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The Separated Enantiomers of 2′-Deoxy-3′-Thiacytidine (BCH 189) Both Inhibit Human Immunodeficiency Virus Replication In Vitro

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Racemic 2′-deoxy-3′-thiacytidine (BCH 189) is a dideoxycytidine analog having a sulfur atom in place of the 3′ carbon. The enantiomers of BCH 189 have been resolved and found to be equipotent in antiviral activity against human immunodeficiency virus types 1 and 2. However, the (−)-enantiomer (3TC) is considerably less cytotoxic than the (+)-enantiomer.

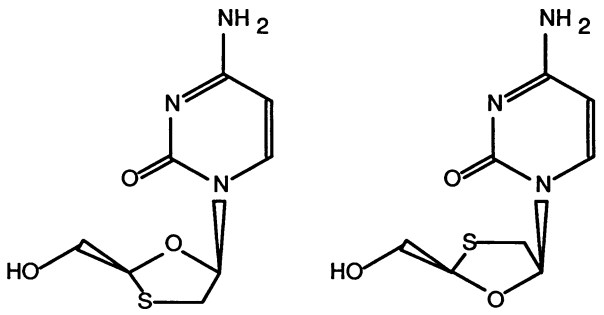
The etiological agent of AIDS is human immunodeficiency virus (HIV) (2, 5). A large number of agents have been reported to inhibit the replication of HIV. Among the most potent of these are 2′,3′-dideoxynucleoside analogs targeted against the viral reverse transcriptase. In cell cultures, the most active dideoxynucleosides are ddC and 3′-azido-3′-deoxythymidine (AZT), the latter being the only drug currently approved for the therapy of AIDS (8, 9). However, anti-AIDS chemotherapy with AZT is associated with significant side effects, in particular, bone marrow suppression and subsequent anaemia (12). Clinical trials with ddC have demonstrated a painful peripheral neuropathy in patients treated with this drug, limiting its use as a single therapeutic agent for the treatment of AIDS (7, 17). It is clear that drugs with antiviral activities equivalent or superior to those of AZT and ddC but with lower toxicities should be sought for the treatment of HIV-infected individuals.

2′-Deoxy-3′-thiacytidine (BCH 189) is a nucleoside analog in which the ribose is replaced by a 1,3-oxathiolane ring. The reported chemical synthesis of BCH 189 yields a racemic mixture of the (+)- and (−)-enantiomers, the absolute configurations of which have been determined by X-ray diffraction (Fig. 1) (15). The racemate of the β-anomer BCH 189, has previously been reported to be active against HIV type 1 (HIV-1) in vitro (3, 14).

Biological activities of nucleosides usually reside in a single enantiomer, as has been reported for the anti-HIV activity of carbocyclic-2′,3′-didehydro-2′,3′-dideoxyguanosine (carbovir [4, 16]), and enzymes are often used for the resolution of racemic nucleosides (see, for example, reference 13). To determine which of the enantiomers of BCH 189 has antiviral activity, we have separated the enantiomers of BCH 189 by chiral high-pressure liquid chromatography and determined the anti-HIV activity and cytotoxicity in vitro of both the (+) and (−) forms.

Separation of enantiomers was achieved on a Cyclobond I acetyl (acetylated β-cyclodextrin) column (250 by 4.6 mm). The mobile phase was prepared by titration of a 0.2% (vol/vol) solution of triethylamine in water to pH 7.2 by the addition of glacial acetic acid. Elution was effected at 1.0 ml/min and monitored at 270 nm. The (−)-enantiomer had a retention time of 6.3 min and was obtained 97.9% pure (Fig. 2A). The (+)-enantiomer had a retention time of 6.7 min and was obtained 95.7% pure (Fig. 2B).

The separated enantiomers were tested for anti-HIV activities in MT-4 cells infected with HIV essentially as described by Pauwels et al. (10, 11), and the activities were compared with those of AZT and ddI. Surprisingly, we found that the two compounds had very similar potencies against HIV-1 (Fig. 3). The concentrations resulting in 50% inhibition of virus cytopathic effects (IC_{50}s) were, on the basis of the experiment shown, 1.13 ± 0.22 μM for the (+)-enantiomer and 0.61 ± 0.31 μM for the (−)-enantiomer. These IC_{50}s are higher than the IC_{50} of AZT (0.11 ± 0.09 μM) and lower than that of ddI (8.47 ± 4.98 μM). That both enantiomers of BCH 189 have antiviral activity was confirmed in other assays with HIV-1 strain GB8 and HIV-2 strain ROD and in the cell lines JM and C8166. Virus replication was measured by syncytium formation and the production of p24 antigen (Table 1). Cells were infected at a multiplicity of infection of 0.001 infectious units of HIV per



(+) enantiomer (-) enantiomer (3TC)

FIG. 1. Chemical structure of BCH 189.

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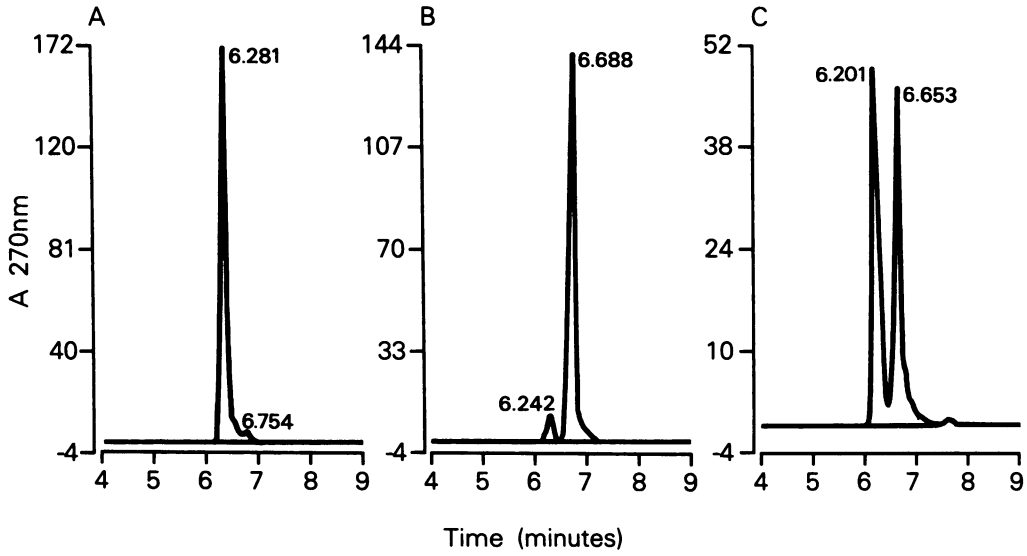

FIG. 2. Resolution of (−)-enantiomer (A), (+)-enantiomer (B), and racemate (C) by high-pressure liquid chromatography.

cell, and virus was adsorbed for 1 h at room temperature. The cells were washed free of unadsorbed virus, and aliquots of cells were placed in 24-well plates containing serial dilutions of test compounds. After incubation at 37°C for 4 to 5 days in 5% CO_2–air, the cells were examined for HIV-induced syncytia, and these were counted. By reference to untreated infected controls, the IC_{50}s were calculated. For determination of p24 antigen levels, cells were infected in the same manner as in the syncytium formation assays and incubated for 4 days at 37°C. Supernatant fluid from each well was decanted, cleared by centrifugation, and assayed for p24 antigen with an HIV-1 p24 enzyme-linked immunosorbent assay kit from Abbott Laboratories, (North Chicago, Ill.). The procedures described by the manufacturer were followed exactly. In all experiments, both enantiomers had similar IC_{50}s, ranging from 2 nM to 1.2 μM (Table 1).

Although the differences seen in anti-HIV activity between the enantiomers was statistically significant, the difference was lower than a factor of 2. A much larger difference was seen in the toxicity of these compounds for human lymphocytic cell lines in cultures. Aliquots (50 μl) of each compound were dispensed into flat-bottomed 96-well microtiter plates, to which 150 μl of a cell suspension (2.4×10^5 cells per ml) in RPMI 1640 growth medium was added. The

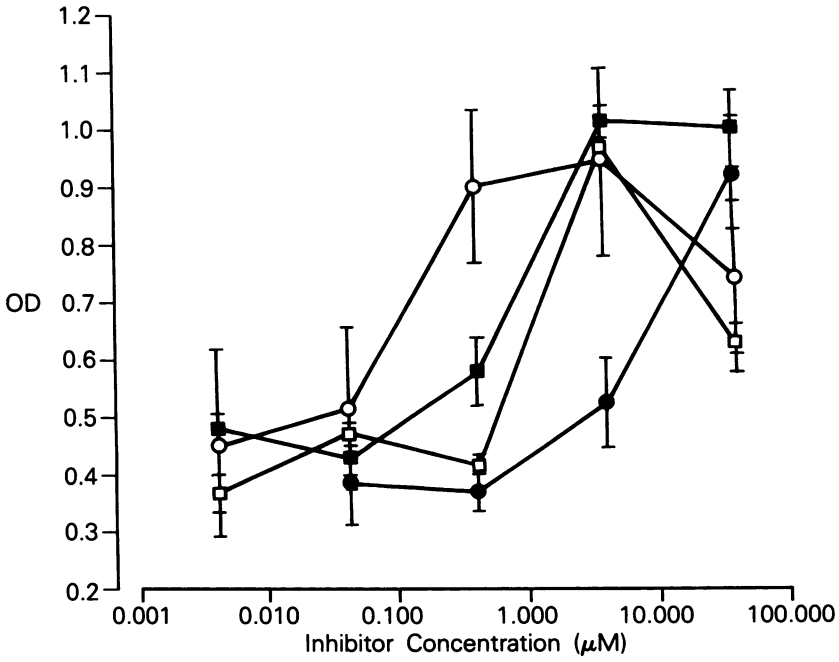

FIG. 3. Inhibition of cytopathic effects of HIV-1 strain RF-infected MT-4 cells by (−)-enantiomer (■), (+)-enantiomer (□), AZT (○), and ddI (●). Error bars represent standard errors of means. OD, optical density.

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TABLE 1. Effect of enantiomers on the replication of HIV in a range of T-lymphocyte cell lines

Enantiomer	Antiviral activity[a] in the following assay with the indicated cells (virus):								
	Formazan, MT-4 (HIV-1 RF)[b]	Inhibition of syncytium formation[c]					Inhibition of HIV-1 p24 synthesis[c]		
		CEM (HIV-2 ROD)	CEM (HIV-1 RF)	CEM (HIV-1 U455)	JM (HIV-1 GB8)	C8166 (HIV-1 RF)	C8166 (RF)	JM (GB8)	MT-4 (RF)
(+)	1.22 ± 0.024	0.19	0.3	0.03	0.13	0.22	0.09	0.14	0.004
(−)	0.87 ± 0.33	0.24	0.18	0.04	0.22	0.04	0.07	0.07	0.002

[a] Reported as IC_{50} (micromolar).
[b] The results were derived from four independent antiviral assays performed in duplicate and are reported as mean ± standard error.
[c] The results are the means of two determinations.

plates were incubated at 37°C in 5% CO_2–air for 5 days. Following incubation, the cells were thoroughly resuspended, 50-μl samples were removed and mixed with an equal volume of trypan blue, and viable cell counts were determined with a hemacytometer. By reference to untreated control cultures, the doses resulting in 50% of the cell numbers found in the control (ID_{50}s) were calculated. In CEM cells, the ID_{50} of the (+)-enantiomer was 3.84 ± 0.26 μM, whereas that of the (−)-enantiomer was >363 ± 102 μM (determined from three independent experiments, one of which is shown in Fig. 4). The cytotoxicity seen with the racemate (ID_{50}, 10.73 ± 0.22 μM) could be reproduced by mixing the enantiomers in a 1:1 ratio (Fig. 4). A similar differential cytotoxicity was also seen in H9, JM, U937, and C8166 cells. In all cases, the (−)-enantiomer was 20- to 100-fold less toxic than the (+)-enantiomer (data not shown).

Our observation that the enantiomers of BCH 189 are both potent inhibitors of HIV-1 and HIV-2 was unexpected, since the antiviral activity of nucleosides is usually found associated with only one enantiomer. Balzarini et al. (1) recently reported that both enantiomers of carbocyclic 5-(2-bromovinyl)-2′-deoxyuridine and carbocyclic 5-iodo-2′-deoxyuridine are inhibitors of herpes simplex virus types 1 and 2, but in these cases the (−)-enantiomers were 10- to 14-fold less active than the (+)-enantiomers. A direct comparison between these two studies is not feasible, since no quantitative data on the purity of the enantiomers of either carbocyclic 5-(2-bromovinyl)-2′-deoxyuridine or carbocyclic 5-iodo-2′-deoxyuridine were provided. The antiviral activities of the (+)- and (−)-enantiomers in our study cannot, however, be explained by an incomplete separation of the enantiomers, since the purity of each was >95%, but the antiviral activities differed by less than twofold. Mansuri et al. (6) also reported anti-HIV activity with the unnatural L-β-enantiomer of ddC, but the activity was ~15-fold lower than that of the natural D-β-enantiomer.

The two enantiomers of BCH 189 were not, however, biologically equivalent in all respects, since the (−)-enantiomer was substantially less toxic for cultured human lymphocytes than the (+)-enantiomer. Work is now under way to determine the mode of action and mechanism(s) of toxicity of both the (+)- and (−)-enantiomers of BCH 189.

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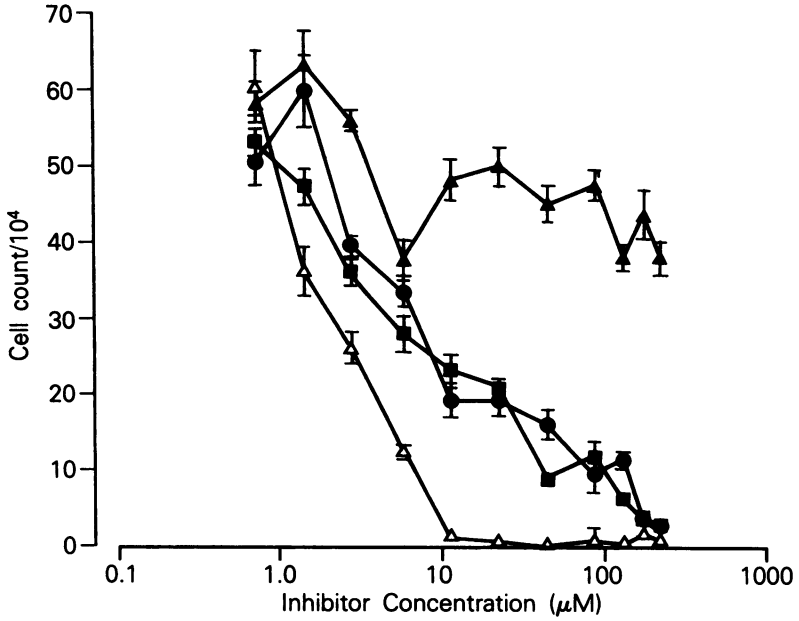

FIG. 4. Effect of (−)-enantiomer (▲), (+)-enantiomer (△), racemate (■), and a 1:1 mixture of the enantiomers (●) on the proliferation of CEM cells. Error bars represent standard errors of means.

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