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Mechanisms of Sensitivity and Natural Resistance to Antifolates in a Methylcholanthrene-Induced Rat Sarcoma

WEI-WEI LI, JAMES T. LIN,[1] BARRY I. SCHWEITZER, and JOSEPH R. BERTINO[2]
Laboratory of Molecular Pharmacology, Memorial Sloan-Kettering Cancer Center, New York, New York 10021
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SUMMARY

A methylcholanthrene-induced rat sarcoma that can be propagated *in vitro* or *in vivo* was evaluated for resistance to antifolates and was found to be relatively resistant to methotrexate and 10-ethyl-10-deazaaminopterin but sensitive to trimetrexate. Rat sarcoma cell extracts contained low levels of dihydrofolate reductase activity, the target enzyme of methotrexate, and inhibition of this enzyme by these three antifolates was similar. Transport studies showed poor uptake of both methotrexate and 10-ethyl-10-deazaaminopterin. In contrast, trimetrexate achieved high intracellular levels. The poor uptake of methotrexate was not due to lack of polyglutamylation. Thus, the basis for natural resistance to methotrexate and 10-ethyl-10-deazaaminopterin, compared with trimetrexate, in this rat sarcoma cell line was due to decreased transport of these drugs.

MTX is used to treat acute lymphocytic leukemias, diffuse lymphomas, head and neck cancer, breast cancer, and osteogenic sarcoma but has little activity against soft tissue sarcoma. Acquired resistance to MTX has been reported to be due to one or more of five mechanisms, 1) increase in DHFR due to gene amplification (1–3), 2) an alteration in DHFR resulting in decreased binding of MTX (4, 5), 3) decreased thymidylate synthase activity (6), 4) reduced formation of MTX polyglutamates (7–9), or 5) a decrease in the uptake of drug into cells (10, 11). However, relatively little information is available on the mechanisms of natural resistance and, in particular, the mechanisms for intrinsic MTX resistance in soft tissue sarcoma.

New antifolates such as 10-EDAM and TMTX have been developed with improved antitumor properties in experimental tumors, compared with MTX (12, 13). 10-EDAM is a classical antifolate that is a potent inhibitor of DHFR and is more effectively transported and polyglutamated than MTX in many tumor cells (14, 15). The nonclassical inhibitor TMTX is also a potent inhibitor of the enzyme DHFR, is not transported by the reduced folate carrier, and does not undergo intracellular polyglutamylation. TMTX has recently been reported to have some activity in patients with soft tissue sarcoma (16). We investigated the effects of MTX, 10-EDAM, and TMTX, using a methylcholanthrene-induced rat sarcoma as a model system, to correlate antitumor effects with biochemical determinants of sensitivity to MTX. This tumor can be propagated both *in vivo* and *in vitro*, thus allowing the opportunity to compare results obtained in cells from both conditions. The data presented in this paper indicate that this rat sarcoma is relatively resistant to MTX and 10-EDAM, as a consequence of poor uptake of these drugs, whereas it is sensitive to TMTX because of the high intracellular drug levels achieved.

Materials and Methods

Chemicals. MTX was obtained from Lederle Laboratories. TMTX, as the glucuronate salt, was supplied by Warner-Lambert/Parke-Davis (Ann Arbor, MI). 10-EDAM was obtained from CIBA-GEIGY. [5-^{3}H] 2′-Deoxyuridine, [^{3}H]MTX (20 Ci/mmol), and [3′,5′,7(N)-^{3}H]LV were purchased from Moravek Biochemicals (Brea, CA). NADPH was obtained from Sigma Chemical Co. (St. Louis, MO). Dihydrofolate was prepared by dithionite reduction of folic acid, as described by Blakley (17). Purified DHFR enzyme from HCT-8 cells was stored at −70°. Sephadex G-25 was purchased from Pharmacia Inc. (Piscataway, NJ). MTX polyglutamate standards were obtained from B. Schircks Laboratories (Jona, Switzerland). All other chemicals were of the highest purity and obtained from standard commercial sources.

Media, sera, and antibiotics for tissue culture were purchased from GIBCO (Grand Island, NY).

Tumor cell culture. Rat sarcoma cells were cultured using a modification of the method described by Nagashima *et al.* (18). A methylcholanthrene-induced sarcoma, obtained from Dr. M. F. Brennan (Memorial Sloan-Kettering Cancer Center), was maintained in CDF-1 rats. Tumor was excised and minced, incubated for 2 hr at 37° in RPMI 1640 medium with 10% FBS, 0.6% collagenase II (Worthington Biochemical), and 0.002% deoxyribonuclease I (Sigma), passed through a 100-mesh screen, washed twice with medium, and plated into

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[1]Norman and Rosita Winston Foundation Clinical Scholar.
[2]American Cancer Society Professor of Medicine and Pharmacology.

ABBREVIATIONS: MTX, methotrexate; 10-EDAM, 10-ethyl-10-deazaaminopterin; TMTX, trimetrexate; LV, leucovorin; DHFR, dihydrofolate reductase; FBS, fetal bovine serum; HPLC, high performance liquid chromatography; FMTX, fluoromethotrexate.

25-cm flasks. After two *in vitro* passages, tumor cells were cloned by the two-layer agar method (19). Twelve days later, one colony was picked, suspended in RPMI 1640 with 10% FBS, and cultured at 37° in a humidified atmosphere containing 5% CO_2. Cells were grown as a monolayer in a RPMI 1640 medium supplemented with 10% FBS, 100 units/ml penicillin, and 100 units/ml streptomycin and were subcultured once a week. The doubling time of exponentially growing cultured sarcoma cells was 20.4 hr. All drug treatments were performed on exponentially growing cells. A cloned subline of the human T lymphoblast cell line CCRF-CEM (20) was grown in RPMI 1640 supplemented with 10% FBS.

Clonogenic assay. The clonogenic assay was performed using a modification of the method of Hamburger and Salmon (19). Sarcoma cells were trypsinized for 2 min, and single-cell suspensions were obtained by passing the cells through a 26-gauge needle. After cells were exposed to a range of drug concentrations for 4 hr, the cells were harvested by centrifugation, washed twice with complete medium, resuspended in drug-free medium containing 10% dialyzed FBS, and cloned at a final cell concentration of 2×10^3 cells/ml, in 0.3% soft agar over 0.5% soft agar medium, in triplicate. For continuous exposure, cells were cloned in soft agar in the presence of known concentrations of the drugs. CCRF-CEM cell viability was measured by the same procedure as described above, but the cells were cloned in 0.1% soft agar over 0.5% soft agar medium. After 12 days of incubation, colonies were counted, and the results are expressed as a percentage of untreated control colony counts.

MTX resistance screening assay. The whole-cell *in situ* thymidylate synthesis assay (21), as modified by Rodenhuis *et al.* (22), was used. Cells were suspended at $1–2 \times 10^6$ cells/ml in complete medium, and six 1.5-ml aliquots were incubated for 3 hr at 37° in the presence of no drug, 0.1 μM MTX, 1.0 μM MTX, 1.0 μM 10-EDAM, 0.1 μM TMTX, or 5 μM FMTX. [5-^{3}H]2′-Deoxyuridine was added to a final concentration of 2 μCi/ml. A blank sample containing [5-^{3}H]2′-deoxyuridine in medium without cells and drugs was used for background subtraction. Samples (100-μl) were taken at 15-min intervals, placed in 200 μl of 15% actived charcoal suspension in 4% trichloroacetic acid to stop the reaction, and centrifuged for 5 min at 16,000 × *g*, a 100-μl aliquot was added to 5 ml of scintillation fluid, and the radioactivity was quantitated. Five aliquots were incubated for 4 hr in the presence of no drug, 1.0 μM MTX, 1.0 μM 10-EDAM, 1.0 μM TMTX, or 5.0 μM FMTX, washed twice in prewarmed complete medium, and resuspended in drug-free complete medium for another 4 hr. After this time period, [5-^{3}H]2′-deoxyuridine was added and the test was performed as described above. The results of the scintillation counting were analyzed by calculation of the slope of the ^{3}H release curve, using linear regression, and were expressed as a percentage of the slope of untreated control cells. Cells were considered resistant to drug for each particular condition if the corrected thymidylate synthesis activity was >20% of control activity.

DHFR activity assay. Logarithmically growing sarcoma cells in 150-cm^2 flasks were harvested by scraping with a rubber policeman, washed twice with cold phosphate-buffered saline, and resuspended in 50 mM Tris-HCl (pH 7.5) containing 100 mM KCl, 10% glycerol, 2 mM dithiothrietol, and 1 mM phenylmethylsulfonyl fluoride. Crude extracts were obtained by freeze-thawing the cells three times, followed by centrifugation at 16,000 × *g* for 30 min at 4°. The supernatant was used for the enzyme assays. For comparison studies, partially purified DHFR from CDF-1 rat liver was obtained by modification of the procedure of Rothenberg and Iqbal (23). Approximately 8 g of liver were minced and homogenized with 3 volumes of 0.1 M potassium phosphate buffer (pH 7.4). After centrifugation, the extract was treated with 2% protamine sulfate and zinc sulfate (16 mM), followed by ammonium sulfate precipitation (50–80% fraction) and chromatography on a Baker SPE-PEI anion exchange column. Protein concentration was measured by the Bio-Rad assay.

DHFR activity was determined spectrophotometrically, as described (24). Briefly, the decrease in absorbance at 340 nm that occurs when NADPH and dihydrofolate are converted to NADP and tetrahydrofolate, respectively, was measured at 37° using a Beckman DU-65 spectrophotometer (Beckman Instruments, Fullerton, CA). ED_{50} values were obtained by inhibition assays in which the drug was added at various concentrations to the standard assay mixture described above, which contained the enzyme (enzyme concentration was 3.2 nM and 3.9 nM for sarcoma and rat liver, respectively). From the inhibition curve for MTX, the picomoles of enzyme per 10^7 cells were calculated by extrapolating to the *x*-intercept, assuming a 1:1 stoichiometric binding of MTX to enzyme (25).

Drug uptake studies. Logarithmically growing cells were harvested by centrifugation. Single-cell suspensions were prepared as described, suspended in RPMI 1640 medium with 10% filtered FBS at a density of about 1×10^7 cells/ml, and incubated at 37° in the presence of 1 μM MTX, 10-EDAM, or TMTX. At the indicated times, 100-μl aliquots of cells were removed, added to 900 μl of ice-cold 0.9% saline solution in duplicate, centrifuged, and washed twice with ice-cold 0.9% NaCl. The pellets were then resuspended in 500 μl of 0.9% NaCl, boiled for 5 min, and centrifuged for 2 min at 16,000 × *g*. The clear supernatant fraction was then analyzed for drug content by DHFR enzyme inhibition (26). Uptake of [^{3}H]MTX or [^{3}H]LV in CCRF-CEM cells, as well as in sarcoma cells, was determined essentially as described by Pizzorno *et al.* (8).

Separation of DHFR-bound and free intracellular MTX. Sarcoma cells were grown to near-confluence, in 150-cm tissue flasks and in 30 ml of medium. The medium was removed, 30 ml of complete medium containing either 1 μM or 10 μM [^{3}H]MTX were added, and the cells were incubated for indicated times at 37°. At the end of the incubation, the cells were washed once with phosphate-buffered saline and resuspended in 750 μl of sodium phosphate buffer (0.1 M, pH 6.0) at 4°. The cells were then frozen and thawed three times and centrifuged at 16,000 × *g* for 30 min at 4°, and 500 μl of the supernatant were applied to a 2.2-cm × 8.5-cm Sephadex G-25 column equilibrated with 0.1 M sodium phosphate buffer, pH 6.0 at 4°. Protein-bound drug was eluted in the first peak, with 8 ml of the same buffer (1 ml/min). Column fractions (1 ml) were collected, added to 5 ml of Ecolume scintillation fluid, and counted, and the counts were converted to picomoles of MTX per 10^7 cells.

Analysis of intracellular levels of MTX polyglutamates. [^{3}H]MTX and unlabeled MTX were added to flasks of exponentially growing cells to yield the desired final concentration. Cell extracts for analysis of MTX polyglutamates were prepared by the method of Pizzorno *et al.* (8). At the indicated times, cells were harvested by scraping, transferred to glass tubes on ice, centrifuged (4°), and washed twice with ice-cold 0.9% NaCl. The cell pellet was suspended in 1 ml of boiling 50 mM sodium phosphate, pH 5.5, and boiled for 5 min. Cellular debris was removed by centrifugation at 20,000 × *g* for 10 min at 4°. The supernatant was stored at −20° until analysis by HPLC.

HPLC analysis of cell extracts was performed using a modification of the method of Cashmore *et al.* (27). Separations were done on a Spectra Physics 8800 HPLC gradient pump, using an Absorbosphere C-18 column (5-μm particle size; 4.6 mm × 250 mm) equipped with a 1-cm guard cartridge column of the same material from Alltech Asso-

TABLE 1

Inhibitory effects of MTX, 10-EDAM, and TMTX on colony formation in rat sarcoma and CCRF-CEM cells

The values are means of two or three different experiments. See Materials and Methods for details.

Drug	IC_{50}			
	Sarcoma		CCRF-CEM	
	4-hr	Continuous	4-hr	Continuous
	μM			
MTX	0.52	0.018	0.09	0.005
10-EDAM	0.12	0.005	0.04	0.003
TMTX	0.05	0.001	0.04	0.002

TABLE 2

Detection and classification of antifolate resistance in rat sarcoma cells

Values are mean ± standard error of three different experiments. The cells were assayed for thymidylate synthesis activity as described in Materials and Methods.

Cell line	Tritium release								
	3-hr incubation					4-hr efflux			
	MTX (0.1 μM)	MTX (1.0 μM)	10-EDAM (1.0 μM)	TMTX (0.1 μM)	FMTX (5.0 μM)	MTX (1.0 μM)	10-EDAM (1.0 μM)	TMTX (1.0 μM)	FMTX (5.0 μM)
	% of control								
Sarcoma	95.3 ± 3.0	60.7 ± 14.0	33.2 ± 3.4	17.0 ± 6.3	68.0 ± 5.0	101.5 ± 12.0	67.3 ± 2.0	25.3 ± 3.3	78.0 ± 8.9
CEM	11.3 ± 9.3	2.0 ± 1.4	0.3 ± 0.2	1.5 ± 1.2	0.8 ± 0.5	14.8 ± 12.0	1.4 ± 1.3	17.9 ± 9.6	106.5 ± 37.0

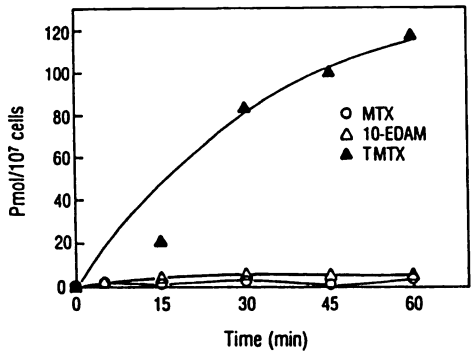

Fig. 1. Uptake of MTX, 10-EDAM, and TMTX in cultured sarcoma cells. The cells were incubated in the presence of 1.0 μM MTX, 10-EDAM, or TMTX. Intracellular drug concentrations at the time points indicated were analyzed by DHFR enzyme inhibition (27).

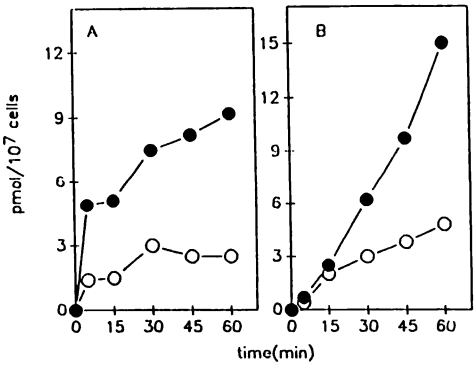

Fig. 2. Uptake of MTX and LV in cultured sarcoma and CCRF-CEM cells. Cells were exposed to 1 μM [^{3}H]MTX (A) or 1 μM [^{3}H]LV (B). At the indicated times, the cells were assayed for radioactivity as described in Materials and Methods. ○, Cultured sarcoma cells; ●, CCRF-CEM cells.

TABLE 3

Determination of DHFR-bound and free MTX in rat sarcoma cells

Cells were incubated with 1 or 10 μM [^{3}H]MTX for the indicated times, the cells were washed, and the amount of intracellular drug was separated into DHFR-bound and free fractions by using a Sephadex G-25 column, as described in Materials and Methods.

	MTX		
	Total	Bound	Free
	pmol/10⁷ cells		
1-hr exposure			
1 μM	5.0	5.0	0
10 μM	13.8	6.2	7.6
4-hr exposure			
10 μM	26.5	6.4	20.1

ciates. A 100-μl sample was injected. The mobile phase consisted of 0.1 M sodium acetate buffer (pH 5.5) and a linearly increasing gradient of acetonitrile (1–10%) over 40 min, at a flow rate of 1 ml/min; elution was then continued isocratically for another 10 min. The radioactivity in each 1-ml fraction was measured by using a Beckman LS5801 liquid scintillation counter, and these values were converted to picomoles of product formed per 10^7 cells. Sufficient chemically synthesized MTX polyglutamates were added to radiolabeled samples to provide an internal standard. The effluent was monitored at 315 nm with a Waters 99 photodiode array detector.

Results

Effects of MTX, 10-EDAM, and TMTX on colony formation of cells. Cultured sarcoma cells were exposed to MTX, 10-EDAM, or TMTX for 4 hr or continuously, and cytotoxicity was determined by the soft agar cloning assay. The results are shown in Table 1. The ED_{50} values for MTX and 10-EDAM after a 4-hr exposure were 0.52 and 0.12 μM, respectively, which were 6-fold and 3-fold higher than those in CCRF-CEM cells. In contrast, ED_{50} values for TMTX were similar in sarcoma and CCRF-CEM cells. Compared with the 4-hr exposure, continuous exposure to the three antifolates resulted in a more marked cell kill and, again, higher ED_{50} values were observed for MTX (0.018 μM) and 10-EDAM (0.005 μM), compared with TMTX (0.001 μM). In contrast, all three antifolates were equally potent against the CCRF-CEM cell line (0.002–0.005 μM).

Screening of MTX resistance. Using a screening system to detect resistance to MTX (22), the mechanisms of relative intrinsic MTX resistance were classified by measurement of the effects of MTX, 10-EDAM, and TMTX on whole-cell thymidylate synthesis activity (Table 2). CCRF-CEM cells showed marked sensitivity to all four antifolates after a 3-hr incubation in the presence of drug. After a 4-hr exposure to these drugs and then resuspension of the cells for 4 hr in drug-free medium, thymidylate synthesis activity was still inhibited by MTX, 10-EDAM, and TMTX, but not by FMTX. This latter compound was used as a positive control (28). In contrast, the sarcoma cells showed a different pattern of inhibition; after a 3-hr drug exposure period marked inhibition was only produced by TMTX, and after a 4-hr efflux period inhibition was maintained only in TMTX-treated cells.

DHFR activity. The level of DHFR enzyme activity in logarithmically growing sarcoma cells was 0.10 μmol/hr/mg of protein. The IC_{50} values for MTX, 10-EDAM, and TMTX were 3.95 ± 1.8, 3.60 ± 0.3, and 3.12 ± 1.8 nM (three experiments), respectively, which were comparable to the results from inhibition of normal rat liver DHFR by MTX, 10-EDAM, and TMTX (IC_{50} values were 2.70 ± 0.9, 3.12 ± 0.4, and 0.79 ± 0.2 nM, respectively). The amount of enzyme, based on extrapola-

TABLE 4

Intracellular concentration of MTX and MTX polyglutamates in rat sarcoma and CCRF-CEM cells

Logarithmically growing cells were exposed to [^{3}H]MTX, at 1 or 10 μM concentrations, for 4 or 24 hr. Intracellular metabolites were determined by HPLC. Glu_1 is MTX.

Cell line	MTX	4-NH_2-10-CH_3PteGlu$_n$								
		$n=1$	$n=2$	$n=3$	$n=4$	$n=5$	$n=6$	$n=7$	Total, $n=1$–7	Total, $n=3$–7
	μM	pmol/10^7 cells								
4-hr incubation										
Sarcoma	10	21.1	1.4	3.0	0.7	<0.1	<0.1	<0.1	26.2	3.7 (14%)
CEM[a]	10	24.0	11.8	21.1	3.4	1.4	1.0	0.9	63.6	27.8 (44%)
24-hr incubation										
Sarcoma	1	1.2	0.2	0.5	1.4	1.0	0.1	0.1	4.5	3.2 (69%)
	10	31.0	2.0	4.7	12.7	9.5	1.0	<0.1	60.9	29.9 (49%)
CEM[a]	10	72.9	46.2	142.1	28.1	15.4	2.5	1.7	308.9	190.2 (61%)

[a] From Ref. 8.

tion from the MTX inhibition curves, was 6.0 ± 0.1 pmol/10^7 cells.

Uptake of MTX, 10-EDAM, TMTX, and LV. At an extracellular concentration of 1.0 μM, the amount of drug uptake, as assessed by the enzyme inhibition assay, was only 4.2 pmol/10^7 cells for MTX and 5.5 pmol/10^7 cells for 10-EDAM, after a 60-min incubation. In contrast, the uptake of TMTX at the same concentration reached a level of 117 pmol/10^7 cells without reaching a plateau (Fig. 1). Uptake of [^{3}H]MTX and [^{3}H]LV was 2.5 and 4.8 pmol/10^7 cells, respectively, at 60 min in sarcoma cells, about 3–4-fold lower than the levels detected in CCRF-CEM cells (9.2 and 15.0 pmol/10^7 cells, respectively) (Fig. 2). Even when sarcoma cells were exposed to 1 μM [^{3}H]MTX for 4 hr, accumulation of MTX was only 4.4 pmol/10^7 cells.

DHFR-bound and intracellular free MTX. The studies described above indicate that the cause of MTX intrinsic resistance was the lack of ability of sarcoma cells to achieve a sufficient intracellular concentration to completely inhibit DHFR activity. To further establish this, the distribution of bound and free intracellular MTX after a 1-hr exposure to 1.0 μM [^{3}H]MTX was determined using G-25 Sephadex chromatography (Table 3). All of the [^{3}H]MTX was bound to protein, consistent with the fact that the amount of MTX entering into cells in 1 hr was less than the binding capacity of DHFR. When cells were incubated in 10 μM MTX for either 1 or 4 hr, free MTX was detected. The amount of protein-bound MTX was 6.2–6.4 pmol/10^7 cells, consistent with the level of DHFR measured by extrapolation of MTX inhibition curves.

MTX polyglutamate formation. MTX polyglutamate formation was assessed after exposure to two different concentrations of MTX (1 and 10 μM) for 4 and 24 hr (Table 4). After a 4-hr exposure at 10 μM, most of the drug remained in the parent form, with only 3.7 pmol/10^7 cells (14%) in the triglutamate or longer chain forms. After a 24-hr exposure at 1 μM, most of the intracellular drug was metabolized to polyglutamate forms, with the tetra- and pentaglutamate forms predominating. However, the total amount of MTX and its metabolites was still only 4.5 pmol/10^7 cells, less than the total amount of DHFR in the cells. Even after a 24-hr exposure at 10 μM, the total amount of MTX in these cells was 5.1-fold lower than that in CCRF-CEM cells incubated under the same conditions, although the distribution of MTX and its polyglutamates (Glu_{3-7}) was similar to that obtained in sensitive CCRF-CEM cells (8).

Discussion

The sarcoma cells propagated *in vitro* used in our experiments were obtained from a methylcholanthrene-induced sarcoma grown in rats. Cytotoxicity results indicated that these cells were relatively insensitive to MTX, compared with the CCRF-CEM cells. Thymidylate synthesis measured in intact cells was not inhibited completely, even in the presence of 1.0 μM MTX, and recovery occurred rapidly once the drug was removed. In contrast, these cells were highly sensitive to TMTX, an antifolate that enters cells through a pathway independent from that of MTX. Results obtained with 10-EDAM indicated that this drug was more effective than MTX, but not as effective as TMTX. In studies reported elsewhere (29), *in vivo* studies with maximally tolerated doses of these drugs confirmed the *in vitro* studies, in that the order of effectiveness for tumor regression was TMTX ≫ 10-EDAM > MTX.

Based on the screening assay for MTX resistance (22), these sarcoma cells appeared to be resistant on the basis of decreased uptake and possibly also decreased polyglutamylation. To confirm and distinguish between these mechanisms, specific assays were performed. The level of DHFR measured in these cells was similar to that in a sensitive CCRF-CEM cell line reported by Pizzorno *et al.* (8). Inhibition of DHFR by MTX, 10-EDAM, and TMTX was similar to that of normal rat liver DHFR; therefore, the differences in toxicity could not be explained by altered binding of these antifolates to DHFR. Long-chain MTX polyglutamates (Glu_{3-5}) were formed in these cells after a 24-hr exposure to this drug even at a 1 μM concentration, suggesting that impaired MTX polyglutamylation was also not a cause of natural resistance to MTX.

The following suggests that intrinsic MTX resistance was due to poor uptake of this drug into these cells: 1) the 1-hr uptake of MTX at a 1 μM concentration was only 2.5–4.2 pmol/10^7 cells; even after 24 hr, the amount of intracellular MTX was still lower than the level of DHFR in these cells (6.0–6.4 pmol/10^7 cells), consistent with the observed partial inhibition of thymidylate synthesis activity at this concentration; 2) uptake of MTX was about 3.7-fold lower in sarcoma cells than in sensitive CCRF-CEM cells; 3) these cells had relatively low uptake of 10-EDAM and were also relatively resistant to 10-EDAM, which also utilizes the reduced folate carrier used by MTX (10, 30); 4) sarcoma cells also had decreased uptake of the reduced folate LV, compared with sensitive CCRF-CEM cells; and 5) the sarcoma cells were sensitive to TMTX, which

enters cells independently of the reduced folate transport system (31–33).

Although decreased uptake of MTX may also be a result of decreased polyglutamylation of MTX (34, 35), this does not appear to be the case in these sarcoma cells, because a high percentage of intracellular MTX was metabolized to long-chain polyglutamate forms after a 24-hr exposure to drug. Although controversy remains regarding the presence of other possible folate transport routes (11, 36), results from various laboratories suggest that MTX and reduced folate compounds enter cells via a low affinity, high capacity, transport system (37–39). Uptake of LV at the 1 μM concentration in these sarcoma cells is also 3.1-fold lower than that found in CCRF-CEM cells, suggesting that these cells have either a decreased amount of folate transport protein or an altered protein with decreased affinity for both natural folates and classical antifolates such as MTX and 10-EDAM.

These results encourage the use of these methods to assess the relative responsiveness of human sarcoma cells to folate antagonists, with the hope of predicting response.

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Send reprint requests to: Joseph R. Bertino, 601 RRL, 430 E. 67th Street, Memorial Sloan Kettering Cancer Center, New York, NY 10021.