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INHIBITION OF NUCLEIC ACID SYNTHESIS IN LEUKEMIA 1210 CELLS BY ANTIMETABOLITES OF COENZYME Q_{10}

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SUMMARY

Thirteen diversified antimetabolites of coenzyme Q_{10} which have antitumor activity in vivo were tested for inhibition of uptake of tritiated thymidine and uridine into DNA and RNA, respectively, of L1210 cells grown in tissue culture. Eight of these antimetabolites have inhibitory activities of the same order of magnitude as the used anticancer drugs, rubidazone and ellipticine. 5-ω-Phenylpropylmercapto-2,3-dimethoxy-1,4-benzoquinone was particularly potent to inhibit nucleic acid synthesis; ED_{50} for DNA = 2.1 μM and ED_{50} for RNA = 4.0 μM.

INTRODUCTION

The anthracycline quinones, adriamycin and daunorubicin, are very prominent in the combined modality of new drugs for treatment of cancer in man at the present time. Adriamycin has been clinically used for the treatment of leukemias (1,2), solid tumors (3,4), and Ewing's sarcoma (5). It is believed that the antineoplastic activities of these quinones stem from their interaction with nucleic acids (6,7), and it has been proposed that intercalation of the anthraquinone moiety between base pairs of the DNA helix occurs with subsequent inhibition of DNA replication and/or RNA biosynthesis.

Many coenzyme Q_{10} analogs with a variety of quinone nuclei and side chains have been found to be potent inhibitors of mitochondrial respiration (8,9,10). Consequently, it was considered important to test selected analogs of coenzyme Q_{10} for inhibition of DNA and RNA biosynthesis. Fourteen diversified antimetabolites of coenzyme Q_{10}, all of which have antitumor activity except NSC 276023, were tested for inhibition of uptake of tritiated thymidine and uridine into DNA and RNA, respectively, of L1210 cells grown in tissue culture.

MATERIALS AND METHODS

L1210 cells were grown in RPMI 1640 medium containing 10% heat inactivated fetal bovine serum (FBS) and were maintained at a density of 1 - 4 x 10^6/ml (11). All compounds were tested for possible inhibition of the incorporation of ^3H-thymidine into TCA precipitable material. The compounds were weighed on a microbalance immediately before use and were dissolved in the 1640 medium containing 10% FBS and 20 mM HEPES, pH 7.2 buffer. 1% DMSO was added to improve

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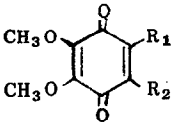
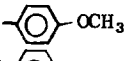
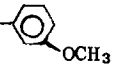
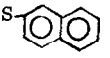
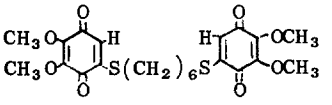
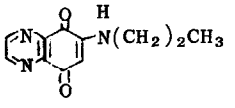
TABLE I. INHIBITION OF NUCLEIC ACID SYNTHESIS IN L1210 CELLS

	NSC No. NCI/NIH	ED_{50} in L1210 Cells DNA Synthesis	RNA Synthesis
Daunorubicin	82151	0.4	0.2
Adriamycin	123127	0.7	0.3
Rubidazone	164011	4.1	1.2
Actinomycin D	3053	0.2	0.004
Ethidium chloride	--	32	10
Ellipticine	71745	5.6	2.5
5-Flourouracil	19893	>100	96
			
R_1 = Cl; R_2 = $S(CH_2)_7CH_3$	252188	8.46	7.44
R_1 = Cl; R_2 = $S(CH_2)_{11}CH_3$	220334	16.8	18.6
R_1 = OH; R_2 = phytyl	277818[a]	163.8	142.5
R_1 = H; R_2 = S-phytyl	276371[a]	109.4	62.7
R_1 = H; R_2 = $S(CH_2)_3$- 	258835	2.1	4.0
R_1 = Cl; R_2 = $S(CH_2)_3$- 	265479	8.9	6.6
R_1 = Cl; R_2 = $SCH_2CH_2CH(CH_3)_2$	265469	7.4	5.3
R_1 = H; R_2 = S- 	277807	5.4	5.1
R_1 = H; R_2 = S- 	290814	5.74	5.06
R_1 = H; R_2 = S- 	234214	6.9	7.3
R_1 = Cl; R_2 = S- 	247511	18.4	13.3
R_1 = OH; R_2 = $(CH_2)_7CH=CHCH_2-CH=CH(CH_2)_4CH_3$	276023	176.4	168.1
	292681	24.6	16.9
	268930	4.18	2.70

a) This compound formed a suspension.

solubility. Insoluble compounds were sonicated briefly to produce a fine suspension. Typically, drugs were assayed at final concentrations of 100, 30, 10, 3, 1, 0.3 and 0.1 μM. Less active compounds were also tested at higher

concentrations. All groups of assays included a standard of daunorubicin as well as untreated control cells. One ml of L1210 cells at a concentration of 2 x 10^6/ml was added to 1-ml dilutions of the test solutions and incubated for 3 hr at 37° in a reciprocating shaker bath. Then, each assay of cells and test compounds was exposed for 1 hr to 0.5 μCi/ml of ^{3}H-thymidine (40 Ci/mole), and TCA precipitable radioactivity was determined. The concentration producing a 50% inhibition of ^{3}H-thymidine incorporation into TCA precipitable material as compared with untreated controls (ED_{50}) was calculated for each compound. Appropriate concentrations in the range of the initial ED_{50} were selected for each compound, and assays were repeated. A similar technique using ^{3}H-uridine provided ED_{50} values for inhibition of RNA synthesis.

RESULTS AND DISCUSSION

Fourteen diversified synthetic antimetabolites of coenzyme Q_{10}, all of which have antitumor activity *in vivo* except one, were tested for inhibition of the uptake of tritiated thymidine and uridine into DNA and RNA, respectively, of L1210 grown in tissue culture (11). The data are in Table I. Eight of these analogs had inhibitory activities of the same order of magnitude as the anticancer drugs, rubidazone and ellipticine. One antimetabolite was particularly potent, and it is 5-ω-phenylproplmercapto-2,3-dimethoxy-1,4-benzoquinone (NSC 258835); the ED_{50} for DNA was 2.1 μM and the ED_{50} for RNA was 4.0 μM. Against Walker carcinosarcoma 256 in rats, this antimetabolite at 6.25 mg/kg resulted in 3/6 cures and a T/C of 789.

Two other analogs of coenzyme Q_{10}, which were effective *in vivo* against Walker carcinosarcoma 256 in rats, 6-phytyl-5-hydroxy-2,3-dimethoxy-1,4-benzoquinone (NSC 277818; 4/4 cures, % T/C = 923 at 50 mg/kg) (12) and 5-phytylmercapto-2,3-dimethoxy-1,4-benzoquinone (NSC 276371; 3/6 cures, % T/C = 789 at 0.78 mg/kg) (12), showed no significant inhibition to L1210 cells. 6-Octylmercapto-5-chloro-2,3-dimethoxy-1,4-benzoquinone (NSC 252188), an analog active in this assay (ED_{50} = 8.46 for DNA; ED_{50} = 7.44 for RNA), was recently found to be a particularly potent inhibitor of two human cell lines of leukemia (13) and was also active *in vivo* in Walker carcinosarcoma 256 (6/6 cures, % T/C = 584 at 3.13 mg/kg) (12).

The "bis-quinone", bis-1,6-(2,3-dimethoxy-1,4-benzoquinonyl)-hexanedithiol (NSC 292681), was approximately as active as ethidium chloride to L1210 cells.

All of these antimetabolites except one, NSC 268930, are 1,4-benzoquinones with diversified side chains and ring substituents. Replacement of the hydrogen atom in position 5 by a chloro group resulted in a sharp decrease in activity as evidenced by the activities of 5-ω-phenylpropylmercapto- and 5-β-naphthylmercapto-2,3-dimethoxy-1,4-benzoquinones (NSC 258835 and NSC 234214) versus those of 6-ω-phenylpropylmercapto- and 6-β-naphthylmercapto-5-chloro-2,3-dimethoxy-1,4-benzoquinones (NSC 265479 and NSC 247511), respectively. Increasing the side-chain length also brought about a similar decline in inhibitory activity. In general, incorporation of tritiated uridine into RNA was slightly more sensitive

to inhibition by these analogs than was the incorporation of tritiated thymidine into DNA.

These data support the interpretation that such analogs of coenzyme Q_{10} may exert their antitumor activity through mechanisms including inhibition of DNA and RNA biosynthesis, although their antitumor activity may also be due to other mechanisms. Studies (10,12) *in vitro* have demonstrated inhibition of coenzyme Q_{10}-enzyme systems, succinoxidase and NADH-oxidase, by such analogs and indicate that antitumor activity may also include inhibition of mitochondrial respiration.

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