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Collateral Sensitivity to Methotrexate and Dichloromethotrexate of Cultured Lymphocytic Leukemia L1210 Cells Resistant to 6-Mercaptopurine[1] (41401)

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Abstract. Cultured cell populations, derived from lymphocytic leukemia L1210 resistant to 6-mercaptopurine (6-MP; L1210/6-MP) and sensitive lymphocytic leukemia (L1210/0), were propagated in Dulbecco's modified Eagle's minimum essential medium supplemented with 10% horse serum and 5 μm 2-mercaptoethanol. The population doubling times (mean ± SD) were 16.6 ± 1.9 hr (L1210/0) and 16.1 ± 1.8 hr (L1210/6-MP) between cell densities of $7 \times 10^4 - 1.0 \times 10^5$ and 2.0×10^6 cells/ml. Cultured L1210/6-MP cell populations were significantly resistant to 6-MP (1.7 to 170 μg/ml for exposure periods of 24 or 48 hr). Cultured L1210/6-MP cell populations were also collaterally sensitive to methotrexate (0.055 to 5.5 μg/ml for an exposure period of 24 hr) or to dichloromethotrexate (1.0–100 μg/ml for an exposure period of 24 hr). The results indicate that resistance to 6-MP and collateral sensitivity to the folate analogs are properties of the L1210/6-MP cells and are independent of any host factors.

A frequent occurrence with drug treatment of leukemias and solid tumors is that initially drug-sensitive tumors become progressively less responsive and finally fail to respond during continuing treatment (1). Studies with murine tumor models have indicated that a principal reason for these treatment failures is the overgrowth of drug-resistant tumor cells during chronic treatment (2). In an attempt to circumvent the potential overgrowth of drug-resistant tumor cells, a strategy is being developed in that drug-resistant tumor cells would be treated with drugs that are more lethal for the resistant tumor cells than they would be for the parent drug-sensitive tumor cells (1).

Several reports (1, 3, 4) have indicated that lymphocytic leukemia L1210 resistant to 6-mercaptopurine (6-MP) (L1210/6-MP) are significantly more sensitive to methotrexate (MTX) and dichloromethotrexate (DCMTX) when compared to 6-MP nonresistant L1210 lymphocytic leukemia (L1210/0). In order to determine if the collateral sensitivity to these folic acid analogs was a cellular property independent of host factors, we compared the sensitivity of cultured L1210/0 and L1210/6-MP cell populations to these antitumor agents.

Materials and Methods. The L1210/6-MP lymphocytic leukemia developed by Law *et al.* (3) and the L1210/0 lymphocytic leukemia were obtained from the tumor bank of the Division of Drug Cancer Treatment, National Cancer Institute, Bethesda, Maryland. Cultured cell populations derived from L1210/0 and L1210/6-MP lymphocytic leukemias transplanted in DBA/2 mice were propagated in Dulbecco's modified eagle's minimum essential medium (DMMEM) supplemented with 10% horse serum with 5 μM 2-mercaptoethanol (growth medium) (5). The cell lines were maintained in stationary suspension cultures. Under these conditions, the cell populations exponentially proliferate with doubling times of 16.6 ± 1.9 hr (L1210/0; mean ± SD) and 16.1 ± 1.8 hr (L1210/6-MP) between cell densities of $7 \times 10^4 - 1 \times 10^5$ and 2×10^6 cells/ml.

To determine the effect of 6-MP, MTX, or DCMTX on the viability of replicating populations of L1210/0 and L1210/6-MP cells, cultures were initiated at a cell density of 7.0×10^4 to 1.0×10^5 cells/ml in a total volume of 100 ml medium in spinner flasks (Bellco Glass, Inc., Vineland, N.J.) 48 hr

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prior to the addition of the drug. The spinner cultures were incubated at 37° on a magnetic stirrer (120 rpm). Proliferating cell populations were exposed to either 6-MP (1.7–170 μg/ml) for 24 or 48 hr, or to MTX (0.055–5.5 μg/ml), or DCMTX (1.0–100 μg/ml) for 24 hr. The cells were harvested by sedimentation, washed in a balanced salts solution, and the number of cells surviving drug treatment was determined by colony formation in semisolid medium (5).

The semisolid medium employed was DMMEM supplemented with 20% horse serum, 50 μmole 2-mercaptoethanol, 100 μg/ml sodium pyruvate, and a final concentration of 0.15% agarose (Sigma Chemical Co., St. Louis, Mo., Type II). Cells from the drug-treated cultures were assayed for colony formation at 1.0×10^4, 1.0×10^3, and 1.0×10^2 cells/tube in quadruplicate, and the cells from the control cultures were assayed for colony formation at 1.0×10^2 cells/tube in a series of eight tubes. The tubes were incubated 10–12 days at 37° in 5% CO_2. Only colonies with 50 or more cells were counted (6); colony-forming efficiencies of control cultures were 50 ± 16% (L1210; mean ± SD) and 77 ± 12% (L1210/6-MP).

The sensitivities of cultured leukemia L1210/0 and L1210/6-MP cell populations were compared by χ^2 analysis. The standard error of the ratios of surviving fractions of L1210/0 and L1210/6-MP cell populations were calculated as described by Kendall and Stuart (7).

Drug concentrations were selected on a basis of not exceeding the theoretical maximum concentration (TMC) estimated to be attainable in the body fluids of mice, assuming rapid equal distribution in all fluids following the administration of a drug in a single dose lethal for 10% of the animals (8). The TMC for each agent is as follows: 6-MP, 440 μg/ml; MTX, 55 μg/ml; DCMTX, 2,040 μg/ml.

6-MP was dissolved in a minimum volume of 1 *N* NaOH and diluted with growth medium to the desired concentrations. MTX was dissolved in a minimum volume of 1 *N* NaOH, diluted with growth medium, and the pH adjusted to 7.0 with 1 *N* HCl. The MTX solution was then diluted to the desired concentrations with growth medium. DCMTX was dissolved in a minimum volume of 0.01 *N* NaOH and diluted to the desired concentrations with growth medium. Stock solutions of DCMTX were shielded from light, and all experiments with DCMTX were conducted in subdued light.

Results and Discussion. Cultured L1210/6-MP cells were significantly resistant to 6-MP (1.7–170 μg/ml) when the cells were exposed to this agent for 24 or 48 hr (Table I). For example, when proliferating L1210/0 cells were exposed to 1.7 μg/ml of 6-MP for 48 hr, a 99.9% reduction in viability of the cell population was observed, whereas this concentration and exposure period had no effect on the viability of the L1210/6-MP cell population. Even when both cell populations were exposed to 170 μg/ml of 6-MP for 24 or 48 hr, the surviving fractions of the L1210/6-MP cell populations were significantly greater than the surviving fractions of the L1210/0 cell populations.

Cultured L1210/6-MP cells were also collaterally sensitive to MTX (0.055–5.5 μg/ml) or to DCMTX (1.0–100 μg/ml) (Table II). It should be noted that these are drug concentrations estimated to be attainable in the body fluids of mice. For instance, the surviving fractions of L1210/0 cells exposed to 0.55 μg/ml of MTX or to 100 μg/ml of DCMTX for 24 hr were 67- and 19-fold greater than the surviving fractions of the L1210/6-MP cells exposed to the same concentrations of these agents.

Resistance to a chemotherapeutic agent with concomitant sensitivity to a second antitumor agent has been known for many years and this has been reported for 6-MP resistant L1210 lymphocytic leukemia with respect to MTX (3, 4). However, several reports have indicated that other L1210/6-MP lymphocytic leukemias are either cross-resistant or retain the same sensitivity as the L1210/0 lymphocytic leukemia to MTX (4). Browman and Csullog (9) have recently reported that they could only demonstrate collateral sensitivity of L1210/

TABLE I. SENSITIVITY OF CULTURED L1210/6-MP AND L1210/0 CELLS TO 6-MERCAPTOPURINE FOR 24- AND 48-hr EXPOSURE PERIODS[a]

6-MP[b] (μg/ml)	Hours	S_f[c]		Ratio ± SE[d] S_f L1210/6-MP	
		L1210/6-MP	L1210/0	S_f L1210/0	*P* value[e]
1.7	24	400/400	148/40,000	270 ± 22	<0.001
17	24	400/400	88/40,000	455 ± 48	<0.001
170	24	232/400	8/40,000	2900 ± 1033	<0.001
1.7	48	400/400	36/40,000	1111 ± 185	<0.001
17	48	360/400	8/40,000	4500 ± 1593	<0.001
170	48	260/4000	8/40,000	325 ± 117	<0.001

[a] Proliferating cultured L1210/6-MP or L1210/0 cell populations were exposed to 6-MP (1.7–170 μg/ml) for 24 or 48 hr and the number of cells surviving drug treatment was estimated by colony formation in semisolid medium as described under Materials and Methods.
[b] The TMC for 6-MP is approximately 440 μg/ml.
[c] Surviving fraction.
[d] Standard error of the ratio was estimated as described in Ref. (7).
[e] S_fs for each exposure period were compared by use of χ^2 analysis.

6-MP lymphocytic leukemia to MTX in an *in vivo* system and not in cell culture.

Cells resistant to 6-MP are deficient in hypoxanthine–guanine phosphoribosyltransferase and consequently are unable to convert hypoxanthine and guanine to inosine monophosphate and guanosine monophosphate in the presence of phosphoribosylpyrophosphate (10). Thus cells dependent only upon *de novo* purine synthesis would be more sensitive to the lethal effects of MTX and DCMTX (Table II). Work is in progress to delineate the biochemical basis for this collateral sensitivity. Preliminary results indicate that although L1210/6-MP cells accumulate less MTX than L1210/0 cells, and the sensitivity of the dihydrofolate reductase enzyme to MTX is the same in both the L1210/6-MP and L1210/0 cell populations, the level of dihydrofolate reductase activity in the L1210/6-MP cells is only about 30% of the enzyme activity found in the L1210/0 cells (J. Strobel-Stevens, personal communication). Whether increased sensitivity of the L1210/6-MP cell populations to MTX is at

TABLE II. SENSITIVITY OF CULTURED L1210/6-MP AND L1210/0 CELL POPULATIONS TO METHOTREXATE OR DICHLOROMETHOTREXATE FOR A 24-hr EXPOSURE PERIOD[a]

Agent	μg/ml[b]	S_f[c]		Ratio ± SE[d] S_f L1210/0	
		L1210/0	L1210/6-MP	S_f L1210/6-MP	*P* value[e]
MTX	0.055	264/400	144/4,000	18.3 ± 1.6	<0.001
	0.55	352/4,000	52/40,000	67.7 ± 10.0	<0.001
	5.5	28/40,000	4/40,000	7.0 ± 3.7	<0.001
Dichloro-MTX	1.0	556/4,000	636/40,000	8.7 ± 0.5	<0.001
	10.0	240/4,000	184/40,000	13.0 ± 1.3	<0.001
	100.0	536/40,000	28/40,000	19.1 ± 3.7	<0.001

[a] Proliferating cultured L1210/6-MP or L1210/0 cell populations were exposed to either MTX (0.055–5.5 μg/ml) or dichloro-MTX (1.0–100.0 μg/ml) for 24 hr and the number of cells surviving drug treatment was estimated by colony formation in semisolid medium as described under Materials and Methods.
[b] The TMC for MTX is approximately 55 μg/ml and for dichloro-MTX it is about 2040 μg/ml.
[c] Surviving fraction.
[d] Standard error of the ratio was estimated as described in Ref. (7).
[e] S_fs for each exposure period were compared by use of χ^2 analysis.

least partially due to decreased dihydrofolate reductase activity remains to be defined.

The cultured L1210/6-MP cell populations derived from L1210/6-MP lymphocytic leukemia have been propagated in culture in the absence of 6-MP for 200 population doublings and are resistant to 6-MP and collaterally sensitive to MTX or DCMTX (Tables I and II). We conclude that resistance to 6-MP and collateral sensitivity to the folate analogs in this system are properties of the L1210/6-MP cells and that the collateral sensitivity to the folate analogs are independent of any host factors.

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