. Hematol. **18**:1094–1096.
10. **Parker, W. B., B. J. Bowdon, L. M. Rose, S. C. Shaddix, R. Vince, W. M. Shannon, and L. L. Bennett, Jr.** 1992. Metabolism of carbovir in CEM cells. Antiviral Res. **17**(Suppl. 1):68.
11. **Parker, W. B., S. C. Shaddix, C.-H. Chang, E. L. White, L. M. Rose, R. W. Brockman, A. T. Shortnacy, J. A. Montgomery, J. A. Secrist III, and L. L. Bennett, Jr.** 1991. Effects of 2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)adenine on K562 cellular metabolism and the inhibition of human ribonucleotide reductase and DNA polymerases by its 5'-triphosphate. Cancer Res. **51**:2386–2394.
12. **Parker, W. B., E. L. White, S. C. Shaddix, L. J. Ross, R. W. Buckheit, Jr., J. M. Germany, J. A. Secrist III, R. Vince, and W. M. Shannon.** 1991. Mechanism of inhibition of human immunodeficiency virus type 1 reverse transcriptase and human DNA polymerases α, β, and γ by the 5'-triphosphates of carbovir, 3'-azido-3'-deoxythymidine, 2',3'-dideoxyguanosine, and 3'-deoxythymidine. A novel RNA template for the evaluation of antiretroviral drugs. J. Biol. Chem. **266**:1754–1762.
13. **Shannon, W. M.** 1990. Antiretroviral activity of carbocyclic nucleoside analogs, p. 75–95. *In* R. B. Diasio and J.-P. Sommadossi (ed.), Advances in chemotherapy of AIDS. Pergamon Press, Inc., New York.
14. **Solter, A. W., and R. E. Handschumacher.** 1969. A rapid quantitative determination of deoxyribonucleoside triphosphates based on the enzymatic synthesis of DNA. Biochim. Biophys. Acta **174**:585–590.
15. **Sommadossi, J.-P., R. Carlisle, and Z. Zhou.** 1989. Cellular pharmacology of 3'-azido-3'-deoxythymidine with evidence of incorporation into DNA of human bone marrow cells. Mol. Pharmacol. **36**:9–14.
16. **Starnes, M. C., and Y.-C. Cheng.** 1987. Cellular metabolism of 2',3'-dideoxycytidine, a compound active against human immunodeficiency virus in vitro. J. Biol. Chem. **262**:988–991.
17. **Vasquez-Puada, M. A., M. C. Starnes, and Y. C. Cheng.** 1990. Incorporation of 3'-azido-3'-deoxythymidine into cellular DNA and its removal in a human leukemic cell line. Cancer Commun. **2**:55–62.
18. **Vince, R., and J. Brownell.** 1990. Resolution of racemic carbovir and selective inhibition of human immunodeficiency virus by the (−) enantiomer. Biochem. Biophys. Res. Commun. **168:** 912–916.
19. **Vince, R., M. Hua, J. Brownell, S. Daluge, F. Lee, W. M. Shannon, G. C. Lavelle, J. Qualls, O. S. Weislow, R. Kiser, P. G. Canonico, R. H. Schultz, V. L. Narayanan, J. G. Mayo, R. H. Shoemaker, and M. R. Boyd.** 1988. Potent and selective activity of a new carbocyclic nucleoside analog (carbovir: NSC 614846) against human immunodeficiency virus *in vitro*. Biochem. Biophys. Res. Commun. **156**:1046–1053.
20. **White, E. L., W. B. Parker, L. J. Macy, S. C. Shaddix, G. McCaleb, J. A. Secrist III, R. Vince, and W. M. Shannon.** 1989. Comparison of the effect of carbovir, AZT, and dideoxynucleoside triphosphates on the activity of human immunodeficiency virus reverse transcriptase and selected human polymerases. Biochem. Biophys. Res. Commun. **161**:393–398.