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LEUCOVORIN RESCUE OF HUMAN CANCER AND BONE MARROW CELLS FOLLOWING EDATREXATE OR METHOTREXATE

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Abstract—We have examined the cytotoxic activities of edatrexate (EDX) and methotrexate (MTX) and their reversal by leucovorin in nine human cancer cell lines and in human bone marrow CFU-GM cells. EDX was 3.7- to 123-fold more toxic than MTX against the cancer cell lines and 25-fold against the bone marrow cells. Lower EDX concentrates generally were needed to inhibit cancer cell growth relative to bone marrow cells, however, whereas bone marrow and cancer cell growth were more often susceptible to the same MTX concentrations. The new antifolate was metabolized to long-chain polyglutamates to a greater extent than MTX in seven cell lines. Leucovorin at 0.2 μM rescued two breast cancer and two non-small cell lung cancer cell lines to a lesser extent following EDX than MTX, but significant rescue was observed in two head and neck cancer cell lines that formed large amounts of polyglutamates. These cell lines also accumulated reduced folates to a greater extent than the other cell lines following leucovorin exposure. Leucovorin rescued bone marrow cells following MTX but only partially following the highest EDX concentrations. EDX may enjoy a better therapeutic index than MTX against some cancer cell lines relative to bone marrow precursor cells, especially after leucovorin rescue.

Key words: edatrexate; methotrexate; leucovorin; human cancer cells; human bone marrow cells

EDX¶ is a novel antifolate which, like MTX, exerts its antitumor effects by inhibiting the enzyme dihydrofolate reductase [1]. The new antifolate is better transported across the cell membrane than MTX through the same reduced folate carrier and also has enhanced affinity for folylpolyglutamate synthetase, the enzyme responsible for the addition of γ-glutamyl residues to folates and antifolates. MTX polyglutamates, particularly those containing a total of three or more polyglutamates, are important for the cytotoxic effects of MTX after short cellular exposures since they are retained intracellular and continue to exert the cytotoxic effects of the drug for prolonged periods after removal of extracellular MTX [2]. The improved transport and polyglutamylation characteristics of EDX explain its increased activity *in vitro* and *in vivo* against murine tumors compared with MTX [1]. However, the increased propensity of EDX for polyglutamylation does not extend to normal murine tissues, thus explaining its improved experimental therapeutic index [1]. Clinical trials have confirmed that the new antifolate is active against non-oat cell lung [3], breast [4], non-Hodgkin's lymphoma [1] and head and neck cancer [5]. The side-effects of EDX consist mainly of leucopenia and mucositis with the latter often being dose-limiting, especially in combination with other chemotherapeutic agents [6]. This had led to laboratory and clinical studies examining EDX administration followed by rescue with the reduced folate leucovorin (5-formyltetrahydrofolate, folinic acid). Early experimental and clinical data have suggested that leucovorin can selectively rescue normal tissue from the toxic effects of EDX without compromising its antitumor activity [7, 8].

We have further examined the selective rescue from the cytotoxic effects of EDX by leucovorin in nine human breast, non-small cell lung and head and neck cancer cell lines and in human bone marrow CFU-GM cells as a measure of normal tissue rescue. Furthermore, we have compared the reversal of the cytotoxic effects of EDX and MTX by leucovorin and correlated these observations with antifolate polyglutamate metabolism and intracellular folate accumulation after leucovorin exposure.

MATERIALS AND METHODS

Chemicals. Unlabelled MTX and leucovorin,

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¶ Abbreviations: EDX, edatrexate; MTX, methotrexate; DMEM, Dulbecco's minimum essential medium; IMDM, Iscove's Modified Dulbecco's Medium; FBS, fetal bovine serum; dFBS, dialysed FBS; CFU-GM, granulocyte-macrophage colony-forming unit; GM-CSF, granulocyte-macrophage colony-stimulating factor; IC_{50}, drug concentrations inhibiting cell growth to 50% of control growth; and CFU-E, late human erythroid marrow progenitor cells.

respectively, were purchased from the Sigma Chemical Co. (St. Louis, MO) and B. Schircks Laboratories (Jona, Switzerland), and EDX was provided by Ciba-Geigy Ltd. (Basel, Switzerland). [3',5',7,9-^{3}H]MTX (19 Ci/mmol), [^{3}H]leucovorin (6RS-5-HCO-H_4PteGlu) (3 Ci/mmol) and [^{3}H]-EDX (15 Ci/mmol) were purchased from Moravek Biochemicals (Brea, CA), purified by reverse phase HPLC [9], and kept at −80° prior to use. All other chemicals were obtained from the Fisher Scientific Co. (Pittsburgh, PA) or Sigma.

Cell lines. The cell lines were chosen to represent tumor sites where EDX has demonstrated clinical antitumor activity. The ZR-75-1 human breast cancer cell line [10] was obtained from the National Cancer Institute (Bethesda, MD), while the MCF-7 and MDA-231 breast cancer cells, the CALU-1 epidermoid and the A549 adenocarcinoma lung cancer cell lines were purchased from the American Type Culture Collection (Rockville, MD). The QU-DB large cell lung carcinoma cell line [11] was obtained from Dr. B. Campling (Kingston, Ontario, Canada) and the SCC-14, -11B and -22B human head and neck cancer cell lines were obtained from Dr. T. E. Carey (University of Michigan, Ann Arbor, MI) [12]. All the cell lines were grown as monolayers. The breast cancer lines were kept in DMEM (Gibco Laboratories, Chagrin Falls, OH) supplemented with 10% FBS (Gibco), penicillin (200 μg/mL) and streptomycin (200 μg/mL) under 5% CO_2 at 37°. The other lines were maintained in RPMI-1640 with the same additives. The dFBS used for the cytotoxicity experiments was obtained by dialysing FBS against phosphate-buffered saline at 4° until less than 1% of the [^{3}H]thymidine added before the procedure remained.

Cell growth inhibition studies. For the cell growth inhibition studies, 1×10^5 cells were plated in petri dishes 24–48 hr prior to drug incubation. Following drug exposure, the cells were washed with drug-free medium and two different growth media were added: 10% dFBS with no further additions to the regular growth medium or with 0.2 μM leucovorin added. This concentration was chosen to achieve 0.1 μM of the biologically active 6*S* isomer of 5-formyltetrahydrofolate in the culture medium, a concentration representative of the reduced folate serum concentrations achieved after oral low-dose (10–15 mg) leucovorin administration [13]. Cells were counted after 2–3 doubling times of untreated cells.

Human bone marrow CFU-GM studies. Normal human bone marrow from cancer patients was made available by the bone marrow transplantation unit at the Netherlands Cancer Institute in Amsterdam from patients who had no cancer involvement in the bone marrow but could not undergo transplantation after the bone marrow had been harvested. After thawing, the mononuclear bone marrow cells were suspended in IMDM supplemented with 10% dFCS and allowed to stand for 1 hr at 37° in a humidified 5% CO_2 incubator prior to drug incubation. Aliquots were then incubated for 3 hr with either MTX or EDX. After incubation, the cells were washed twice in drug-free medium and plated for the CFU-GM assay as previously described by Iscove *et al.* [14, 15] in 0.8% methylcellulose, 20% dFBS and 50 ng/mL recombinant GM-CSF. Leucovorin concentrations in the CFU-GM assay were either 5 nM (control) or

Table 1. Leucovorin rescue from antifolate toxicity in human cancer cell lines

Cell lines	Doubling time (hr)	MTX IC_{50} (μM)		EDX IC_{50} (μM)		MTX_C/EDX_C	MTX_L/EDX_L
		C	L	C	L		
Breast cancer							
ZR-75	35	15 ± 3	67 ± 12	0.24 ± 0.04	0.18 ± 0.09	63	372
MCF-7	40	15 ± 5	CR*	0.66 ± 0.17	4.2 ± 2.0	23	MTX CR*
MDA-231	28	298 ± 34	323 ± 72	66 ± 5	60 ± 11	4.5	5.4
Non-small cell lung cancer							
CALU-1	28	27 ± 2.9	CR	0.22 ± 0.02	0.33 ± 0.04	123	MTX CR
A549	28	4.2 ± 0.9	133 ± 20	0.29 ± 0.09	4.7 ± 0.9	15	28.3
QU-DB	26	19 ± 0.5	42 ± 5	5.1 ± 0.8	8.7 ± 1.5	3.7	4.8
Head and neck cancer							
SCC-11B	38	40 ± 8	150 ± 35	0.45 ± 0.10	4 ± 0.6	89	38
SCC-22B	43	3.5 ± 0.6	10 ± 3	0.07 ± 0.01	0.5 ± 0.1	50	20
SCC-14C	36	850 ± 240	>1000†	70 ± 15	300 ± 70	12	>33†

Growth curves were obtained following 3-hr incubations with various concentrations of either EDX or MTX after which the medium was aspirated, cells were washed, and either standard medium containing 10% dialysed FBS (C) or the same medium with 0.2 μM leucovorin (L) was added. The mean cell doubling times for control untreated cells grown in standard medium are shown for each cell line (N = 6–10). Mean IC_{50} values with standard deviations were determined from 4–6 experiments. The ratios of MTX to EDX IC_{50} values in control experiments (MTX_C/EDX_C) and following leucovorin (MTX_L/EDX_L) are also shown.

* CR represents complete leucovorin rescue to control growth rates and MTX CR complete rescue following MTX.

† The IC_{50} could not be determined because of the high antifolate concentrations.

0.2 μM (rescue). After 12–14 days of culture, CFU-GM, defined as granulocytic, monocytic or eosinophilic aggregates of more than 40 cells, were counted.

Antifolate polyglutamate determinations. MTX and EDX polyglutamates were assayed using the HPLC system previously described for MTX polyglutamates [16]. Retention times for MTX and EDX were similar, and tritiated peaks following EDX exposure had similar retention times to MTX polyglutamates using synthesized standards purchased from B. Schircks Laboratories. Since no EDX polyglutamates were available as standards, their chain length was estimated from their elution order following EDX and in comparison to the MTX polyglutamate standards.

Intracellular folate determinations. Cells were plated as for the growth inhibition studies and exposed for 3 hr to either EDX or MTX or to no antifolate. The cells were then washed and exposed for 2 days to 0.2 μM [^{3}H]leucovorin in the same medium used for the growth inhibition studies. Thereafter, they were washed three times in ice-cold phosphate-buffered saline, and scraped in water to lyse the cells. Total intracellular folate accumulation was determined in the lysate by scintillation counting and protein content by the Lowry assay.

RESULTS

Leucovorin rescue from antifolate toxicity in human cancer cell lines. The results of the growth inhibition studies are shown in Table 1. The two antifolate-sensitive breast cancer cell lines (ZR-75 and MCF-7) were respectively, 63- and 23-fold more sensitive to EDX than to MTX and both were rescued by leucovorin to a lesser extent after exposure to EDX than to MTX. The MDA-231 breast cancer cells were relatively resistant to antifolates and only 4.5-fold more sensitive to EDX but were not rescued by leucovorin after either antifolate. Two non-small cell lung cancer cell lines (CALU-1 and A549) were, respectively, 123- and 15-fold more sensitive to EDX and also less likely to be rescued by leucovorin after exposure to EDX. The QU-DB lung cancer cells were only 3.7-fold more sensitive to EDX than to MTX and were rescued similarly after exposure to either antifolate. The two drug-sensitive head and neck cell lines (SCC-11B and SCC-22B) were 89- and 50-fold more sensitive to EDX but, contrary to the breast and non-small cell lung cell lines, both were rescued to a greater extent following EDX than MTX. The SCC-14C cells were highly antifolate resistant but still 12-fold more sensitive to EDX and could be rescued by leucovorin after either antifolate.

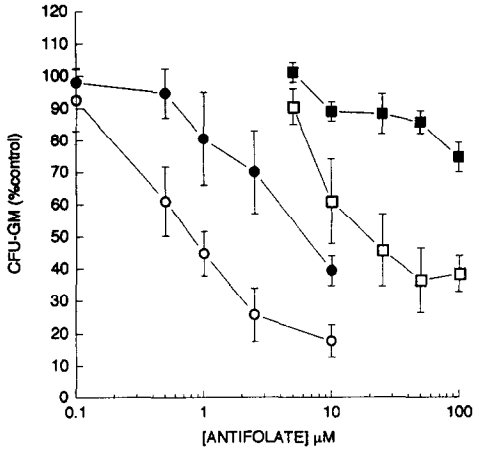

Fig. 1. Leucovorin rescue from antifolate toxicity in human bone marrow granulocyte-monocyte colony-forming cells. Human bone marrow samples were exposed to either MTX or EDX for 3 hr, and cytotoxicity was determined by counting colonies growing in agar. Values are the means ± SEM of results from three different bone marrows. There were, respectively, 99, 119 and 266 control CFU-GM colonies from 10^5 plated mononuclear cells in each bone marrow. Key: (○) colonies growing in 5 nM leucovorin and (●) colonies growing in 0.2 μM leucovorin following EDX exposure; (□) colonies growing in 5 nM leucovorin and (■) colonies growing in 0.2 μM leucovorin following MTX exposure.

Leucovorin rescue from antifolate toxicity in human bone marrow granulocyte-monocyte colony-forming cells. As illustrated in Fig. 1, EDX was 25-fold more toxic than MTX to CFU-GM cells in human bone marrow. Leucovorin at 0.2 μM almost completely rescued the marrow from up to 100 μM MTX, whereas there was only partial rescue from higher EDX concentrations. The EDX IC_{50} in the marrow CFU-GM assay was 0.8 μM, a value higher than the EDX IC_{50} for six of the seven antifolate-sensitive cancer cell lines. On the other hand, the MTX IC_{50} (20 μM) was in the same range as the corresponding IC_{50} values for five of the seven sensitive cancer cell lines. In a parallel experiment performed in two of the bone marrow samples, we observed that the number of CFU-E cells decreased by 20 and 62% following 3-hr incubations with 100 μM MTX and 10 μM EDX, respectively. However, 0.2 μM leucovorin completely rescued CFU-E from both antifolates.

Antifolate polyglutamate metabolism studies. We next examined antifolate polyglutamate formation and retention following 3-hr incubations with various antifolate concentrations yielding similar and >60% cell growth inhibition in each cell line. Results are shown in Table 2. EDX was metabolized more efficiently to longer chain length (≥3) polyglutamate derivatives than MTX with consequently greater drug retention in all but two cell lines. There was less EDX metabolism in the relatively EDX-resistant QU-DB cells compared with the other antifolate-sensitive cell lines and significantly more metabolism in the two drug-sensitive head and neck cell lines. Polyglutamate metabolism was examined using high antifolate concentrations in the drug-resistant cell lines. No long-chain metabolites were seen in the MDA-231 cells, but there was significant metabolism in the SCC-14C cells although very little polyglutamates could be identified in these cells at lower antifolate concentrations (data not shown).

Intracellular folate accumulation following leucovorin exposure in human cancer cell lines. Total intracellular folate accumulation was also examined following leucovorin exposure, as illustrated in Fig.

Table 2. Antifolate polyglutamate metabolism studies

		Antifolate polyglutamates			
		3-hr Accumulation (nmol/g)		24-hr Retention (nmol/g)	
Cell line	[Antifolate]	$Glu_{\leq 2}$	$Glu_{\geq 3}$	$Glu_{\leq 2}$	$Glu_{\geq 3}$
ZR-75	MTX 50 μM	114 ± 47.7	0	3.97 ± 0.7	0
	EDX 1 μM	23.9 ± 5.6	25.4 ± 5.3	0	10.2 ± 0.7
MCF-7	MTX 50 μM	84.8 ± 18.7	0	3.97 ± 0.3	0
	EDX 1 μM	34.8 ± 4.6	12.7 ± 2.6	0	12.6 ± 2.6
MDA-231*	MTX 500 μM	3002	0	53	0
	EDX 100 μM	1944	0	14.7	0
CALU-1	MTX 50 μM	56.6 ± 12.4	0	4.08 ± 0.5	0
	EDX 1 μM	36.8 ± 3.0	9.20 ± 0.8	3.83 ± 1.0	7.40 ± 1.0
A549	MTX 100 μM	19.8 ± 4.5	8.92 ± 1.6	1.61 ± 0.3	5.24 ± 0.4
	EDX 10 μM	12.3 ± 1.4	23.0 ± 2.2	2.65 ± 0.6	12.6 ± 2.4
QU-DB	MTX 50 μM	151 ± 15.4	12.0 ± 2.0	6.69 ± 1.6	2.38 ± 0.4
	EDX 10 μM	69.0 ± 16.5	2.37 ± 1.1	4.31 ± 1.4	5.62 ± 2.2
SCC-11B	MTX 50 μM	79.0 ± 5.7	116 ± 11.5	30.4 ± 5.7	93.7 ± 10.4
	EDX 1 μM	35.7 ± 8.4	179 ± 16.6	26.2 ± 12.9	122 ± 9.3
SCC-22B	MTX 50 μM	158 ± 20.3	322 ± 35.7	56.6 ± 14.0	192 ± 18.3
	EDX 1 μM	78.2 ± 18.4	579 ± 74.3	41.0 ± 10.1	342 ± 32.1
SCC-14C*	MTX 1000 μM	130.5	62.6	9.1	11.0
	EDX 100 μM	150	139	40.6	93.9

MTX and EDX polyglutamates were assayed by HPLC after 3-hr [^{3}H]EDX or [^{3}H]MTX incubations at various concentrations (3-hr accumulation) and after an additional 24 hr following drug removal (24-hr retention). The various antifolate concentrations [antifolate] used for the metabolism studies were chosen because they yielded similar and >60% cell growth inhibition in each cell line. $Glu_{\leq 2}$ represents the mean sum and standard deviation derived from three experiments of the metabolites containing up to a total of 2 glutamyl residues, while $Glu_{\geq 3}$ represents metabolites containing 3 residues and more.

* Only one experiment could be performed in the MDA-231 and SCC-14C cell lines because of the large quantities of radiolabelled material needed to perform polyglutamate determinations at equitoxic antifolate concentrations.

2. Intracellular labelled folates were measured after 2-day incubations with 0.2 μM tritiated leucovorin in untreated control cells or following 3-hr exposures to either MTX or EDX. The concentrations of unlabelled antifolates used were the same as those for the experiments reported in Table 2. The breast cancer MDA-231 cells had markedly decreased folate intracellular accumulation, thus explaining the lack of leucovorin rescue observed following both MTX and EDX. Conversely, the two antifolate-sensitive head and neck cancer cell lines (SCC-11B and -22B) had markedly increased folate accumulation compared to the other breast and lung cancer cell lines. This can probably account for the leucovorin rescue observed in these cell lines despite their extensive antifolate polyglutamate metabolism and retention.

DISCUSSION

EDX was more cytotoxic than MTX in all the cell lines examined. This is consistent with the published experimental data [1] and confirms the enhanced cytotoxic potential of the new antifolate over MTX in a series of human cancer cell lines representative of tumor sites where EDX has demonstrated clinical activity [3–5]. EDX was also more toxic to human bone marrow precursor cells than MTX with leucovorin reversing the toxicity except at the highest EDX concentrations. These data, along with the clinical experience suggesting enhanced mucositis following EDX, suggest that, contrary to the murine data [1], normal human tissue EDX polyglutamylation may also be increased relative to MTX. However, lower EDX concentrations were generally needed to inhibit cancer cell growth relative to bone marrow GM-CSF cells, whereas bone marrow and cancer cell growth were more often susceptible to the same MTX concentrations. These data suggest that, despite its greater bone-marrow toxicity, EDX may enjoy a better therapeutic index than MTX against some cancer cell lines relative to bone marrow precursor cells.

Antifolate polyglutamates are thought to be important determinants of tumor cell sensitivity after short-term antifolate exposures by acting as active retainable forms of the drug after its extracellular disappearance [2]. Our cytotoxicity and metabolism experiments, done simultaneously in nine human cell lines, permitted a reevaluation of this concept. Using different but roughly equitoxic MTX and EDX drug concentrations, we interestingly could not confirm the presence of any consistent correlation between cytotoxicity and extent of metabolism and

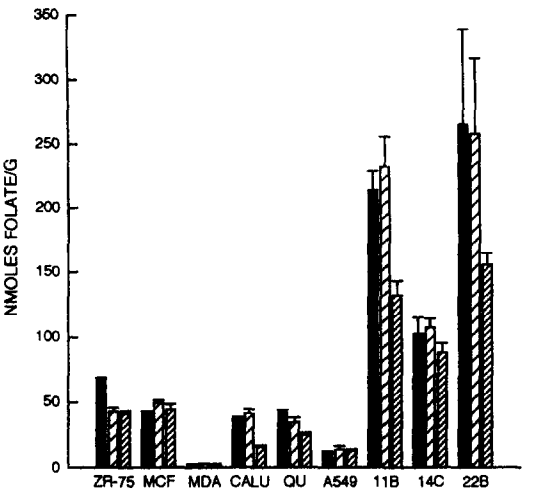

Fig. 2. Intracellular folate accumulation following leucovorin exposure in human cancer cell lines. Cells were plated as for the growth curve studies and exposed for 3 hr to either MTX (solid bars), EDX (coarsley hatched bars), both at the same concentrations as for the polyglutamate studies (Table 2), or to no antifolate (finely hatched bars). Total intracellular folate accumulation was determined following an additional 2-day exposure to 0.2 μM [^{3}H]-leucovorin. Results are expressed in nanomoles folate per gram total protein and are the means ± SD of three experiments.

polyglutamate retention in these cell lines. While we are unable to explain this observation, these data highlight the fact that polyglutamate metabolism is not the sole factor involved in antifolate cytotoxicity, and that other determinants such as the levels of dihydrofolate reductase, thymidylate synthase and the folate-dependent enzymes of *de novo* purine synthesis and possible modifications in their expression following antifolate exposure [17] probably all have to be accounted for to explain antifolate cytotoxicity in a given cell line.

The higher MTX concentrations used compared with EDX for the metabolism experiments could have prevented polyglutamate metabolism since high substrate concentrations have been shown to impair elongation of the polyglutamate chain by folylpolyglutamate synthetase *in vitro* [18]. Previous data obtained in the breast cancer cell lines revealed, however, that little MTX polyglutamylation can be seen after short (<6 hr) exposures to lower (2 μM) MTX concentrations [16]. The extensive metabolism of EDX observed after only 3-hr drug incubations in these cell lines demonstrates its enhanced potential for polyglutamylation.

The reversibility of antifolate cytotoxicity by leucovorin is also thought to be largely dependent on the extent of antifolate metabolism to polyglutamate derivatives. MTX polyglutamates prevent leucovorin rescue by directly competing at dihydrofolate reductase, thymidylate synthase and the folate-dependent enzymes of *de novo* purine synthesis with the reduced folates accumulating intracellularly following leucovorin exposure [19, 20]. Tumor cells with long-chain antifolate polyglutamate formation and retention are rescued competitively only by higher leucovorin concentrations, whereas normal tissues, such as human bone marrow precursor cells, can be rescued by lower leucovorin concentrations since they metabolize MTX to polyglutamates to a considerably lesser extent than many tumor cell lines [21]. Larger EDX polyglutamate concentrations should have consistently reduced the potential for leucovorin to rescue the tumor cells following exposure to the new antifolate. Our results are consistent with this in four of the antifolate-sensitive cancer cell lines examined (breast MCF-7 and ZR-75, non-small cell CALU-1 and A549). The QU-DB non-small cell lung cancer cells showed little but similar leucovorin rescue and similar amounts of retained polyglutamates after either antifolate. The patterns of antifolate polyglutamate retention were not consistent with the outcome of leucovorin rescue, however, in the two head and neck antifolate-sensitive cell lines. These had the most extensive long-chain antifolate polyglutamylation and retention of all the cell lines studied, probably because of a higher folypolyglutamate synthetase activity [22] compared with the ZR-75 cells [23], the only other cell line of the panel in which this activity has been measured.

The folate accumulation experiments following leucovorin exposure yielded some insights into the reasons behind leucovorin rescue in these cells and the lack of rescue in the MDA-231 MTX-resistant cell lines. The two head and neck antifolate-sensitive cell lines accumulated intracellular folates to a much greater extent than the other drug-sensitive cell lines after leucovorin exposure. These experiments cannot define the mechanism behind the varying levels of folate uptake between the cell lines since intracellular folate accumulation depends both on folate transport and its intracellular metabolism. They suggest, however, that the conditions facilitating antifolate metabolism and accumulation in these cell lines also enhanced reduced folate accumulation, thus negating the potential regulatory effects of antifolate polyglutamates on leucovorin rescue. It remains unclear why more leucovorin rescue was seen following EDX than MTX in these cell lines. The breast cancer MDA-231 cells had markedly decreased folate intracellular accumulation, thus explaining the lack of leucovorin rescue observed following both MTX and EDX.

The effectiveness of high-dose therapy followed by leucovorin rescue in antifolate-resistant cell lines thus depends on the underlying mechanism of resistance. This therapeutic approach is potentially most useful in the MDA-231 cells with defects in both antifolate and leucovorin intracellular accumulation in which high-dose antifolate therapy overcomes drug resistance while leucovorin cannot rescue the tumor cells. The SCC-14C head and neck cells, however, present a prominent impairment in polyglutamylation due to decreased folyl-polyglutamate synthetase activity with no significant defect in the reduced folate carrier compared with the two antifolate-sensitive head and neck cancer cell lines [22, 24]. This explains their high level

resistance to the short drug incubations used and the success of leucovorin rescue.

There is only limited experimental and clinical data on the use of leucovorin rescue following EDX. Early evidence of selective rescue in experimental murine tumors [7] led to recent studies in which high dose EDX followed by leucovorin rescue greatly increased the therapeutic index of EDX against a number of murine tumors *in vivo* [25]. Preliminary human studies have also suggested that high dose EDX with leucovorin rescue can be tolerated by patients although they seem to present a higher incidence of mucositis than seen with similar high dose MTX protocols [26].

In conclusion, EDX was more cytotoxic than MTX in nine human cancer cell lines and human bone marrow precursor cells. Lower EDX concentrations generally were needed to inhibit cancer cell growth relative to bone marrow GM-CSF cells compared with MTX, suggesting that EDX may enjoy a better therapeutic index than MTX in certain cancer cell lines relative to bone marrow precursor cells. The new antifolate was also metabolized to polyglutamates to a greater extent than MTX in almost all the cell lines. These metabolites prevented rescue from low concentrations of leucovorin in two breast cancer and two non-small cell lung cancer cell lines but not in the head and neck cancer cell lines, which efficiently accumulated reduced folates following leucovorin exposure. Thus, EDX may enjoy a better therapeutic index than MTX against some cancer cell lines relative to bone marrow precursor cells especially after leucovorin rescue.

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