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Influence of Stereochemistry on Antiviral Activities and Resistance Profiles of Dideoxycytidine Nucleosides

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β-L-2′,3′-Dideoxycytidine (β-L-ddC) and β-L-5-fluoro-2′,3′-dideoxycytidine (5-F-β-L-ddC) were prepared and shown to have potent activity against human immunodeficiency virus type 1 (HIV-1) and hepatitis B virus (HBV). These compounds were compared with β-D-2′,3′-dideoxycytidine (β-D-ddC) and two β-L-oxathiolane nucleosides (β-L-3′-thio-2′,3′-dideoxycytidine and β-L-5-fluoro-3′-thio-2′,3′-dideoxycytidine) in terms of anti-HIV and anti-HBV activity, cytotoxicity, and development of HIV-1 resistance. Compared with β-D-ddC, the β-L-dideoxycytidine nucleosides had similar anti-HIV-1 activities, significantly greater anti-HBV activities, and decreased toxicities to a B-cell line, T-cell lines, and human bone marrow progenitor cells. HIV-1 strains resistant to β-D-ddC were susceptible to the β-L-ddC analogs. Compared with the β-L-oxathiolane nucleosides, β-L-ddC and 5-F-β-L-ddC had similar anti-HIV-1 activities, decreased anti-HBV activities, and greater toxicities to B- and T-cell lines and bone marrow progenitor cells. There were similarities between the β-L-ddC and β-L-oxathiolane nucleosides in the rate of development and pattern of resistant HIV-1 selection. While the in vitro activity and cytotoxicity profiles of the β-L-ddC nucleosides differed from those of the β-D-ddC and β-L-oxathiolane nucleosides, the data presented herein suggest that the sugar configuration of a dideoxynucleoside analog may play a major role in the rate of development and the pattern of HIV-1 resistance.

The striking differences reported in antiviral activity, cytotoxicity, and human immunodeficiency virus type 1 (HIV-1) resistance selection between β-D-2′,3′-dideoxycytidine (β-D-ddC) and oxathiolane nucleosides β-L-3′-thio-2′,3′-dideoxycytidine (3TC) and β-L-5-fluoro-3′-thio-2′,3′-dideoxycytidine (FTC) have led us to investigate the roles of sugar structure and stereochemistry on the biological activities of dideoxycytidine nucleosides (2, 3, 5, 16, 21). We prepared β-L-2′,3′-dideoxycytidine (β-L-ddC) and β-L-5-fluoro-2′,3′-dideoxycytidine (5-F-β-L-ddC) and evaluated these compounds in a variety of in vitro biological assays. β-D-ddC is structurally similar but enantiomeric to β-L-ddC and 5-F-β-L-ddC (Fig. 1). Conversely, 3TC and FTC are stereochemically identical to β-L-ddC and 5-F-β-L-ddC but have a significantly different sugar moiety. We wished to determine whether the biological profiles of the β-L-analogs were influenced by the nucleoside structure (e.g., the presence or absence of the 3′ sulfur) or the stereochemical configuration (β-L-ddC versus β-D-ddC). While this work was in progress, a report on the anti-HIV activity of β-L-ddC was published (14). The present report details the antiviral activity, cytotoxicity, deoxycytidine kinase activity, and HIV-1 resistance profiles of β-L-ddC and 5-F-β-L-ddC and compares their in vitro biological activities with those of β-D-ddC, 3TC, and FTC.

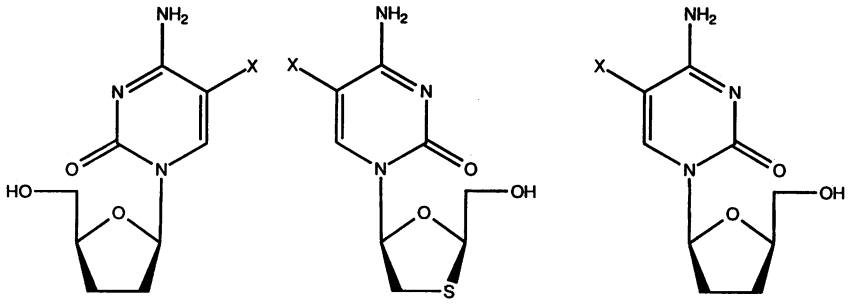

FIG. 1. Structures of β-D-ddC, 3TC, FTC, β-L-ddC, and 5-F-β-L-ddC.

β-L-ddC and 5-F-β-L-ddC were prepared by the method reported elsewhere (25) and assessed for the ability to inhibit the replication of HIV-1 (1) and hepatitis B virus (HBV) (9) in MT4 and 2.2.15 cells, respectively (Table 1). The cellular toxicities of these compounds were determined in growing

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TABLE 1. Antiviral activities and cellular toxicities of 2',3'-dideoxycytidine nucleosides[a]

Compound	IC_{50} (μM)		CC_{50} (μM)						
	HIV-1[b]	HBV[c]	MT4[b]	2.2.15[c]	IM-9[b]	CEM[b]	Molt-4[b]	CFU-GM[d]	BFU-E[d]
β-L-ddC	3.6	0.25	>200	>20	33	58	22	13 ± 3	3 ± 2
5-F-β-L-ddC	0.2	0.1	>20	42	>100	24	29	12 ± 2	10 ± 2
β-D-ddC	2.2	6.9	>100	>200	70	6	10	1.6 ± 0.3	0.7 ± 0.1
3TC	3.2	0.017	>33	>200	>100	>100	>100	250 ± 8[e]	180 ± 2[e]
FTC	0.5	0.038	>200	>200	>100	>100	>100	300 ± 40[e]	220 ± 8[e]

[a] IC_{50}s and 50% cytotoxic concentrations (CC_{50}s) were determined on the basis of five compound concentrations encompassing the IC_{50} and CC_{50}, respectively. Calculations were performed as described previously (22).
[b] Data are means of three replicate samples.
[c] Data are means of four replicate samples.
[d] Data are values (mean ± standard deviation) from several assays (β-L-ddC, $n = 4$ [4 donors]; 5-F-β-L-ddC, $n = 8$ [7 donors]; β-D-ddC, $n = 11$ [10 donors]; 3TC and FTC, $n = 6$ [6 donors]).
[e] Values from reference 5.

cultures of MT4 cells, 2.2.15 cells, human B-cell line IM-9, and two human T-cell lines, CEM and Molt 4 (1). The anti-HIV-1 and anti-HBV activities of β-D-ddC, 3TC, and FTC are included in Table 1 for comparison. The β-L-dideoxy-, β-D-dideoxy-, and oxathiolane-cytidine analogs could not be differentiated from one another on the basis of anti-HIV-1 activity (Table 1). However, the oxathiolane nucleosides appeared to be the most potent inhibitors of HBV replication followed by the β-L-analogs (50% inhibitory concentrations [IC_{50}s] of 0.017 to 0.038 μM versus 0.1 to 0.25 μM, respectively). Additionally, β-L-ddC and 5-F-β-L-ddC were at least 100-fold more potent than the β-D-analog. Differentiation between these classes of compounds on the basis of cellular toxicity to various human leukemic cell lines was also observed. β-L-ddC and 5-F-β-L-ddC were not as toxic to CEM cells as was β-D-ddC but were more toxic than 3TC and FTC were to IM-9, CEM, and Molt 4 cells. On the basis of the anti-HBV and cytotoxicity data, β-L-ddC and 5-F-β-L-ddC differed from both the β-D-dideoxy and β-L-oxathiolane analogs. To extend the comparison, these compounds were examined as inhibitors of human bone marrow progenitor stem cell growth, as substrates for deoxycytidine kinase, and for the potential to select for HIV-1-resistant strains.

β-L-ddC and 5-F-β-L-ddC were moderately potent inhibitors of CFU granulocyte-macrophage (CFU-GM) and burst-forming unit erythroid (BFU-E) colonies, while 3TC and FTC were essentially nontoxic in this assay (Table 1) (4). β-D-ddC was a potent inhibitor of CFU-GM and BFU-E colony proliferation. Thus, the β-L-nucleosides were again dissimilar to either β-D-ddC or to the β-L-oxathiolane compounds.

β-L-ddC and 5-F-β-L-ddC were efficient substrates for deoxycytidine kinase (Table 2) (10). The apparent K_m values for β-L-ddC and 5-F-β-L-ddC were the same or twice the values determined for the oxathiolane nucleosides but were one-third to one-sixth that for β-D-ddC. Of these three classes of compounds, the β-L-dideoxy nucleosides were the most efficient substrates.

Since the introduction of nonnucleoside reverse transcriptase (RT) inhibitors and the discovery of their ability to rapidly select resistant virus (15, 17, 19), new drug candidates have been examined for their potential to select resistant virus. Traditional nucleoside HIV RT inhibitors such as zidovudine (AZT) and β-D-ddC select resistant virus relatively slowly (6, 7, 12, 13, 23). In contrast, 3TC and FTC, like the nonnucleoside inhibitors, select resistant HIV rapidly (6, 18, 20, 24). In addition, determination of the cross-resistance profile of these viral strains is important in assessing the potential value of new RT inhibitors. In general, resistant viruses are cross-resistant to analogs with a similar sugar moiety but remain susceptible to nucleosides containing dissimilar sugar moieties. For example, the dideoxyinosine (ddI)-resistant virus strain is cross-resistant to β-D-ddC but remains susceptible to AZT (11, 23).

The rates at which β-L-ddC and 5-F-β-L-ddC selected resistant mutants and the cross-resistance profiles of these viral strains were investigated to address the question of similarity between the β-D-dideoxy analogs and either β-D-ddC or the β-L-oxathiolane nucleosides. If resistant strains were difficult to isolate in vitro and were cross-resistant to ddI and β-D-ddC, the β-L-dideoxycytidine analogs could be considered similar to traditional dideoxy nucleosides. However, if high-level resistance developed rapidly and cross-resistance to oxathiolane-resistant virus was observed, β-L-ddC and 5-F-β-L-ddC would resemble 3TC and FTC.

5-F-β-L-ddC, the more potent anti-HIV agent of the two β-L-dideoxy nucleosides, was chosen to determine the rate of resistant HIV-1 virus selection. Wild-type HXB2 virus was exposed to increasing concentrations (two- to fourfold) of 5-F-β-L-ddC in MT4 cells, and the sensitivity of virus to the compound was determined at each passage by a plaque reduction assay in HT4 LacZ-1 cells (11). After four passages of virus, the IC_{50} for 5-F-β-L-ddC increased more than 500-fold (Table 3). Genetic analysis of RT in the region from codons 170 to 215 from this resistant virus indicated a change at codon 184 which has also been reported to produce high-level resistance to 3TC and FTC (6, 20, 24) and low-level resistance to ddI and ddC (6, 8). With the 5-F-β-L-ddC-resistant variants, this codon was changed from ATG to ATA,

TABLE 2. Velocities and binding constants of cytidine analogs with calf thymus deoxycytidine kinase

Compound	% Relative V_{max}[a]	Apparent K_m (μM) (mean ± SD)	Substrate efficiency (V_{max}/K_m)
β-L-ddC	40	40 ± 1	1
5-F-β-L-ddC	71	19 ± 1	3.7
β-D-ddC	16	120 ± 5	0.13
3TC[b]	8	20 ± 1	0.4
FTC	12	23 ± 1	0.5
2'-deoxycytidine[c]	100	1.6 ± 0.3	5

[a] V_{max} value for 2'-deoxycytidine was 0.002 mmol/min/μg of protein.
[b] Values from reference 5.
[c] Values from reference 10.

TABLE 3. Susceptibility of HIV-1 strains to 2′,3′-dideoxycytidine analogs and AZT

Compound	IC_{50} (μM)[a] (mean ± SD) (no. of assays)			
	HXB2 (wild type)	74V (β-D-ddC^r)	184V (3TC^r FTC^r)	RTMC (AZT^r)
β-L-ddC	4 ± 2 (4)	6.3 ± 0.3 (2)	180 (2)	4 ± 2 (2)
5-F-β-L-ddC	0.4 ± 0.2 (4)	0.8 ± 0.8 (2)	>200 (2)	1.0 (1)
β-D-ddC	0.38 ± 0.05 (3)	3.1 ± 0.9 (2)	0.8 ± 0.2 (3)	0.65 (1)
FTC	0.09 ± 0.03 (7)	0.12 (1)	>500 (5)	0.4 ± 0.2 (4)
AZT	0.02[b]	0.01[c]	0.03[b]	1.26[b]

[a] Antiviral activity determined by plaque reduction in HT4 LacZ-1 cells (11) using four or five compound concentrations in duplicate. Results are from individual assays or from two or more assays run in parallel. IC_{50}s were determined as described previously (22).
[b] Values from reference 24. Assays were run with the same cells and virus strains.
[c] Value from reference 17. Assays were run with the same cells and virus strains.

a change from methionine to isoleucine. It is interesting that 5-F-β-L-ddC induced a change to isoleucine, as seen with 3TC (24), and not to valine, as seen with FTC (24), even though 5-F-β-L-ddC and FTC are both 5-fluoro nucleosides.

The β-D-ddC-resistant mutant (74V) showed a sevenfold reduction in sensitivity to β-D-ddC (Table 3) but was only marginally less sensitive to β-L-ddC and 5-F-β-L-ddC than was the wild-type virus (HXB2). In contrast, a high level of cross-resistance to β-L-ddC and 5-F-β-L-ddC was seen with the 3TC- and FTC-resistant virus (184V), but only low-level cross-resistance (approximately twofold) was observed with β-D-ddC. The rapid emergence of resistant strains, coupled with the cross-resistance pattern, suggests that β-L-ddC and 5-F-β-L-ddC (β-L-dideoxy nucleosides) interact with HIV RT in a fashion similar to 3TC and FTC (β-L-oxathiolane nucleosides).

Conclusion. A comparison of the antiviral activity, cytotoxicity, and development of HIV-1-resistant virus of β-L-ddC, β-D-ddC, and 3TC nucleoside analogs has been completed. On the basis of the antiviral activity and cytotoxicity observed with these three classes of compounds, both the absolute stereochemistry and the structure of the nucleoside are important in determining the biological profile of a cytidine nucleoside analog. While all compounds had similar anti-HIV-1 activity, it is apparent that the β-L-configuration of β-L-ddC and 5-F-β-L-ddC gives enhanced anti-HBV activity and lower cytotoxicity relative to β-D-ddC. Furthermore, the 3′ sulfur present in compounds 3TC and FTC confers greater anti-HBV activity and significantly lower cytotoxicity relative to the 2′,3′-dideoxy analogs. These results indicate that the β-L-dideoxycytidine nucleosides are dissimilar to both β-D-ddC and to β-L-oxathiolane nucleosides. However, the HIV resistance pattern of 5-F-β-L-ddC was different from that observed for β-D-ddC but closely mimicked that seen with 3TC and FTC. Both the β-L-dideoxy and the oxathiolane nucleosides rapidly selected highly resistant virus, and the HIV-1 oxathiolane-resistant strain was cross-resistant with β-L-ddC and 5-F-β-L-ddC. The studies presented herein suggest that the absolute configuration of a dideoxynucleoside analog may play a major role in the rate of development and the cross-resistance pattern of HIV-1-resistant virus.

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