[CANCER RESEARCH 55, 3008–3011, July 15, 1995]

Advances in Brief

Anticancer Activity of β-L-Dioxolane-cytidine, a Novel Nucleoside Analogue with the Unnatural L Configuration[1]

Kristie L. Grove, Xin Guo, Shwu-Huey Liu, Zhiling Gao, Chung K. Chu, and Yung-Chi Cheng[2]

Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06510 [K. G., X. G., S-H. L., Y-C. C.], and the Department of Medicinal Chemistry, School of Pharmacy, University of Georgia, Athens, Georgia 30602 [Z. G., C. C.]

Abstract

Naturally occurring nucleosides and all anticancer nucleoside analogue drugs are in the β-D configuration. L-(−)-dioxolane-cytidine [(−)-OddC] is the first L-nucleoside analogue ever shown to have anticancer activity. This compound was converted within cells to its mono-, di-, and triphosphate metabolites and was incorporated into DNA. As with cytosine arabinoside, conversion to the monophosphate was catalyzed by cellular deoxycytidine kinase, which was essential for cytotoxicity. However, unlike cytosine arabinoside, (−)-OddC was not susceptible to degradation by deoxycytidine deaminase. Because (−)-OddC inhibited the growth of hepatocellular and prostate tumors that are generally difficult to treat, it is a promising candidate for additional testing. Our results indicate that there is a great deal of variability in the chiral specificities of cellular enzymes and demonstrate how these differences can be exploited in the design of better anti-viral and anticancer drugs.

Introduction

Because naturally occurring nucleosides are in the β-D configuration, most nucleoside analogues designed for the treatment of cancer and viral diseases have also been synthesized in this stereochemical form. Until recently, it was thought that the corresponding L-enantiomers would not be recognized by the metabolic enzymes required for their activation and would, therefore, be ineffective. The discovery that L-(−)-SddC[3] had more potent anti-HBV and anti-HIV activity and less cytotoxicity than its D-enantiomer was the first indication that L-nucleoside analogues could have therapeutic potential (1–4). In the past 2 years, several other L-nucleoside analogues with antiviral activity have been identified. These include L-(−)-5-fluoro-2′,3′-dideoxythiacytidine (5, 6), L-(−)-ddC (7, 8), and L-(−)-5-fluoro-2′,3′-dideoxycytidine (8, 9) which are active against HBV and HIV, and L-(−)-2′-fluoro-5-methyl-arabinofuranosyluridine (10), which has anti-HBV and anti-EBV activity. Like (−)-SddC, these compounds have no significant cellular or mitochondrial toxicity. We were, therefore, surprised to find that the related compound L-(−)-OddC (Fig. 1) not only had potent anti-HBV and anti-HIV activity but was also extremely cytotoxic (11). Because this was the first L-nucleoside analogue ever shown to have potent cytotoxicity, we decided to explore its anticancer potential in comparison to that of AraC, the most effective drug clinically available for the treatment of acute leukemias. Because the metabolic enzymes dCK and dCD play a crucial role in determining the efficacy of deoxycytidine analogues such as AraC, their interactions with (−)-OddC were also investigated.

Materials and Methods

Cell Culture and Cytotoxicity Studies. All cells were maintained in RPMI 1640 supplemented with 10% dialyzed fetal bovine serum and incubated under humidified air with 5% CO_2 at 37°C. For cytotoxicity studies, cells in logarithmic growth were plated at a density of 5000 cells/ml/well in 24-well plates (Corning Glass Works, Corning, NY). Drugs were added to cells at different dosages, and cultures were maintained for a period of three generations. At the end of this time, methylene blue assays were performed (12), and/or cell numbers were counted using a hemacytometer. IC_{50}s were determined through extrapolation of the plotted data.

Enzyme Assays. dCK was purified from BL21(de3) bacteria (Novagen) containing the PET-3d expression vector into which the cDNA of the dCK gene cloned from KB cells has been inserted. This sequence is identical to that published for the gene cloned from Molt-4 cells (13). The protein was purified as described previously (14). dCD was purified from human liver by fast protein liquid chromatography using anion exchange, cation exchange, and hydrophobic columns, in that order. The dCK reaction mixture contained 0.1 M Tris-HCl (pH 7.5), 2 mM DTT, 6 mM $MgCl_2$, 6 mM ATP, 0.2 mM tetrahydrouridine, 1 unit creatine kinase, 6 mM creatine phosphate, 7 mM NaF, 0.3 mM dCyd, and 0.1 μCi of [^{14}C]dCyd in a total volume of 0.1 ml. The assays were performed as described previously (14). The same procedure was used for kinetic studies, with the exception that reaction mixtures contained the different substrates ranging in concentration from 0.1 to 216 μM, with a radiospecificity of 56 mCi/mmol. The dCD reaction mixture contained 0.1 M Tris-HCl (pH 8.0), 5 mM dCyd, and 0.1 μCi [^{14}C]dCyd in a final volume of 0.1 ml; assays were performed as described previously (1). For kinetic studies, the reaction mixture contained 400 μM dCyd, AraC, or (−)-OddC in a final volume of 0.5 ml. Reaction mixtures were incubated for 1 h or longer, and reactions were then terminated by adding 0.1 ml of acetonitrile. The precipitated protein was removed by centrifugation, and the supernatant was lyophilized to dryness. The samples were reconstituted with 5% methanol and analyzed by HPLC using an Alltech RP-C_{18} column.

Analysis of Acid-soluble Metabolites. HepG2 cells were incubated in 1.0 μM [^{3}H](−)-OddC (200 mCi/mmol) for 4 h. Cells were then harvested, washed with cold PBS, and extracted with 0.5 M perchloric acid on ice. The acid-soluble material was neutralized and analyzed by HPLC, using an anion exchange column (Whatman Partisil-SAX). Fractions from the column were collected at 1-min intervals, and radioactivities were quantitated by scintillation counting.

Cesium Sulfate Density Gradients. HepG2 cells were incubated with 1.0 μM [^{3}H](−)-OddC (300 mCi/mmol) or 1.0 μM [^{14}C]AraC (60 mCi/mmol) for 24 h. Nucleic acids were extracted into buffer containing 10 mM Tris-HCl(pH 7.6), 1 mM EDTA, 100 mM NaCl, 0.5% sarcosyl, and 0.5% SDS. The extract was treated with proteinase K (6 mg/ml), and nucleic acids were extracted again using phenol:chloroform (24:1) and ether. The nucleic acids were then placed on a cesium sulfate solution (1.548 g/cm^3) and centrifuged at 25,000 rpm for 65 h. Fractions of the gradient were collected, and the radioactivity and density of each fraction were determined.

***In Vivo* Studies.** Three- to 6-week-old NCr nude mice (Taconic immunodeficient mice and rats) were inoculated s.c. in each flank with 2×10^6 HepG2 or DU-145 cells, and tumors were allowed to grow. Treatment was started

Received 4/17/95; accepted 6/1/95.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Supported by USPHS Grants CA 44358 and AI 33655.

[2] To whom requests for reprints should be addressed, at Department of Pharmacology, Yale University School of Medicine, SHM B-313, 333 Cedar Street, New Haven, CT 06510-8066.

[3] The abbreviations used are: (−)-SddC, L-(−)-2′,3′-dideoxythiacytidine; HBV, hepatitis B virus; (−)-OddC, L-(−)-dioxolane-cytidine; dCK, deoxycytidine kinase; dCD, deoxycytidine deaminase; (−)-ddC, L-(−)-2′,3′-dideoxycytidine; EBV, Epstein-Barr virus; AraC, arabinoside cytosine; dCyd, deoxycytidine.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/55/14/3008/2457248/cr0550143008.pdf by guest on 03 December 2024

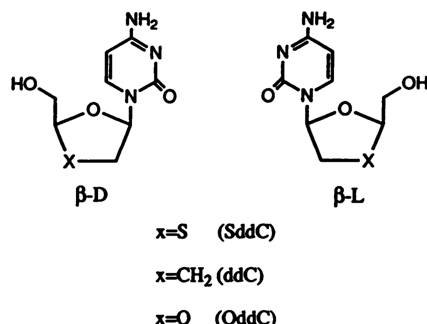

Fig. 1. Structures of dideoxycytidine analogues. Because nucleosides have two chiral carbon atoms at the 1′ and 4′ positions on the sugar ring, they have four possible enantiomers. The two β diastereomers have the 1′ and 4′ substituents in a *cis* relationship, and the α diastereomers have them in *trans*. The D and L designations represent absolute configuration, while (+) and (−) are experimental values of optical rotation. Naturally occurring nucleosides and all nucleoside analogues available for anticancer therapy are in the β-D configuration.

Table 1 *In vitro cytotoxicity of AraC and (−)-OddC*

Cell line	IC_{50} (ηM)[a]		Specific activity (ηmol/min/mg)[b]	
	AraC	(−)-OddC	dCyd kinase	dCyd deaminase
KB	152 ± 10	48 ± 21	0.093 ± 0.019	5.136 ± 0.321
DU-145	170 ± 35	24 ± 20	0.158 ± 0.014	6.122 ± 0.154
PC-3	200 ± 78	56 ± 39	0.139 ± 0.010	5.454 ± 0.014
HepG2	185 ± 24	110 ± 50	0.131 ± 0.014	0.101 ± 0.018
2.2.15	145 ± 7	110 ± 11	0.140 ± 0.020	0.133 ± 0.011
CEM	30 ± 10	25 ± 30	0.158 ± 0.038	0.095 ± 0.023
CEM/dCK-	15500 ± 1000	9250 ± 500	0.009 ± 0.005	0.090 ± 0.027

[a] Values represent the means ± SD of five experiments, with each data point done in duplicate.
[b] Values represent the means ± SD of three experiments, with each data point done in triplicate.

when the tumors were 100–250 mg as determined by caliper measurement and calculated according to the formula

$$\text{Tumor weight (mg)} = \frac{\text{Length (mm)} \times \text{width (mm}^2)}{2}.$$

Drugs were given at the indicated doses on days 0 through 4, and tumor sizes were measured every several days. The tumor growth curves were generated as described previously (15). Toxicity was evaluated by changes in body weight.

Results and Discussion

In cell growth inhibition assays (Table 1), (−)-OddC was significantly more potent than AraC in the nasopharyngeal carcinoma KB line and in the two prostate carcinoma lines DU-145 and PC-3. The two compounds had similar activity against the hepatocellular carcinoma lines HepG2 and 2.2.15 and in the leukemic CEM line. The rate-limiting step in the conversion of AraC to AraCTP is monophosphorylation catalyzed by dCK. An important enzyme in the catabolism of AraC is dCD, which converts AraC to AraU, a comparatively inactive compound. The relative activities of these two enzymes within the cell play a crucial role in determining the amount of AraCTP formed, thereby regulating the extent to which DNA synthesis is inhibited. Because dCK and dCD are so critical in deciding the efficacy of many deoxycytidine analogues, their levels in crude cell extracts were determined. The KB, DU-145, and PC-3 cell lines, in which (−)-OddC was much more potent than AraC, had significantly higher levels of dCD than the lines in which AraC and (−)-OddC were similarly effective. In addition, CEM cells that lack dCK activity (CEM/dCK−) were resistant to (−)-OddC as well as AraC. Since (−)-OddU is not toxic, these results suggested that while (−)-OddC may be activated by the same metabolic pathway as AraC, it may not be susceptible to deamination by dCD. Kinetic studies done with partially purified human dCK and dCD verified that (−)-OddC could be phosphorylated by dCK with a K_m similar to that of the natural substrate (Table 2). However, (−)-OddC was not a substrate for dCD. The resistance of (−)-OddC to deamination gives it a significant advantage over AraC as an anticancer agent and may explain why it is effective in cells where AraC is not.

To determine if (−)-OddC was phosphorylated intracellularly, HepG2 cells were exposed to radiolabeled (−)-OddC, and the acid-soluble metabolites were analyzed using HPLC. Fig. 2A shows that (−)-OddC was converted to its mono-, di-, and triphosphate forms, with the diphosphate being the major metabolite. This finding is unusual in that most nucleoside analogues occur predominantly in their mono- or triphosphate forms. Although (−)-OddCTP was not the major metabolite, its intracellular levels were between 1.0 and 10.0 μM. This concentration is high enough to inhibit human DNA

Table 2 *Interaction of AraC and (−)-OddC with human dCK and dCD*

Substrate	dCK		dCD
	K_m (μM)[a]	Relative V_{max}	Relative V
dCyd	10.57 ± 2.25	1.00	1.00
AraC	28.57 ± 2.78	5.43	0.74
(−)-OddC	14.21 ± 2.59	0.22	<.001

[a] Values are means ± SD of three experiments, with each data point done in triplicate.

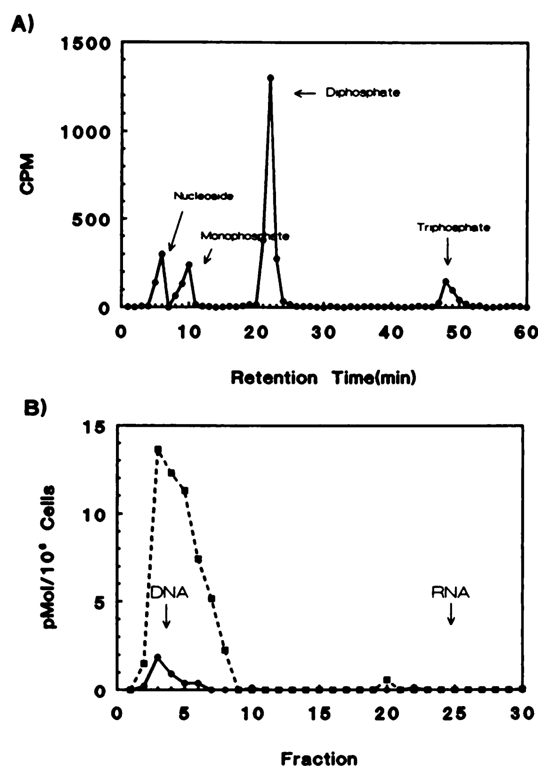

Fig. 2. Intracellular metabolism of (−)-OddC. *A*, HPLC analysis of acid-soluble metabolites. HepG2 cells were incubated in 1.0 μM [^{3}H](−)-OddC (200 mCi/mmol) for 4 h. Cells were then harvested, and the acid-soluble material was extracted and analyzed by HPLC using a Whatman Partisil anion exchange column. The peaks of radioactivity correspond to (−)-OddC nucleoside, monophosphate, diphosphate, and triphosphate, as determined by analysis of chemically synthesized (−)-OddC metabolite standards. *B*, incorporation of (−)-OddC into DNA. To determine if (−)-OddC was incorporated into DNA or RNA, HepG2 cells were incubated with 1 μM [^{3}H](−)-OddC (300 mCi/mmol) or 1 μM [^{14}C]AraC (60 mCi/mmol) for 24 h. DNA and RNA were extracted and analyzed by cesium sulfate density centrifugation. The peak of radioactivity in the gradient was detected at a density of 1.42 g/ml, which represents the DNA fraction; ●, (−)-OddC; ■, AraC.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/55/14/3008/2457248/cr0550143008.pdf by guest on 03 December 2024

polymerases α, β, γ, and δ *in vitro*.[4] To determine if (−)-OddC was being incorporated into DNA or RNAs, HepG2 cells were exposed to radiolabeled (−)-OddC, and nucleic acids were extracted and subjected to cesium sulfate density gradient centrifugation (Fig. 2). Radioactivity was found in the DNA fraction indicating that (−)-OddC metabolites do get incorporated into DNA. Digestion of this DNA with phosphodiesterase, followed by alkaline phosphatase and subsequent HPLC analysis of the digestion products, verified that (−)-OddC had been present in the DNA. Because (−)-OddC acts as a chain-terminator, it was incorporated to a much lesser extent than AraC, which can support continued synthesis and is multiply integrated.

Once the *in vitro* cytotoxicity of (−)-OddC had been established, we investigated its *in vivo* activity in a nude mouse model. NCr immunodeficient mice were inoculated s.c. with either HepG2 or DU-145 cells, and treatment was started when the tumors were in an advanced stage of growth.

The tumor growth curves are shown in Fig. 3. Although the *in vitro* toxicity of AraC was similar to that of (−)-OddC, AraC was ineffective in the animal model. Enzymatic analysis of tumor extracts indicated that this was not due to increased dCD activity or decreased dCK activity but may have been the result of extensive AraC metabolism in the liver which had high dCD levels. Unlike AraC, (−)-OddC was effective in both HepG2 and DU-145 xenografts. The net cell kill (log 10) calculated for HepG2 tumors were 0.67 and 0.87 for i.p. and oral treatment, respectively. The DU-145 tumors became smaller in size, with one-half of them regressing completely by day 15. The tumors did begin to reappear about 25 days after the last treatment, but growth stopped again after day 47. On day 60, the animals were sacrificed, and tumors were removed. The tumors had a necrotic morphology, with very few of the cells being able to exclude trypan blue. In addition, no enzyme activities could be detected in this tissue. The doses of AraC and (−)-OddC given were equally toxic, as shown by weight loss of the animals (Fig. 3*C*), and toxicity experiments indicated that a 5-day treatment with (−)-OddC at 50 mg/kg was tolerated, but the same dosage of AraC was lethal to 4 of 5 animals. A protocol in which drug is administered on an intermittent basis may prove to be even more effective. (−)-OddC was also shown to be effective in two murine tumor models. It inhibited the growth of solid colon 38 tumors and significantly increased the life span of mice bearing p388 leukemia cells, producing several complete cures.

The *in vitro* and *in vivo* data shown here demonstrate that (−)-OddC has significant anticancer potential and has unique properties which make it an interesting candidate for further testing. Not only is it the first L-nucleoside analogue ever shown to have anticancer activity, but it is also the first true chain-terminator capable of inhibiting tumor growth. Although its unnatural stereochemistry does not prevent (−)-OddC from being activated by metabolic enzymes or from being incorporated into DNA, it may be a factor that protects this compound from degradation by dCD. (−)-OddC is also unique in that it is active in solid tumors that are usually unresponsive to nucleoside analogue therapy. The drug 2′,2′-difluorodeoxycytidine (gemcitibine), which is currently undergoing clinical evaluation for the treatment of solid tumors, is still susceptible to inactivation by dCD (16). Because the elevation of dCD levels is a mechanism by which cells become resistant to dCyd analogues such as AraC (17), (−)-OddC may be useful in the treatment of patients who have become unresponsive to these drugs. It would be interesting to compare (−)-OddC

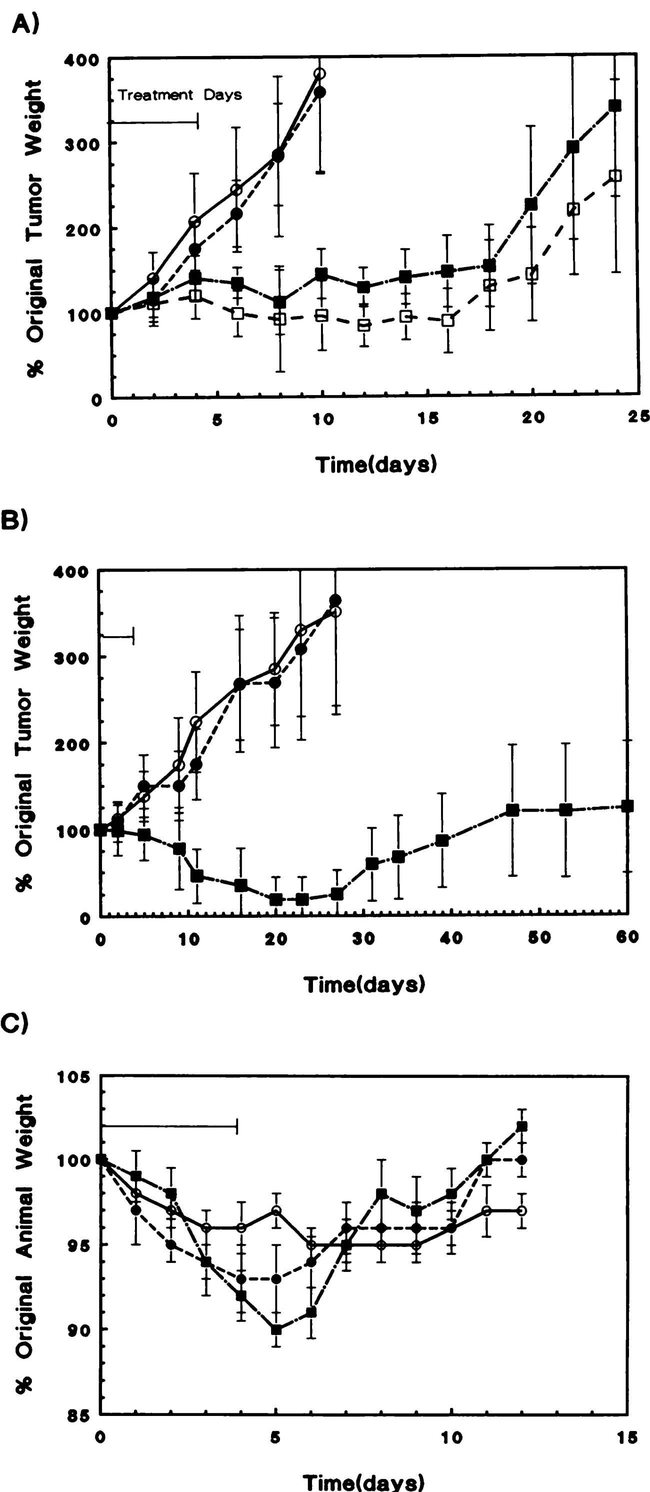

Fig. 3. Inhibition of human tumor growth by (−)-OddC. Three- to 6-week-old NCr nude mice were inoculated s.c. in each flank with 2×10^6 HepG2 or DU-145 cells. Treatment was started when the tumors were in an advanced stage of growth. Drugs were administered twice a day on days 0 through 4, and tumor sizes were measured on the indicated days. *A* and *B*, the drug effects on HepG2 tumors and DU-145 tumors, respectively. *C*, weight loss of the animals over the course of treatment: ○, control; ●, 25 mg/kg AraC, i.p.; □, 25 mg/kg (−)-OddC, p.o.; ■, 25 mg/kg (−)-OddC, i.p.. Each point represents the mean of 10 tumors in *A*, 6 tumors in *B*, and 6 animals in graph *C*; *bars*, SD.

with dCD-resistant analogues such as Ara-azacytosine (Fazarabine), which do not cause immediate chain-termination.

The results reported here also raise many interesting questions regarding the relationship of substrate stereochemistry to enzyme

[4] M. Kukhanova, S-H. Liu, and Y-C. Cheng. L and D enantiomers of 2′,3′-dideoxycytidine-5′-triphosphate analogues as substrates for human DNA polymerases: implications for the mechanism of toxicity, submitted for publication.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/55/14/3008/2457248/cr0550143008.pdf by guest on 03 December 2024

catalysis. One of the reasons that (−)-SddC is a better antiviral agent than its D-enantiomer is that (−)-SddCTP is a much less potent inhibitor of human DNA polymerases α and δ than (+)-SddCTP, thereby making the L-isomer less cytotoxic (20). Simply changing the atom at the 3′ sugar position from a sulfur to an oxygen makes (−)-OddCTP a very potent inhibitor of the same polymerases. The enzyme dCK may not have much chiral specificity, since the K_ms of (−)-OddC, (−)-SddC, and (+)-SddC (18) are all virtually the same as that of the natural substrate. However, in the case of dCD, stereochemistry seems to be important since (+)-SddC is a substrate but (−)-OddC and (−)-SddC are not (1). These differences in chiral specificity create an entirely new class of nucleoside analogues that can be explored for anticancer potential.

References

1. Chang, C. N., Doong, S. L., Zhou, J. H., Beach, J. W., Jeong, L. S., Chu, C. K., Tsai, C. H., and Cheng, Y. C. Deoxycytidine deaminase-resistant stereoisomer is the active form of (±)-2′,3′-dideoxy-3′-thiacytidine in the inhibition of hepatitis B virus replication. J. Biol. Chem., *267:* 13939–13942, 1992.
2. Beach, J. W., Jeong, L. S., Alves, A. J., Pohl, D., Kim, H. U., Chang, C. N., Doong, S. L., Schinazi, R. F., Cheng, Y. C., and Chu, C. K. Synthesis of enantiomerically pure (2′*R*,5′*S*)-(−)- 1-[2-(hydroxymethyl)-oxathiolan-5-yl] cytosine as a potent antiviral agent against hepatitis B virus (HBV) and human immunodeficiency virus (HIV). J. Org. Chem., *57:* 2217–2219, 1992.
3. Coates, J. A. V., Cammack, N., Jenkinson, H. J., Mutton, I. M., Pearson, B. A., Storer, R., Cameron, J. M., and Penn, C. R. The separated enantiomers of 2′-deoxy-3′-thiacytidine (BCH-189) both inhibit human immunodeficiency virus replication *in vitro*. Antimicrob. Agents Chemother., *36:* 202–205, 1992.
4. Schinazi, R. F., Chu, C. K., Peck, A., McMillan, A., Mathis, R., Cannon, D., Jeong, L. S., Beach, J. W., Choi, W. B., Yeola, S., and Liotta, D. C. Activities of the four optical isomers of 2′,3′-dideoxy-3′-thiacytidine (BCH-189) against human immunodeficiency virus type 1 in human lymphocytes. Antimicrob. Agents Chemother., *36:* 672–676, 1992.
5. Furman, P. A., Davis, M., Liotta, D. C., *et al.* The anti-hepatitis B virus activities, cytotoxicities and anabolic profiles of the (−) and (+) enantiomers of cis-5-fluoro-1-[2(hydroxymethyl)-1,3-oxathiolan-5-yl]-cytosine (FTC). Antimicrob. Agents Chemother., *36:* 2686–2692, 1992.
6. Schinazi, R. F., McMillan, A., Cannon, D., *et al.* Selective inhibition of human immunodeficiency viruses by racemates and enantiomers of *cis*-5-fluoro-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-cytosine. Antimicrob. Agents Chemother., *36:* 2423–2431, 1992.
7. Mansuri, M. M., Farina, V., Starrett, J. E., Benigni, D. A., Brankovan, V., and Martin, J. E. Preparation of the geometric isomers of DDC, DDA, D4C, and D4T as potential anti-HIV agents. Bioorg. Med. Chem. Lett., *1:* 65–68, 1991.
8. Lin, T. S., Luo, M. Z., Liu, M. C., Pai, B., Dutschman, G. E., and Cheng, Y. C. Antiviral activity of 2′,3′-dideoxy-B-L-5-fluorocytidine (B-L-FddC) against hepatitis B virus and human immunodeficiency virus type 1 *in vitro*. Biochem. Pharmacol., *47:* 171–174, 1994.
9. Gosselin, G., Mathe, C., Bergogne, M. C., Aubertin, A. M., Kirn, A., Schinazi, R. F., Sommadossi, J. P., and Imbach, J. L. Enantiomeric 2′,3′-deoxycytidine derivatives are potent human immunodeficiency virus inhibitors in cell cultures. C. R. Acad. Sci. Paris Sci. Vie, *317:* 85–89, 1994.
10. Chu, C. K., Ma, T., Shanmuganathan, K., Wang, C., Xiang, Y., Pai, S. B., Yao, G. Q., Sommadossi, J-P., and Cheng, Y-C. 2′-Fluoro-5-methyl-B-L-arabinofuranosyluridine (L- FMAU) as a novel antiviral agent for hepatitis B and Epstein-Barr virus. Antimicrob. Agents Chemother., *39:* 979–981, 1995.
11. Kim, H. O., Shanmuganathan, K., Alves, A. J., Jeong, L. S., Beach, J. W., Schinazi, R. F., Chang, C. N., Cheng, Y. C., and Chu, C. K. Potent anti-HIV and anti-HBV activities of (−)-L-B-Dioxolane-C and (+)-D-B-Dioxolane-T and their asymmetric syntheses. Tetrahedron Lett., *33:* 6899–6902, 1992.
12. Finlay, G. J., Baguley, B. C., and Wilson, W. R. A semiautomated microculture method for investigating exponentially growing carcinoma cells. Anal. Biochem., *139:* 272–277, 1984.
13. Chottiner, E. G., Shewach, D. S., Datta, N. S., Ashcraft, E., Gribbin, D., Ginsburg, D., Fox. I. H., and Mitchell, B. S. Cloning and expression of human deoxycytidine kinase cDNA. Proc. Natl. Acad. Sci. USA, *88:* 1531–1535, 1991.
14. Cheng, Y. C., Domin, B., and Lee, L. S. Human deoxycytidine kinase purification and characterization of the cytoplasmic and mitochondrial isozymes derived from blast cells of acute myelocytic leukemia patients. Biochim. Biophys. Acta, *481:* 481–492, 1977.
15. Ball, C. R., and Double, J. A. Transplantable colon tumors as chemotherapy screening models. Cancer (Phila.), *36:* 2437–2440, 1975.
16. Bouffard, D. Y., Laliberte, J., and Momparler, R. L. Kinetic studies on 2′,2′-difluorodeoxycytidine (gemcitibine) with purified human deoxycytidine kinase and cytidine deaminase. Biochem. Pharmacol., *45:* 1857–1861, 1993.
17. Ometto, M., Momparler, L. F., and Gyger, M. *In vitro* biochemical tests to evaluate the response to therapy of acute leukemia with cytosine arabinoside or 5-aza-2′-deoxycytidine. Semin. Oncol., *14:* 231–237, 1987.
18. Chang, C-N., Skalski, V., Zhou, J. H., and Cheng, Y-C. Biochemical pharmacology of (+) and (−)-2′,3′-dideoxy-3′-thiacytidine as anti-hepatitis B virus agents. J. Biol. Chem., *267:* 22414–22420, 1992.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/55/14/3008/2457248/cr0550143008.pdf by guest on 03 December 2024