Biochemical Pharmacology, Vol. 44, No. 10, pp. 1921–1925, 1992.
Printed in Great Britain.

0006-2952/92 $5.00 + 0.00

COMPARISON OF CYTOTOXICITY OF THE (-)- AND (+)- ENANTIOMER OF 2',3'- DIDEOXY-3'-THIACYTIDINE IN NORMAL HUMAN BONE MARROW PROGENITOR CELLS

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(Accepted 1 October 1992)

ABSTRACT-The effects of racemic *cis*-2',3'-dideoxy-3'-thiacytidine [(±)-BCH-189] and its two enantiomers on human myeloid and erythroid colony-forming cells were studied by clonogenic assays. The (+)-isomer was the most toxic with a median inhibitory concentration approximating 2 μM in both cell lineages. In contrast, concentrations of the (-)-isomer required for 50% inhibition of granulocyte macrophage colony-forming units (CFU-GM) and erythroid burst-forming units (BFU-E) were 33.9 ± 15.1 and 169.4 ± 87.9 μM, respectively. The racemic BCH-189 was quite toxic to these cells, but to a lesser extent than observed with 3'-azido-3'-deoxythymidine and 3'-fluoro-3'-deoxythymidine (positive controls).

2',3'-Dideoxynucleoside analogues continue to represent a major chemotherapeutic approach to the treatment and prevention of acquired immunodeficiency syndrome (AIDS). In the search for highly selective novel nucleosides, Belleau and his colleagues have synthesized racemic *cis* 2',3'-dideoxy-3'-thiacytidine [(±)-BCH-189] which is characterized by the presence of a heteroatom of sulfur at the 3'-position of the furanose ring¶. The racemic compound was reported to be active *in vitro* against HIV-1 with a decreased toxicity as compared to 3'-azido-3'-deoxythymidine (AZT) (1). Of interest, racemic BCH-189 was also demonstrated to be effective and selective against human hepatitis B virus in a human hepatoblastoma-derived cell line (cell line 2215) that continuously synthesizes this virus (2).

Recently, we reported that the two *cis*-isomers of BCH-189 were both active *in vitro* against HIV-1 in a human established cell line (CEM) and primary human peripheral blood mononuclear (PBM) cells, while no substantial antiviral activity was detected with the *trans*-isomers (3). Of particular significance was the finding that the (-)-analogue [(-)-BCH-189, 3-TC] was approximately 40-fold less toxic than the (+)-compound in CEM cells, while no difference was observed in PBM and Vero cells (3). These results were confirmed independently by other groups using CEM cell growth to evaluate drug cytotoxic effects (4, 5). Although it is claimed that the (-)-isomer also has similar decreased toxicity in other established cell lines such as H9, JM, U937 and C 8166 (5), no major difference was detected in MT-2 cells (4). Bone marrow toxicity has been shown to be the limiting toxicity of certain nucleosides (6), and we have demonstrated the potential of *in vitro* clonogenic assays to predict *in vivo* bone marrow suppression (7).

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¶Belleau B, Dixit D, Nguyen-Ba N and Kraus L, Design and activity of a novel class of nucleoside analogs effective against HIV-1. Abstr. T.C.O. 1, p. 515. Abstr. 5th Int. Conf. AIDS, Montreal, Canada, 4-9 June 1989.

The substantial differences observed in the cytotoxicity among cell lines for (-)-BCH-189 and its current clinical evaluation in patients infected with HIV† have prompted us to assess its effects on human bone marrow progenitor cells and compare it with the (+)-enantiomer and the racemic mixture.

MATERIALS AND METHODS

Chemicals. The BCH-189 analogues described herein were synthesized in our laboratories and were characterized by proton nuclear magnetic resonance by using techniques such as the nuclear Overhauser effect to confirm the assigned structure of the compound. The detailed synthesis and the optical rotations of these compounds have recently been published (3,8-10). AZT and 3'-fluoro-3'-deoxythymidine (FLT) were also synthesized in our laboratories and 2'-3'-dideoxycytidine (DDC) was purchased from the Sigma Chemical Co. (St. Louis, MO).

Human CFU-GM and BFU-E clonogenic assays. Human bone marrow cells were collected by aspiration from the posterior iliac crest of normal healthy volunteers, according to a protocol approved by the Institutional Review Board Committee at the University of Alabama at Birmingham. Cells were treated with heparin, and the mononuclear population was separated by Ficoll-Hypaque gradient centrifugation, as described previously (11). Cells were washed twice in Hanks' Balanced Salt Solution and counted with a hemocytometer, and their viabilities were >98%, as assessed by trypan blue exclusion. The culture assays were performed by using a bilayer soft agar or methylcellulose method (11). McCoy's 5A nutrient medium supplemented with 15% dialyzed fetal bovine serum (heat-inactivated at 56^0 for 30 min) (GIBCO Laboratories, Grand Island, NY) was used in all experiments. This medium was devoid of thymidine and uridine.

Human recombinant granulocyte/macrophage colony-stimulating factor (50 U/mL; Genzyme, Boston, MA) or erythropoietin (1 U/mL; Connaught, Swiftwater, PA) was used as a colony-stimulating factor. After 14-18 days of incubation at 37^0 in a humidified atmosphere of 5% CO_2 in air, colonies (>50 cells) were counted by using an inverted microscope. The clonogenic efficiency was between 0.1 and 0.2% in all experiments.

Data Analyses. The median inhibitory concentration (IC_{50}) and the 90% inhibitory concentration (IC_{90}) were derived from least-squares linear regression analysis of the logarithm of drug concentration versus CFU-GM or BFU-E survival fraction.

RESULTS AND DISCUSSION

The presence and activity of intracellular enzymes which phosphorylate 2',3'-dideoxynucleosides are highly dependent on species (human or animal), cell type and cell cycle stage, a fact that illustrates the importance of an adequate *in vitro* test for antiviral activity and toxicity in using physiologically relevant cells. The (-)-isomer of BCH-189 has been reported to have a high degree of selectivity against HIV and HBV *in vitro* due in part to its low cytotoxicity as compared to the racemic mixture in some cell lines (3-5,9,12).

†Pluda J, Ruedy J, Levitt N, Cooley T, Berard P, Rubin M and Yarchoan R, Phase I/II study of 3-TC in patients with advanced ARC or AIDS. Abstr. POB3026, 2, p. B91. Abstr. 8th Int. Conf. AIDS, Amsterdam, The Netherlands, 19-24 July 1992.

TABLE 1. Cytotoxic effects of racemic BCH-189 and its enantiomers compared to other nucleoside analogues on human myeloid (CFU-GM) and erythroid (BFU-E) progenitor cells using clonogenic assays.*

Compound	Cells	% of Control (± SD)† at: 0.1 μM	1.0 μM	10 μM	100 μM	IC_{50} ± SD (μM)	TI‡
(±)-BCH-189	CFU-GM	104 (9)	87 (9)	45 (7)	3 (4)	7.6 ± 2.5	127
	BFU-E	95 (10)	87 (10)	53 (8)	8 (3)	9.8 ± 3.6	163
(-)-BCH-189 (3-TC)	CFU-GM	96 (12)	95 (10)	79 (12)	29 (5)	33.9 ±15.1	16,950
	BFU-E	98 (13)	104 (7)	83 (9)	54 (7)	169.4 ± 87.9	84,700
(+)-BCH-189	CFU-GM	94 (12)	64 (7)	2 (2)	0 (0)	1.7 ± 0.4	8.5
	BFU-E	93 (9)	78 (12)	1 (1)	0 (0)	2.3 ± 0.6	11.5
2',3'-dideoxycytidine (DDC)	CFU-GM	98 (10)	96 (6)	67 (13)	24 (15)	21.7± 10.6	1,100
3'-azido-3'-deoxythymidine (AZT)	CFU-GM	85 (10)	57 (10)	30 (9)	0 (0)	1.8 ± 1.4	475
	BFU-E	67 (13)	46 (4)	33 (6)	0 (0)	0.7 ± 0.8	150
3'-fluoro-3'-deoxythymidine (FLT)	CFU-GM	76 (12)	43 (10)	2 (2)	0 (0)	0.55 ± 0.37	275
	BFU-E	36 (5)	7 (6)	1 (1)	0 (0)	0.04 ± 0.01	20

*The BCH-189 analogues were tested blindly; following completion of assays, the identity and purity of each derivative were assessed by chiral HPLC analysis using analytical methodology described in Ref. 12.

†Results are mean values of three separate experiments using different donors performed in triplicate.

‡TI: Therapeutic index as calculated by the ratio of toxicity (IC_{50}) to bone marrow cells and anti-HIV activity measured in human PBM cells obtained from Ref. 3 and unpublished data (R.F. Schinazi).

Although myelosuppression consisting of anemia and neutropenia has been a limiting factor in the treatment of AIDS with some nucleoside analogs (6,7), no *in vitro* data have been reported on the effects of (-)-BCH-189 in human bone marrow cells. Table 1 illustrates the effects of (-)-BCH-189 and related compounds as compared to AZT and FLT (positive controls) on these cells. Racemic BCH-189 was approximately 5- and 10-fold less toxic than AZT in human CFU-GM and human BFU-E, respectively. This difference was even greater when racemic BCH-189 was compared to FLT, which was by far the most toxic compound in both of the CFU-GM and BFU-E assays. Most significantly, (-)-BCH-189 required a concentration about 5-fold greater than the racemic BCH-189 to suppress 50% CFU-GM colony formation. An even larger difference of approximately 15-fold was displayed between (-)-BCH-189 and racemic BCH-189 in erythroid precursor cells. In contrast, the (+)-isomer was the most toxic of the three BCH-189 analogues evaluated against either CFU-GM or BFU-E. While the IC_{50} of the (+)-isomer was in the same range as that of AZT for both marrow cell lineages, its IC_{90} value (7 μM) approximated that calculated for FLT and was 10-fold lower than AZT, further demonstrating the high degree of toxicity of that isomer. DDC was included in these assays, as it is a related analogue of (+)-BCH-189. It is particularly interesting to note that DDC, which differs only by a methylene group at the 3'-position, was at least 10-fold less toxic than (+)-BCH-189 in human CFU-GM.

The therapeutic index, measured in terms of the ratio of toxicity (IC_{50}) to granulocyte-macrophage precursor cells to the median effective anti-HIV-1 (LAV) concentration in human PBM cells for (-)-BCH-189, was almost 17,000 which is orders of magnitude greater than AZT and FLT, with a therapeutic index of 475 and 275, respectively. The therapeutic index for DDC was intermediate with a value of 1,100. For erythroid precursor cells, the therapeutic index was over 84,000 for (-)-BCH-189. Based on the results obtained in human myeloid and erythroid cells, it would be important to determine the acceptable percentage of purity of the drug for clinical use. Therefore a highly stereospecific synthesis of these compounds is required (8,9,13). In general, compounds in our clonogenic assay with IC_{50} values of more than 10 μM usually do not express bone-marrow toxicity in humans on long-term usage of the drug (7).

While stereospecificity has been shown to increase the selectivity of several classes of drugs (14), it is the first time that such a concept may lead us to discover antiviral nucleosides devoid of the untoward effects which have been a major drawback in the development of these therapeutic agents. In that context, it will be important to determine the cellular and molecular factors responsible for the markedly different effects observed on human bone marrow cells between (-)-BCH-189 and its enantiomer (mirror image). While lack of enzymatic deamination was demonstrated with (-)-BCH-189, the (+)-isomer was a substrate for deoxycytidine deaminase (4,15). It is quite unlikely that these data can explain the different toxicity spectrum of the two isomers due to a probable limited phosphorylation of the formed 2',3'-dideoxy-3'-thiauridine as demonstrated for 2',3'-dideoxyuridine (16). More probable differences in the phosphorylation patterns and interactions of the nucleotide isomers with host nucleic acids may account for the increased selectivity of the L-isomer as compared to the D-isomer. Studies are in progress to elucidate these mechanisms, which could provide a rationale for designing other highly selective stereospecific antiviral nucleosides.

ACKNOWLEDGEMENTS-This work was supported by Public Health Service Grants HL-42125 (J-P.S.), RR-00032 (J-P.S.), AI-25899 (R.F.S., C.K.C.) and the Department of Veterans Affairs (R.F.S.). J-P.S. is the recipient of a Faculty Research Award from the American Cancer Society.

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