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Improved Therapeutic Index of Methotrexate with "Leucovorin Rescue" [1]

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SUMMARY

The effect of "leucovorin rescue" on the therapeutic index of methotrexate in epidermoid carcinomas of the head and neck was studied. Sixteen patients received maximally tolerated doses of methotrexate alone as 30-hr i.v. infusions at 2-week intervals and 44% responded. Significant hematological toxicity and 4 drug-related deaths were incurred. Twenty-five patients received higher doses of methotrexate followed by leucovorin rescue as 36- to 42-hr i.v. infusions every 2 weeks. This group experienced a response rate of 60% with fewer cases of severe toxicity. Seventeen of this group received more than 500 mg/sq m as a maximal dose and had a response rate of 76% with two drug-related deaths, while those receiving smaller doses had a response rate of only 25%. Serum methotrexate levels were proportional to the dose of drug. Leucovorin rescue enhances the therapeutic index of high-dose methotrexate infusions in epidermoid carcinomas of the head and neck.

INTRODUCTION

In recent years, the mechanisms of action and the spectrum of diseases in which MTX[6] is therapeutically effective have been studied extensively in both animal model systems and patients. The effectiveness of MTX in the treatment of acute leukemia of childhood (1, 9, 21, 35), choriocarcinoma (30), Burkitt's lymphoma (19), mycosis fungoides (42), and a number of nonneoplastic conditions (12, 40) has been demonstrated. The value of this agent in the therapy of a number of other solid tumors (2, 38) including bronchogenic carcinoma (4, 36), breast carcinoma (13), cervical and vaginal carcinoma (18), and epidermoid carcinomas of the head and neck (6, 27-29, 32, 33, 34, 39) is less well defined. The overall purpose of the current study was to obtain further information concerning the optimal antineoplastic use of MTX.

The demonstration that LR improves the therapeutic index of MTX in mouse leukemias (14, 15, 17) and may have a similar effect in patients (6, 10, 28, 32, 38, 39, 41) prompted this study in which maximally tolerable doses of MTX alone were compared with those achievable when LR was added. Patients with epidermoid neoplasms of the head and neck were chosen for the study because of the relative responsiveness of these tumors to MTX (6, 27-29, 32-34, 38, 41) and the accessibility of these tumors to serial tumor measurements.

MATERIALS AND METHODS

Patient Selection. Patients were selected for this treatment program if they had histologically proven epidermoid carcinoma of the head and neck unsuitable for either radiation therapy or surgery. Inclusion in the protocol required measurable lesions and a Karnofsky performance rating (26) exceeding 30. Patients who had received prior chemotherapy were also excluded. Abnormalities in the following laboratory studies caused exclusion from the study: blood urea nitrogen, serum creatinine, serum alkaline phosphatase, serum bilirubin, serum glutamic-oxaloacetic transaminase, leukocyte or platelet counts (initial), and bone marrow aspirates. Individual patient characteristics are detailed in Table 1.

Drug Regimens. The objective was to compare maximally tolerated doses of MTX alone with MTX + LR using continuous i.v. infusions. On the basis of observations of toxicity in relation to duration of MTX exposure (5), an infusion duration of 30 hr was selected for the patient group receiving MTX alone (MTX). This was extended to 36 or 42 hr in MTX + LR recipients to compensate for the abrupt cessation of action of MTX when LR is instituted (14). This effectively made the MTX exposure durations comparable in the 2 groups because of the slow plasma clearance of the drug in the MTX group (8). Treatment intervals ranged

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[6] The abbreviations used are: MTX, methotrexate; LR, leucovorin rescue.

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Table 1
Patient characteristics

Patient	Sex	Age	Primary site	Disease duration (yr)	Prior radio therapy	Prior surgery	No. of infusions	Highest dose (mg/sq m)	Response 0–+	Duration (wk)
				MTX + LR; MTX dose > 500 mg/sq m						
W. B.	M	72	Tonsil	0.5	0	0	4	540	+	4
A. R.	F	54	Nasopharynx	9.0	+	0	9	660	+	16
W. C.	M	56	Tonsil	2.5	+	+	14	1080	+	32
E. H.	M	76	Larynx	1.3	+	+	5	1080	+	8
A. J.	M	83	Palate	1	+	0	10	1080	+	20
H. M.	M	68	Larynx	1	+	+	3	720	0	
H. W.	M	71	Tongue	0.5	+	+	4	1080	+	12
R. G.	M	46	Tongue	0.6	+	0	8	780	+	8
E. S.	M	55	Tongue	2	+	+	6	1080	0	
K. M.	F	61	Floor of mouth	1.5	+	+	4	960	0	
E. M.	M	59	Tongue	0.7	+	+	6	1080	0	
A. N.	M	62	Lip	1.0	+	+	6	1080	+	6
O. R.	M	83	Maxillary antrum	1.5	+	0	3+	1000	+	4+
N. L.	M	67	Larynx	0.9	+	0	8	1000	+	12
A. M.	M	59	Tongue	0.5	+	0	6	600	+	8
B. D.	M	63	Larynx	0.5	+	+	6	1000	+	12
S. D.	M	74	Floor of mouth	0.5	+	+	4	500	+	8
				MTX + LR; MTX dose < 500 mg/sq m						
A. S.	F	45	Alveolar ridge	0.5	+	+	3	330	0	
K. L.	M	61	Larynx	2	+	0	8	480	0	
E. R.	F	69	Hypopharynx	5	+	0	4	300	+	3
H. B.	M	51	Epiglottis	0.7	+	0	6	480	0	
A. T.	M	45	Tongue	3.5	+	+	2	300	0	
I. Y.	M	52	Unknown	Unknown	+	+	1	240	0	
M. W.	F	54	Soft palate	0.5	+	0	4	480	+	12
F. C.	M	64	Floor of mouth	0.5	+	0	2	480	0	
				MTX without LR						
A. A.	M	65	Larynx	7	+	+	13	180	+	33
J. S.	M	58	Tongue	7	+	+	9	110	0	
J. B.	M	64	Tonsil	1	+	+	4	120	0	
M. L.	F	58	Tonsil	1	+	+	10	140	+	38+
C. P.	M	73	Larynx	3	+	+	3	140	0	
W. C.	M	56	Tonsil	3	+	0	6	200	+	3
N. A.	M	52	Tongue	2	+	0	5	110	+	4
F. D.	M	50	Intraoral	?	+	+	3	140	0	
R. M.	M	74	Tongue	0.7	+	0	3	110	0	
B. G.	M	53	Tongue	0.6	+	0	6	230	+	13
W. B.	M	42	Tonsil	0.7	+	0	3	150	0	
W. L.	M	60	Tongue	0.8	+	0	3	180	0	
G. C.	M	63	Alveolar ridge	4	+	+	1	80	+	7
E. R.	M	80	Hypopharynx	?	+	+	1	120	+	7
C. A.	M	59	Floor of mouth	2	+	+	2	120	0	
H. M.	M	62	Palate	1	+	0	4	120	0	

from 14 to 21 days to allow an evaluation of response and toxicity with each course.

The maximum tolerable drug dose was defined as the dose that produced 1 or more of the following manifestations of toxicity: mucositis, 3+ to 4+; leukocyte count, ≤2500/cu mm; platelet count, ≤100,000/cu mm; or skin rash due to MTX. Patients from the 4 institutions were randomized in a central registry to either the MTX or MTX + LR therapy group. Those in the MTX group received 80 mg/sq m as an initial dose with biweekly increments of 30 mg/sq m until toxicity was attained as defined above. Biweekly therapy was then continued at a dose of 30 mg/sq m less than the toxic dose.

The 1st patients in the MTX + LR group received as an initial dose 240 mg/sq m with biweekly increments of 60 mg/sq m. When it became apparent that the expected toxicity was not being attained, the regimen was adjusted to give an initial dose of 360 mg/sq m with biweekly increments of 360 mg/sq m to a total maximum dose of 1080 mg/sq m. Since even at this dose little toxicity was seen, the duration of MTX infusion was extended to 42 hr in several patients who then developed toxicity.

In the MTX + LR group, leucovorin (N^5-formyltetrahydrofolate, citrovorum factor, folinic acid) was instituted 36 or 42 hr after the beginning of MTX infusion. During the 1st 6 hr, leucovorin, 40 mg/sq m, was given by i.v. infusion to achieve a high initial blood level; 4 additional doses of 25 mg/sq m were then given p.o. at 6 hourly intervals to

complete the course of therapy. Subsequent therapy in this group consisted of biweekly infusions utilizing the dose preceding the maximally tolerated dose of drug.

Patient Evaluation and Regulation of Therapy. During protocol therapy, patients were evaluated weekly. Total leukocyte and differential counts, platelet counts, liver function studies (serum alkaline phosphatase, serum bilirubin, serum glutamic-oxaloacetic transaminase, blood urea nitrogen, and serum creatinine were determined at weekly intervals initially and every 2 weeks after the dose level was established. Serial X-rays were obtained at monthly intervals as indicated. Serum MTX levels were determined according to the method of Bertino and Fischer (3). Tumor response was defined as a greater than 50% reduction in the product of 2 perpendicular diameters of the principal lesion observed during at least 2 consecutive evaluations separated by at least 2 weeks; it was further required that no new lesions or increase in size in other old lesions have occurred during the period of response of the principal lesion.

Drug resistance was defined as the appearance of a new lesion or an increase in the diameter product of a preexisting lesion measured on 2 consecutive evaluations. Therapy was discontinued if toxicity occurred without tumor response or if tumor progression occurred during therapy.

RESULTS

Of the 52 originally randomized patients, 41 were considered evaluable. Three patients were excluded from the study because their original histological diagnoses were changed at review; 8 other patients received only a single course of therapy and were then lost to follow-up and/or voluntarily discontinued therapy. All 6 patients who died of drug-related toxicity after receiving only 1 or 2 courses of therapy were included in the study.

The characteristics of the evaluable patients are outlined in Table 1. Of the 41 patients, 16 received MTX and 25 received MTX + LR. Because of the relative heterogeneity of patients with this category of diseases, a number of their characteristics (including sex, age, anatomic diagnosis, disease duration, and prior therapy) are analyzed in Table 2 to facilitate comparison of the groups. The small numbers of patients precluded statistical analysis of these parameters but no major differences were apparent. The results are summarized in Table 3. Response rates of 44% in the MTX group and 60% in the MTX + LR group were observed. These did not differ significantly. Median response durations were similar. Toxicity was predominantly hematological (Table 4) with less leukopenia ($p < 0.001$) but not less thrombocytopenia (either at the 100,000 or 50,000 level) occurring in those patients who received MTX + LR (Table 5). A number of severe cytopenias and 4 deaths related to drug-induced hematological toxicity were recorded in the MTX group and 2 such deaths occurred in the MTX + LR group. A 5th death in the MTX group occurred after hepatic failure; this may also have been related to MTX toxicity (7, 11). One high-dose MTX + LR patient had hepatic fatty degeneration at postmortem. Thus, in spite of the original objective of regulating therapy using equal predefined toxicity criteria in the 2 experimental groups, hematological toxicity was significantly greater in the MTX group (Table 5). Mucositis, although the limiting factor in a few patients, was not prominent in either group.

Because of significant differences in MTX dosage received by patients who had entered the MTX + LR group early in the study and those who entered after the dosage schedule was augmented, the MTX + LR group was further subdivided into a "high-dose" (high) and a "low-dose" (low) group whose maximum MTX dose was greater or less than 500 mg/sq m, respectively. Response rates were 13 of 17 (76%) and 2 of 8 (25%) in the MTX + LR (high), and MTX + LR (low) groups, respectively (Table 3). These differed significantly ($p < 0.02$) (Table 5). Both drug-related deaths occurred in the MTX + LR (high) group. The probability of a difference in response rate between the MTX and MTX + LR (high) groups approached statistical significance (Table 5).

Serum levels attained with various doses of MTX are shown in Chart 1. Serum concentrations as high as 10^{-5} M were achieved with the highest doses of drug. There was an approximate correlation between the dose of drug administered and the blood level achieved.

DISCUSSION

In the MTX group, the response rate (44%) and mortality

Table 2

Characteristics of patients in the therapeutic groups and subgroups

	Mean age (yr)	Median age (yr)	% males	% with cancer of larynx	% with cancer of tongue	% with cancer of tonsil	% other sites	Mean disease duration (yr)	% prior radiotherapy	% prior surgery	Mean maximal dose (mg/sq m)
MTX (16 patients)	60.6	59.5	85.6	12.5	31.3	25.0	31.3	2.53	100.0	64.3	134.4
MTX + LR (25 patients)	62.1	61.0	80.0	20.0	24.0	8.0	48.0	1.56	96.0	52.0	737.0
MTX + LR (high) (17 patients)	65.2	63.0	88.4	23.5	29.5	11.8	35.2	1.44	94.1	58.9	900
MTX + LR (low) (8 patients)	55.2	53.0	62.5	12.5	12.5	0.0	75.0	1.82	100.0	37.5	386.0
Total (41 patients)	61.5	61.0	85.5	17.2	26.8	14.6	41.4	1.89	97.6	55.7	

Table 3
Results of therapy in epidermoid carcinoma of the head and neck

	No. of patients	Drug-related deaths		Response		Median
		No.	%	No.	%	(wk)
MTX alone	16	4	25	7	44	12
MTX + LR (low)	8	0		2	25	7.5
MTX + LR (high)	17	2	11.8	13	76	11.5
MTX + LR	25	2	8	15	60	11

Table 4
Hematological toxicity of drug treatment

	Leukopenia (≤2,500)	Thrombocytopenia (≤100,000)
MTX	14/15	6/13
MTX + LR	8/25	10/25
MTX + LR (high)	7/17	7/17
MTX + LR (low)	1/8	3/8

incidence (4 of 16; 25%) are comparable to previously reported MTX studies with response rates of 12 to 53% and mortality rates between 2 of 27 (7.5%) and 3 of 24 (12.5%) (27, 29, 33, 34). In none of these studies, however, was MTX given as a continuous infusion. The MTX + LR response rate of 60% and mortality rate of 8% compare favorably with the results of previous studies with LR in which response rates of 22 to 62% and mortality rates of 1 of 21 (4.8%) to 2 of 18 (11%) have been reported (6, 28, 32). The duration of i.v. drug infusion in these prior studies furthermore was only 24 hr compared to 36 to 42 in the current study.

Leucovorin rescue improves the therapeutic index of MTX in this study in that the MTX + LR (high) group showed a significantly lower rate of leukopenia ($p < 0.005$) as well as a higher response rate (which approached statistical significance) than the MTX group (Table 5). The MTX + LR (low) group had a significantly lower response rate than the MTX + LR (high) group ($p < 0.02$) (Table 5) suggesting that suboptimal doses of MTX given with LR compromise response frequency even though they exceed doses of MTX possible without LR. The median response duration of 12 weeks, similar in both groups, did not differ from previously reported results of 28 to 150 days (6).

The serum concentrations of MTX achieved in the MTX + LR group (up to 10^{-5} M) exceed by 5- to 10-fold levels known to achieve 95% inhibition of DNA synthesis in human acute leukemia cells both *in vitro* and *in vivo* (22). The degree of scatter in correlating drug dose and serum concentration (Chart 1) is consistent with the advanced age and generally poor condition of many of the patients and may reflect the variability in renal clearance of the drug. Since renal clearances were not directly measured, however, and blood levels were not determined in every patient at every dose level, toxicity could not be directly correlated with either of these parameters.

Since host toxicity and tumoricidal effect are probably related to both the concentration and duration of exposure to drug (23), it is possible that kinetic differences between the neoplastic and hematopoietic cell populations account for the improved therapeutic index observed in this study. The relatively high proportion of marrow stem cells known to be in resting or G_0 phase of the mitotic cycle support this contention (25, 37) as does the recent demonstration of a short generation time in kinetic studies of squamous cell carcinomas of the head and neck (31). Since sensitivity to antimetabolite chemotherapy is enhanced in rapidly growing neoplastic cells and reduced in advanced plateau-phase tumors (16, 24), unfavorable kinetic relationships with host cells may cause drug resistance.

Table 5
Statistical analysis of differences in response and toxicity in the therapeutic groups

Responses	
MTX *vs.* MTX + LR	N.S.[a]
MTX *vs.* MTX + LR (high)	$< 0.1 > 0.05$
MTX + LR (high) *vs.* MTX + LR (low)	<0.02
Leukopenia (2500)	
MTX *vs.* MTX + LR	<0.001
MTX *vs.* MTX + LR (high)	<0.005
MTX + LR (high) *vs.* MTX + LR (low)	N.S.
MTX *vs.* MTX + LR (low)	<0.001

[a] N.S., not significant.

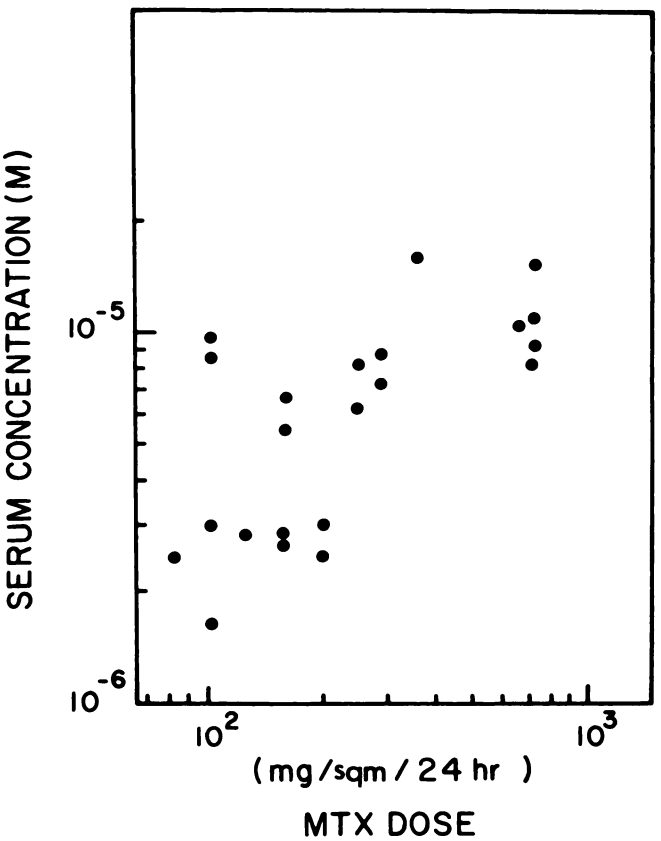

Chart 1. Serum MTX levels midway during 30- or 36-hr MTX infusions.

In this program, the use of LR has permitted the administration of 10-fold higher doses of MTX than those which are otherwise tolerated. It is felt that this confirms the importance of exposure duration to drug relative to drug concentration *per se* in determining cell kill (23) and toxicity.

At the clinical level, this study has both immediate and broad implications. The MTX + LR (high) regimen is characterized by acceptable host toxicity and responses that compare favorably with other drug treatments currently used. However, both the quality and duration of tumor responses in this disease require further improvement in therapy. Pending the development of a more effective single drug, these gains most probably will result from the use of combination chemotherapy (20) programs composed of multiple effective agents, each exploited to achieve the greatest therapeutic advantage.

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