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Effect of β-Enantiomeric and Racemic Nucleoside Analogues on Mitochondrial Functions in HepG2 Cells

IMPLICATIONS FOR PREDICTING DRUG HEPATOTOXICITY

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ABSTRACT. A group of enantiomeric nucleoside analogues with β-D or β-L configuration, which represent potential candidates for the treatment of hepatitis B virus (HBV) infection, were incubated in human hepatoblastoma HepG2 cells at concentrations between 0.1 and 10 μM for 4–14 days. Then the effects on mitochondrial DNA (mtDNA) content, lactic acid production, lipid droplet formation, and mitochondrial morphology were evaluated. No effect on lactic acid production was detected in cells treated with β-L-2′,3′-dideoxy-3′-thiacytidine (3TC), β-L-2′,3′-dideoxy-5-fluoro-3′-thiacytidine (β-L-FTC), β-D-2′,3′-dideoxy-5-fluoro-3′-thiacytidine (β-D-FTC), racemic *cis* 2′,3′-dideoxy-5-fluoro-3′-thiacytidine [(±)-FTC], and 2,4-diamino-7-(2,3-dideoxy-2-fluoro-β-D-arabinofuranosyl)pyrrolo[2,3-*d*]pyrimidine (T70178), whereas a slight increase was associated with β-D-2-hydroxymethyl-5-(2,6-diaminopurin-9-yl)-1,3-dioxolane (β-D-DAPD) and 4-amino-7-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-thiocarboxamide (T70182) at 10 μM. A concentration-dependent increase in lactic acid production was observed in cells exposed to β-D-2′,3′-dideoxy-3′-thiacytidine [(+)-BCH-189], racemic *cis* 2′,3′-dideoxy-3′-thiacytidine [(±)-BCH-189], β-D-2′,3′-dideoxy-5-fluorocytidine (β-D-FddC), β-L-2′,3′-dideoxy-5-fluorocytidine (β-L-FddC), β-D-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3,-dioxolane (β-D-FDOC), 2,4-diamino-7-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)pyrrolo[2,3-*d*]pyrimidine (T70080), and 4-amino-7-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)pyrrolo[2,3-*d*]pyrimidine (T70179). Inhibition on mtDNA content was demonstrated to be concentration-dependent with (+)-BCH-189, β-D-FddC, and T70080, whereas 3TC, (±)-BCH-189, β-L-FTC, β-D-FTC, (±)-FTC, β-L-FddC, β-D-DAPD, T70178, T70179, and T70182 had no effect. β-D-FDOC resulted in a marked inhibition of mtDNA synthesis at 10 μM but not at lower concentrations. Cells treated with 3TC, (±)-BCH-189, β-L-FTC, β-D-FTC, (±)-FTC, β-L-FddC, β-D-DAPD, T70178, T70179, and T70182 did not show morphological changes compared with the control. In contrast, increased cytoplasmic lipid droplets associated with a loss of cristae in mitochondria were detected in cells treated with either β-D-FDOC, β-D-FddC, or T70080. (+)-BCH-189 treatment resulted in loss of cristae in mitochondria. In summary, 3TC, β-L-FTC, β-D-FTC, (±)-FTC, β-D-DAPD, T70178, and T70182 exhibited a relatively safe profile, supporting their further development. BIOCHEM PHARMACOL 52;10:1577–1584, 1996. *Copyright © 1996 Elsevier Science Inc.*

KEY WORDS. nucleoside analogues; hepatitis B; mitochondria; stereoisomerism; hepatotoxicity

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Nucleoside analogues have been the mainstay in the treatment of many viral infections over the past two decades. In recent years, major efforts have been expanded in the

search of effective anti-HIV*** and anti-HBV nucleoside compounds. Although some success has been achieved with this class of drugs, one major problem that can limit their clinical therapeutic use relates to their toxic side-effects. Since long-term therapy is needed to treat HIV and HBV infection, the potential development of toxicity is of paramount importance.

The underlying mechanism(s) for these toxicities is certainly multifactorial [1], and a delayed mitochondrial toxicity has been proposed to be partly responsible for nucleoside-related adverse effects [2]. Previous studies on ddC-induced peripheral neuropathy [3–5]. AZT-induced myopathy [6–8], and 3′-fluoro-3′-deoxythymidine induced hematotoxicity [9] indicated a preferential depletion of mtDNA content in drug-treated cells, which could subsequently damage mitochondrial functions in target organs. Our results on FIAU-induced liver toxicity demonstrated that both FIAU and its *in vivo* metabolite FMAU were incorporated into mtDNA of HepG2 cells and led to marked mitochondrial dysfunction, despite the lack of inhibition on mtDNA synthesis [10]. Similar results with FIAU were also obtained by other groups using several different cell lines [11]. These data suggested that different mechanisms may be involved in mitochondrial damage by certain nucleoside analogues. Furthermore, these studies emphasize the need to routinely evaluate, in pre-clinical studies, newly synthesized antiviral nucleoside compounds for their possible mitochondrial toxicity [12].

Recently, some novel nucleoside analogues with unnatural L-configuration such as 3TC [13–16], β-L-FTC [17–19], and β-L-FddC [20–22] were shown to exhibit potent anti-HBV or anti-HIV activity with minor or no toxicity, providing evidence of a high selectivity index characteristic of these antiviral nucleosides. In the present study, we evaluated the effects of various nucleoside analogues, which are potential candidates for treatment of HBV infection, on mitochondrial functions using a human hepatoblastoma HepG2 cell line.

*** *Abbreviations:* HIV, human immunodeficiency virus; HBV, hepatitis B virus; mtDNA, mitochondrial DNA; AZT, 3′-azido-3′-deoxythymidine; ddC, 2′,3′-dideoxycytidine; FIAU, 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodouracil; FMAU, 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)thymine; 3TC, β-L-2′,3′-dideoxy-3′-thiacytidine; (+)-BCH-189, β-D-2′,3′-dideoxy-3′-thiacytidine; (±)-BCH-189, racemic *cis* 2′,3′-dideoxy-3′-thiacytidine; β-L-FTC, β-L-2′,3′-dideoxy-5-fluoro-3′-thiacytidine; β-D-FTC, β-D-2′,3′-dideoxy-5-fluoro-3′-thiacytidine; (±)-FTC, racemic *cis* 2′,3′-dideoxy-5-fluoro-3′-thiacytidine; β-D-FDOC, β-D-2-hydroxymethyl-5-(5-fluorocytosin-1-yl)-1,3-dioxolane; β-L-FddC, β-L-2′,3′-dideoxy-5-fluorocytidine; β-D-FddC, β-D-2′,3′-dideoxy-5-fluorocytidine; β-D-DAPD, β-D-2-hydroxymethyl-5-(2,6-diaminopurin-9-yl-)1,3-dioxolane; T70080, 2,4-diamino-7-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)pyrrolo[2,3-*d*]pyrimidine; T70178, 2,4-diamino-7-(2,3-dideoxy-2-fluoro-β-D-arabinofuranosyl)pyrrolo[2,3-*d*]pyrimidine; T70179, 4-amino-7-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)pyrrolo[2,3-*d*]pyrimidine; T70182, 4-amino-7-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-thiocarboxamide; HSV, herpes simplex virus; HCMV, human cytomegalovirus; and SSC, 0.15 M sodium chloride + 0.015 M sodium citrate.

MATERIALS AND METHODS

Materials

The HepG2 cell line was obtained from the American Type Culture Collection (Rockville, MD). 3TC, (+)-BCH-189, (±)-BCH-189, β-L-FTC, β-D-FTC, (±)-FTC, β-D-FDOC, and β-D-DAPD were synthesized as published previously [23–25]. The stereoselective synthesis of β-L-FddC has also been reported [26]. β-D-FddC was supplied by Dr. Victor Marquez (National Institutes of Health, Bethesda, MD). T70080, T70178, T70179, and T70182 were synthesized as previously described [27, 28]. Minimum Essential Medium (MEM) with non-essential amino acids, sodium pyruvate, dialyzed fetal bovine serum and 10× trypsin-EDTA were purchased from GIBCO BRL (Grand Island, NY). A lactic acid assay kit was purchased from the Boehringer Mannheim Corp. (Mannheim, Germany). [α-^{32}P]dCTP (3000 Ci/mmol) and [α-^{32}P]dATP (3000 Ci/mmol) were purchased from ICN Biochemical Inc. (Costa Mesa, CA). QuikHyb hybridization solution was purchased from Stratagene (La Jolla, CA). All other chemicals and reagents were of the highest analytical grade available.

Cell Cultures

The HepG2 cells were grown in 75 cm^2 tissue culture flasks in MEM with non-essential amino acids supplemented with 10% heat-inactivated dialyzed fetal bovine serum, 1% sodium pyruvate, and 1% penicillin/streptomycin. The medium was changed every 3 days and cells were subcultured once a week.

Effects of Nucleoside Compounds on Cell Growth and Lactic Acid Production

HepG2 cells (2.5 × 10^4 cells/mL) were plated into 12-well culture clusters and treated with various nucleoside compounds at concentrations of 0.1 to 10 μM. After 4 days of incubation, the cell number in each well was determined with a hemocytometer, and lactic acid content in the medium was measured by using a Boehringer lactic acid assay kit, following the supplier's instructions.

Effects of Nucleoside Compounds on mtDNA Content

After 14 days of incubation, cells (5 × 10^4 per sample) incubated with different nucleoside analogues under various concentrations and no compound (control) were heated at 100° for 10 min in a 0.5-mL mixture of 0.4 M NaOH and 10 mM EDTA, and the DNA was immobilized on a Zeta-Probe membrane with a slot-blot apparatus, following the supplier's instructions (Bio-Rad, Richmond, CA). To detect the mtDNA on the membrane, an [α-^{32}P]dATP-labeled specific human oligonucleotide mitochondrial probe encompassing nucleotide positions 4212-4242 [29] was used in 2.5 × 10^6 dpm/mL. Prehybridization, hybridization, and washes were performed according to the manufacturer's instructions when using QuikHyb hybridiza-

tion solution. After autoradiography, the membrane was washed twice in boiled 0.1 × SSC + 0.1% SDS for 15 min to remove the mtDNA probe. The amount of total cellular DNA loaded on the membrane was standardized with a 625-bp fragment of a human β-actin cDNA plasmid probe, labeled with [α-^{32}P]dCTP (5 × 10^6 dpm/mL). Autoradiograms were scanned by using a CS9000U dual-wavelength flying-spot densitometer (Shimadzu Corp., Kyoto, Japan). The amount of mtDNA on blots was determined as a ratio of oligonucleotide probe radioactive signal to β-actin probe radioactive signal, which was independent of DNA load.

Morphological Evaluation

HepG2 cells (2.5 × 10^4 cells/mL) were plated into 35 × 10 mm cell culture dishes and a 10 μM concentration of tested compounds or no compound (control) was added to each dish. After a 7-day incubation period, cells were fixed in 1% glutaraldehyde for 1 hr, washed with sodium phosphate buffer, and post-fixed in 1% osmium tetroxide for 1 hr. The cells were gradually dehydrated with graded concentrations of ethanol starting with 50% through 100% to propylene oxide and slowly infiltrated and embedded with epon. The cells were then sectioned with a Reichert-Jung ultramicrotome, stained with uranyl acetate and lead citrate. Finally, cells were examined with an Hitachi 7000 electron microscope.

RESULTS AND DISCUSSION

The long-term clinical use of antiviral nucleoside analogues has been limited by toxic side-effects on different organs. The tragedy in the FIAU clinical trial seemed to even shadow the development of this class of compounds [30, 31]. However, some recent studies on a group of L-configuration nucleosides have demonstrated that high selectivity can also be achieved with some of these compounds [13–22], implicating a new era for further developing antiviral nucleoside agents. At the same time, recent reports have indicated that mitochondrial toxicity plays a major role in the nucleoside-related adverse effects [3–11], suggesting that newly developed nucleosides should be cautiously evaluated on their possible anti-mitochondrial activity before clinical investigations [2, 12].

To assess the potential toxicity of some anti-HBV nucleoside candidates with β-D or β-L configuration (Fig. 1), their effects on mitochondrial functions were examined in exponential growth phase HepG2 cells. After a 4-day incubation of HepG2 cells with various tested compounds at concentrations of 0.1 to 10 μM, no effect on lactic acid production was detected in cells treated with 3TC, β-L-FTC, β-D-FTC, (±)-FTC, and T70178, whereas a slight increase was associated with 10 μM β-D-DAPD and T70182 (Table 1). On the other hand, a concentration-dependent increase in lactic acid production was observed in cells exposed to (+)-BCH-189, (±)-BCH-189, β-L-FddC, β-D-FddC, β-D-FDOC, T70080, and T70179, among which (+)-BCH-189 and β-D-FddC showed the strongest influence (Table 1), although to a lesser extent as compared with our previous data obtained with FIAU and FMAU [10]. Of note, in our assay, there was an inversely proportional correlation between cell proliferation and lactic acid production. When cell growth was inhibited, the enhance-

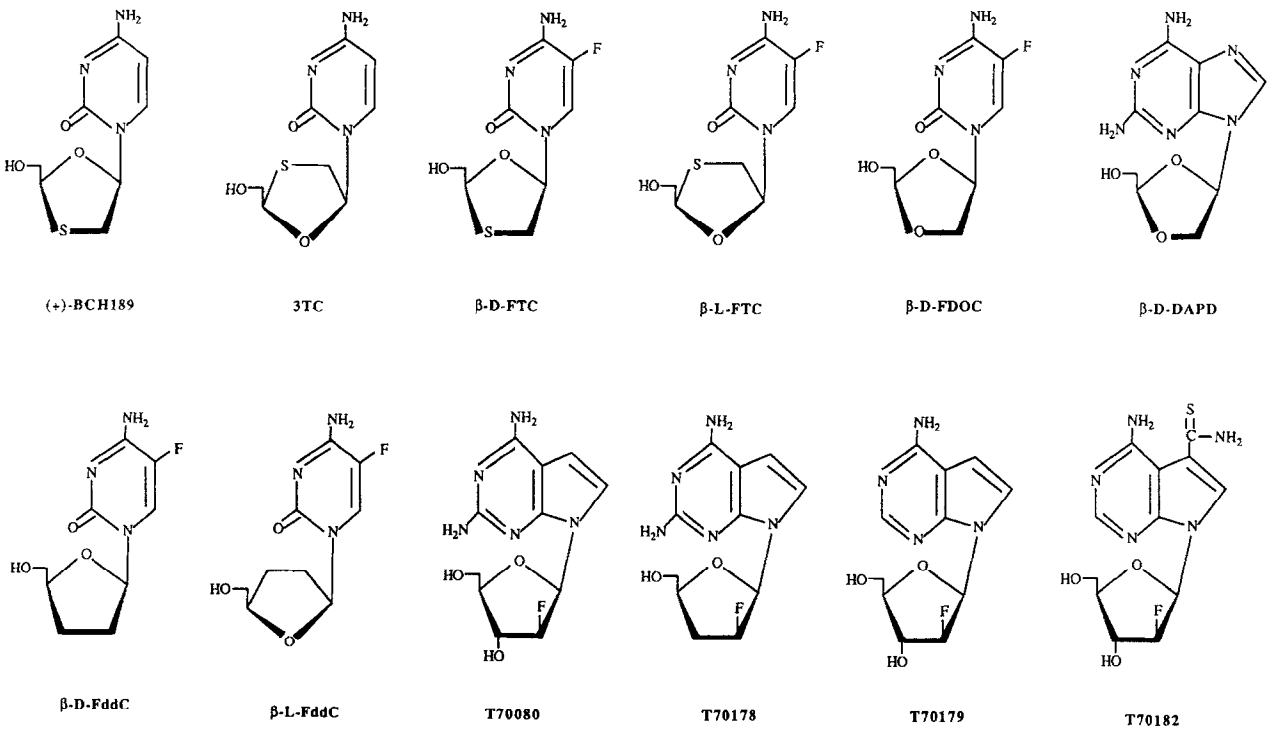

FIG. 1. Chemical structure of the nucleoside analogues studied.

TABLE 1. Effects of nucleoside compounds on cell growth and mitochondrial functions in HepG2 cells

Compound	Concentration (μM)	Cell density (10^4/mL)	Lactic acid formation (mg/10^6 cells)	P*	Ratio to control of mtDNA synthesis (%)
Control	0	16.0 ± 0.9	2.41 ± 0.15		100†
3TC	0.1	16.2 ± 1.1	2.39 ± 0.12 (0%)‡		107 ± 8
	1	15.8 ± 0.7	2.44 ± 0.18 (1%)		96 ± 9
	10	15.9 ± 0.6	2.35 ± 0.08 (0%)	>0.05	98 ± 10
(+)-BCH-189	0.1	15.2 ± 0.8	2.62 ± 0.07 (9%)		84 ± 8
	1	12.1 ± 0.5	3.08 ± 0.08 (28%)		59 ± 5
	10	6.8 ± 0.8	4.31 ± 0.12 (79%)	<0.01	7 ± 3
(±)-BCH-189	0.1	15.7 ± 1.2	2.47 ± 0.06 (3%)		98 ± 17
	1	13.5 ± 0.9	2.82 ± 0.13 (17%)		110 ± 15
	10	9.2 ± 0.3	3.58 ± 0.11 (49%)	<0.01	106 ± 15
β-L-FTC	0.1	16.4 ± 1.4	2.29 ± 0.13 (0%)		108 ± 9
	1	15.8 ± 0.9	2.33 ± 0.11 (0%)		113 ± 5
	10	15.9 ± 0.7	2.37 ± 0.10 (0%)	>0.05	102 ± 7
β-D-FTC	0.1	15.4 ± 0.7	2.31 ± 0.15 (0%)		95 ± 19
	1	15.7 ± 1.3	2.42 ± 0.01 (0%)		92 ± 6
	10	15.4 ± 0.5	2.53 ± 0.07 (5%)	>0.05	85 ± 17
(±)-FTC	0.1	16.1 ± 0.6	2.41 ± 0.11 (0%)		104 ± 31
	1	16.5 ± 1.0	2.46 ± 0.12 (2%)		100 ± 17
	10	15.6 ± 0.5	2.49 ± 0.05 (3%)	>0.05	99 ± 12
β-L-FddC	0.1	16.0 ± 0.4	2.49 ± 0.14 (3%)		101 ± 10
	1	12.5 ± 0.7	2.94 ± 0.68 (22%)		102 ± 16
	10	9.5 ± 1.2	3.69 ± 1.05 (53%)	>0.05	107 ± 8
β-D-FddC	0.1	14.7 ± 0.9	2.63 ± 0.21 (9%)		110 ± 9
	1	10.8 ± 1.1	3.24 ± 0.22 (34%)		81 ± 17
	10	7.0 ± 0.4	4.20 ± 0.27 (74%)	<0.01	39 ± 10
β-D-FDOC	0.1	15.4 ± 1.2	2.56 ± 0.08 (6%)		96 ± 10
	1	12.9 ± 0.5	2.91 ± 0.07 (21%)		87 ± 11
	10	8.6 ± 0.7	3.94 ± 0.09 (64%)	<0.01	14 ± 3
β-D-DAPD	0.1	16.2 ± 0.4	2.30 ± 0.12 (0%)		104 ± 10
	1	15.9 ± 0.8	2.27 ± 0.22 (0%)		100 ± 11
	10	13.2 ± 0.8	2.98 ± 0.14 (24%)	<0.01	92 ± 5
T70080	0.1	15.5 ± 0.6	2.53 ± 0.09 (5%)		100 ± 12
	1	12.2 ± 0.7	3.06 ± 0.16 (27%)		74 ± 7
	10	8.7 ± 0.5	3.74 ± 0.15 (55%)	<0.01	59 ± 2
T70178	0.1	16.4 ± 1.3	2.39 ± 0.06 (0%)		112 ± 24
	1	15.7 ± 0.7	2.45 ± 0.13 (2%)		113 ± 27
	10	16.1 ± 0.9	2.46 ± 0.23 (2%)	>0.05	108 ± 13
T70179	0.1	15.2 ± 0.4	2.55 ± 0.11 (6%)		94 ± 11
	1	11.7 ± 0.5	3.16 ± 0.06 (31%)		91 ± 17
	10	9.0 ± 0.5	3.83 ± 0.12 (59%)	<0.01	130 ± 22
T70182	0.1	15.6 ± 1.1	2.45 ± 0.04 (2%)		97 ± 18
	1	15.9 ± 0.9	2.53 ± 0.16 (5%)		121 ± 26
	10	14.1 ± 0.7	2.73 ± 0.06 (13%)	<0.05	85 ± 19

Values are means ± SD of 3 separate experiments.

* For the lactic acid production assay, a *t*-test was performed for all compounds studied at 10 μM as compared with control; the degree of freedom is 4.

† The control value of mtDNA/nuclear DNA was 1.58 ± 0.15.

‡ The number in parentheses is the percentage of increase in lactic acid production compared with control.

ment of lactic acid production was concomitantly observed. For mtDNA replication, cells treated for 14 days with 3TC, (±)-BCH-189, β-L-FTC, β-D-FTC, (±)-FTC, β-L-FddC, β-D-DAPD, T70178, T70179, and T70182 had no quantitative alteration on mtDNA content as compared with control (Table 1). In contrast, a concentration-dependent decrease on mtDNA content was observed in cells incubated with (+)-BCH-189, β-D-FddC, and T70080. (+)-BCH-189 at a concentration of 10 μM inhibited mtDNA content by as much as 95%. Additionally, 10 μM β-D-FDOC treatment led to a profound reduction on mtDNA synthesis while no substantial inhibition was noted at 0.1 and 1 μM (Table 1).

The increasing interest in the nucleosides with L-configuration results from the fact that a higher selectivity as relative to their D-configuration counterparts may be observed [32]. For instance, although L-thymidine is not recognized by human thymidine kinase, it can serve as substrate for HSV-1 thymidine kinase and has been claimed to have anti-HSV-1 activity [33]. Furthermore, nucleoside L-enantiomers such as 3TC [14–16], β-L-FTC [17–19], and β-L-FddC [20–22] exhibited greater therapeutic indices

TABLE 2. Electron microscopy evaluation on effects of nucleoside compounds on HepG2 cells

Compound	Mitochondrial morphology	Lipid droplet formation
3TC	No change	–
(+)-BCH-189	Loss of cristae	–
(±)-BCH-189	No change	–
β-L-FTC	No change	–
β-D-FTC	No change	–
(±)-FTC	No change	–
β-L-FddC	No change	–
β-D-FddC	Swollen, loss of cristae	+
β-D-FDOC	Loss of cristae	+
β-D-DAPD	No change	–
T70080	Distorted, loss of cristae	++
T70178	No change	–
T70179	No change	–
T70182	No change	–

Cells were incubated with a 10 μM concentration of the compounds studied and compared with control.

against either HIV or HBV *in vitro* than their respective D-enantiomers. In our evaluation on mitochondrial functions, this advantage of low host cell toxicity was confirmed for 3TC as well as β-L-FTC. Unlike 3TC, which showed no mitochondrial toxicity, (+)-BCH-189 led to an increase of lactic acid production and depletion of mtDNA content. (±)-BCH-189, which contains an equal quantity of 3TC and (+)-BCH-189, caused a less marked enhancement of lactic acid production that was probably attributed to the effect of its D-enantiomer. Previous studies using other cell lines have shown similar results with (±)-BCH-189, (+)-BCH-189, and 3TC in terms of their anti-mtDMA synthesis and anti-cell growth activities [18, 34]. In contrast to β-D-FddC, β-L-FddC did not affect mtDNA synthesis, even though both compounds caused an enhancement of lactic acid production. Moreover, 10 μM β-D-FDOC treatment resulted in a substantial depletion of mtDNA content and an increase in lactic acid production, whereas β-D-DAPD, which has a natural configuration, manifested a promising safety profile comparable to that of 3TC and β-L-FTC, with the absence of mitochondrial toxicity. In contrast to toxic (+)-BCH-189, β-D-FTC indicated no effects on mitochondrial functions in HepG2 cells. These data strongly suggest that no general rules are guiding the behavior of each of the nucleoside compounds, and each of them should be recognized and evaluated as a unique entity in regard to their antiviral activity as well as their potential host cell toxicity [1].

FIG. 2. Electron micrograph of control HepG2 cells after 7 days of incubation; magnification 12,000×.

T70080, T70178, T70179, and T70182 are a series of 7-deazaadenosine analogues that contain an arabinosyl fluoro group (Fig. 1). These compounds have been found to be active against HSV and HCMV, and their anti-HBV activity has been reported recently [35]. Early studies illustrated that 2′,3′-dideoxynucleosides such as ddC and AZT are phosphorylated by the cellular enzymes to form their 5′-triphosphate derivatives which can be used as substrates by DNA polymerase γ, an enzyme responsible for mtDNA synthesis. Due to the lack of a 3′-hydroxyl group, their incorporation into mtDNA may result in termination of chain elongation and possibly account for mtDNA depletion [2]. Our data on T70080 and T70178, however, suggested that the lack of a 3′-hydroxyl group on the sugar ring might be neither sufficient nor necessary to cause mtDNA reduction. Possibly, the phosphorylation profile of each nucleoside analogue in cells and the affinity of its 5′-triphosphate on DNA polymerase γ may be more crucial to determine its ultimate action on mtDNA synthesis. Of particular note, T70080, a potential anti-HBV compound [35] that is characterized by a sugar moiety similar to that of FIAU and FMAU, also had major toxic effects on mitochondrial functions, but most likely through different mechanisms as demonstrated by a different pattern of interactions on the various mitochondrial functions studied.

The increase in lactic acid production is usually considered to be a monitoring parameter for mitochondrial functions, because it is often initiated by mitochondrial oxidative phosphorylation disturbance [36]. Although a concentration-related correlation was suggested between increase in lactic acid production and extent of mtDNA inhibition with different nucleoside analogues [37], our results would indicate that this postulate was indeed applicable to (+)-BCH-189, β-D-FddC, β-D-FDOC, and T70080, but not in the case of (±)-BCH189, β-L-FddC, and T70179. On the

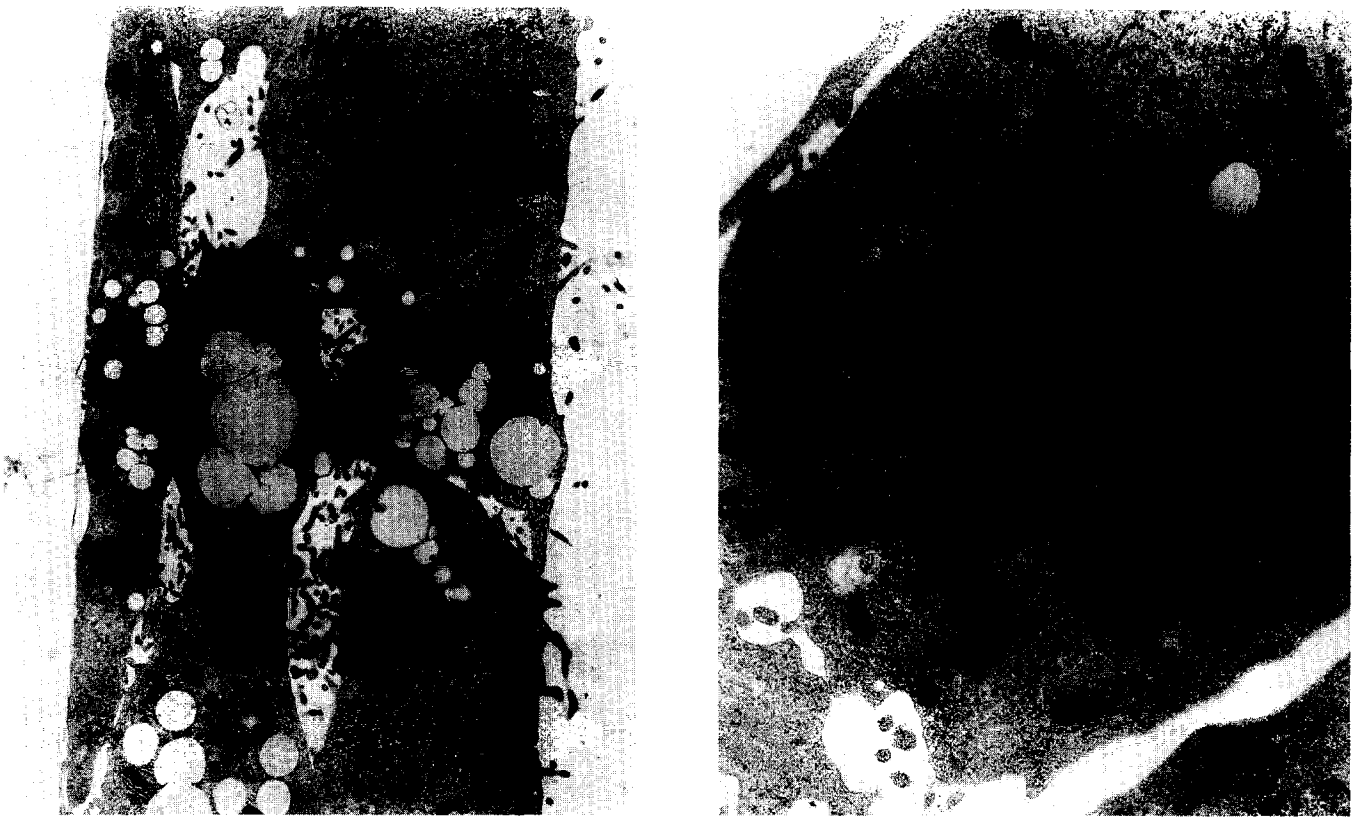

FIG. 3. Electron micrograph of HepG2 cells incubated over 7 days with 10 μM T70080; magnification 12,000× (left panel) and 30,000× (right panel).

other hand, in our experiments, the increase in lactic acid production was closely related to the inhibition of cell growth. It has been known that all the structure proteins and enzymes of mitochondria, except 13 genes of oxidative phosphorylation, are encoded by nuclear DNA [38]. Therefore, when nuclear DNA is affected by nucleoside analogues, mitochondrial function can also be damaged, leading to an increase in lactic acid production as a result of accelerated glycolysis. Overall, the increase of lactic acid production is specific to mitochondrial dysfunction, but it may not necessarily result from direct interaction of nucleoside analogues with mtDNA.

As shown in Table 2, electron microscope examination revealed no morphological changes in cells treated for 7 days with 3TC, (±)-BCH-189, β-L-FTC, β-D-FTC, (±)-FTC, β-L-FddC, β-D-DAPD, T70178, T70179, and T70182 when compared with control (Fig. 2). Increased formation of lipid droplets in the cytoplasm and swelling of the mitochondria with loss of cristae and matrix dissolution were detected in cells treated with β-D-FddC, β-D-FDOC (data not shown), and T70080 (Fig. 3). Loss of cristae in mitochondria was also observed with (+)-BCH-189 treatment (Fig. 4). These ultrastructural data have provided additional evidence that some nucleoside analogues can cause mitochondrial toxicity, although its detailed mechanism(s) needs to be clarified.

In conclusion, 3TC, β-L-FTC, β-D-FTC, (±)-FTC, β-D-DAPD, T70178, and T70182 have no effects on studied

FIG. 4. Electron micrograph of HepG2 cells incubated over 7 days with 10 μM (+)-BCH-189; magnification 30,000×.

mitochondrial functions in HepG2 cells, suggesting that these compounds may be highly selective anti-HBV agents without predictable hepatotoxicity. Studies using a woodchuck animal model would be needed to further confirm the observed *in vitro* selectivity of these nucleoside analogues. Nucleoside analogues can cause mitochondrial toxicity by interacting with either nuclear DNA or mtDNA; however, in those quiescent cells in which the turnover rate of mtDNA is much higher compared with that of nuclear DNA, mtDNA is possibly more vulnerable to being affected, resulting in mitochondrial dysfunction. As nucleoside analogues are still the mainstay in the treatment of various viral infections, a comprehensive understanding of the interactions of these antiviral compounds with mitochondrial functions should provide useful guidance in the discovery and selection of novel selective antiviral candidates.

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