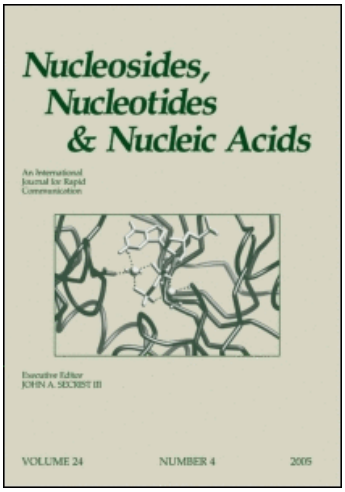

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Beta-L-(-)-dioxolane Cytidine (β-L-(-)-OddC) as a Potent Compound for the Treatment of Cancer

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IV. PRECLINICAL STUDIES OF NUCLEOSIDES AND NUCLEOTIDES

NUCLEOSIDES & NUCLEOTIDES, 16(7-9), 1229-1233 (1997)

BETA-L-(-)-DIOXOLANE CYTIDINE (β-L-(-)-OddC) AS A POTENT COMPOUND FOR THE TREATMENT OF CANCER

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ABSTRACT: L-(-)-OddC is the first nucleoside analog with the unnatural L-configuration and the first chain-terminator shown to have anti-cancer activity. This compound was found to be highly active against solid tumor growth in several human xenograft models. L-(-)-OddC exerts its activity be terminating DNA chain elongation after its incorporation.

Nucleosides are found in nature only in the β-D-conformation. Until recently, it was believed that nucleoside analogs with the unnatural β-L-configuration would not be recognized by the metabolic enzymes required for their activation and would not have biological activity. Therefore, it was surprising to learn that L-nucleoside analogs have potent and selective activity against human immunodeficiency virus (HIV), hepatitis B virus (HBV) and Epstein-Barr virus (EBV) (1-5). Studies of the structure-activity relationships of L-nucleoside analogs indicated that L-(-)-OddC (Figure 1) had more potent activity against HIV and HBV than L-(-)-SddC (3TC) but was also extremely cytotoxic (6).

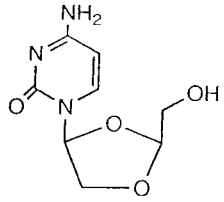

FIGURE 1. Structure of L-(-)-OddC

The *in vivo* anti-cancer activity of L-(-)-OddC was compared to that of cytosine arabinoside (AraC). L-(-)-OddC significantly inhibited the growth of several solid tumors that were completely unresponsive to AraC (Table 1). The two human prostate carcinoma xenografts, DU-145 and PC-3 were most

TABLE 1. *In Vivo* anticancer activity of L-(-)-OddC. 10^6 of the indicated tumor cells were inoculated s.c. into each flank of BDF1 (Colon 38) or NCr Nude (Hepg2, DU-145 and PC-3) mice. Treatment was started when the tumors were in an advanced stage of growth. The dosage used was 25 mg/kg given twice a day for 5 consecutive days. T-C was defined as the time required for the tumors to reach a size of 700 mg. Tumors were considered to have regressed when no tumor mass could be seen or measured. T-C values could not be determined for DU-145 and PC-3 models because the treated tumors never reached a size greater than 150 mg.

Tumor Type	T-C(Days)	Net Cell $Kill_{Log\,10}$	Regressions/Total
Colon 38	5.3	+0.05	0/6
HepG2	23	+1.36	0/6
DU-145	-------	------	6/10
PC-3	--------	------	3/6

sensitive to L-(-)-OddC, with many of the tumors regressing completely.

Biochemical studies indicated that, like AraC, L-(-)-OddC is trasported into cells by both equilibrative sensitive and equilibrative insensitive nucleoside transporters. However, the initial rate of L-(-)-OddC transport is more rapid than that of AraC (7). Inside the cell, L-(-)-OddC is metabolized to its 5'-triphosphate derivative (Figure 2). Monophosphorylation is catalyzed by the enzyme deoxycytidine kinase (7), which also phosphorylates AraC. L-(-)-OddC is not a substrates for mitochondrial thymidine kinase.[1] The formation of L-(-)-OddCDP and L-(-)-OddCTP is inhibited by dCMP and CMP suggesting that L-(-)-OddCMP is phosphorylated by CMP-UMP-dCMP kinase. Unlike AraC, L-(-)-OddC cannot be degraded by the enzyme cytidine deaminase (8).

L-(-)-OddC accumulates within cells as diphosphate. The accumulation of diphosphate is also seen with other L-nucleoside analogs including 3TC (1) and may indicate that nucleoside diphosphate kinases have more chiral specificity than nucleoside kinases. Because other nucleoside analogs that are active against solid tumors, such as

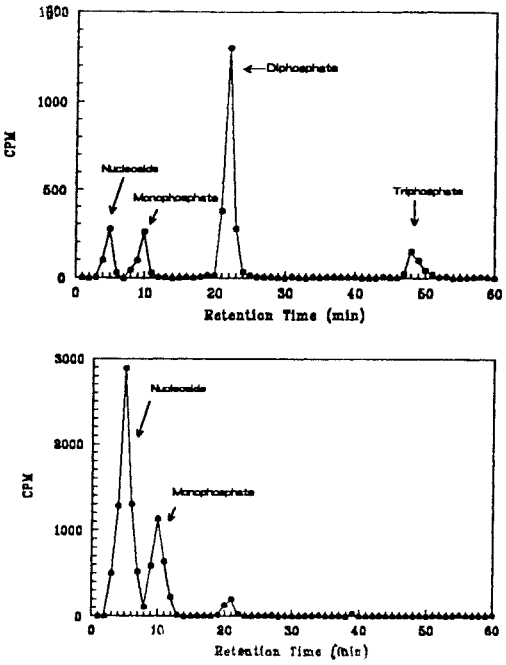

FIGURE 2.A) HPLC analysis of L-(-)-OddC metabolites in CEM cells. B) HPLC analysis of L-(-)-OddC metabolites in CEM extracts in the presence of 1 mM dCMP. Experiments were done as described previously (7). The phosphorylation of L-(-)-OddCMP was inhibited by both dCMP and CMP.

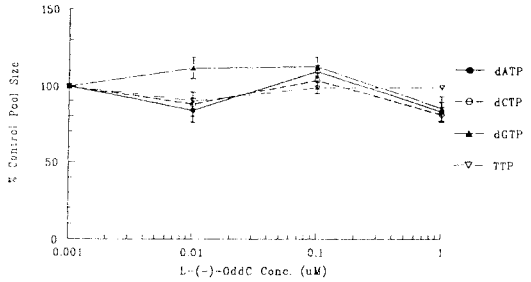

FIGURE 3. Effect of L-(-)-OddC on dNTP pools. DU-145 cells were treated with the indicated concentrations of L-(-)-OddC for 4 hours. Acid-soluble metabolites were then extracted and dNTP pool size determinations were done using a primer-template protocol (11).

[1]S-H. Liu and Y-C. Cheng, unpublished communication

TABLE 2. Incorporation of L-(-)-OddCTP and L-(-)-SddCTP into DNA by human DNA polymerases. Experiments were done as previously described (10).

	K_m (uM)		
Polymerase	dCTP	L-(-)-OddCTP	L-(-)-SddCTP
α	0.6 ± 0.3	1.3 ± 0.5	-------
β	2.6 ± 0.8	2.8 ± 1.2	1.2 ± 0.10
γ	0.1 ± .04	0.7 ± 0.3	0.01 ± 0.002
δ	0.4 ± 0.1	3.5 ± 0.8	-------

gemcitibine, inhibit ribonucleotide reductase (RR) activity (9), it was suspected that L-(-)-OddC might enhance its own anticancer activity by inhibiting RR. However, treatment of cells with L-(-)-OddC had no effect on the size of intracellular dNTP pools (Figure 3).

L-(-)-OddCTP is incorporated into DNA by the human polymerases α, β, γ and δ (Table 2). The inability of the replicative DNA polymerases alpha and delta to utilize L-(-)-SddCTP as a substrate may explain why L-(-)-SddC is not cytotoxic but L-(-)-OddC is.

The cytotoxicity of L-(-)-OddC is directly correlated with its incorporation into DNA (7) suggesting that this is the major mechanism through which it exerts its anticancer activity. Studies of the cellular pharmacodynamics of L-(-)-OddC indicated that its metabolites are retained within cells much longer than AraC metabolites (7). These results, in addition to demonstrating an additional advantage of L-(-)-OddC over AraC,suggested that treatment regimens of higher, less frequent doses might be more effective that those previously used. Figure 4 shows the result of *in vivo* experiments in human DU-145 prostate tumor xenografts using several different treatment regimens. The optimum dosage of L-(-)-OddC was 250 mg/kg given once a day on days one and five. This schedule resulted in complete inhibition of tumor growth. These findings indicate that L-(-)-OddC may be an important agent for treating solid tumors that are unresponsive to conventional therapies.

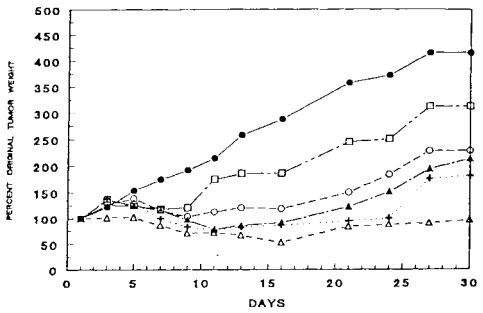

FIGURE 4 Effect of L-(-)-OddC on the growth of DU-145 tumors in nude mice. 10^6 DU-145 cells were inoculated s.c. into each flank of NCr nude mice. Treatment was started when the tumors were at least 100 mg in size as determined by caliper measurement. ●-control, ○-500 mg/kg given on day 1, □-1000 mg/kg given on day 1, ▲-500 mg/kg given on days 1 and 5, Δ-250mg/kg given on days 1 and 5.

The metabolism of L-(-)-OddC is summarized in Figure 5. L-(-)-OddC is a substrate for both ei and es nucleoside transporters. This compound is phosphorylated within cells by the enzyme deoxycytidine kinase. However, it is not a substrate for cytidine deaminase. L-(-)-

OddCMP is further metabolized to its di- and triphosphate derivitives by the enzymes CMP-UMP-dCMP kinase and nucleoside diphosphate kinase respectively (7). Although L-(-)-OddCDP accumulates within cells, it has no effect on ribonucleotide reductase activity.

L-(-)-OddCTP competes with dCTP for incorporation into DNA where it acts as a chain-terminator. L-(-)-OddCMP residues can be slowly removed from DNA (6). However, this removal occurs much more slowly than that of AraCMP. While AraCMP residues are removed from DNA by DNA polymerase epsilon-associated exonuclease, this enzyme cannot remove L-(-)-OddCMP residues. The exonuclease responsible for the removal of L-(-)-OddCMP has not yet been identified.

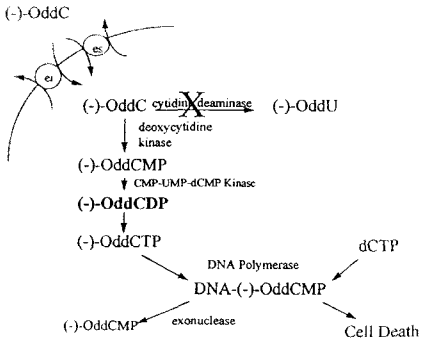

FIGURE 5. Metabolism of L-(-)-OddC.

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