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Alleviation by leucovorin of the dose-limiting toxicity of edatrexate: potential for improved therapeutic efficacy*

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Summary. Edatrexate (10-ethyl-10-deaza-aminopterin; CGP 30 694) is a methotrexate (MTX) analogue that shows promise against non-small-cell lung cancer (NSCLC) and other tumors. Since edatrexate's mechanism of action is the same as that of MTX, we used leucovorin in an attempt to alleviate its dose-limiting toxicity, stomatitis. In four patients with NSCLC who had experienced significant stomatitis after treatment with edatrexate, cyclophosphamide, and cisplatin, we observed a remarkable reduction in stomatitis following the administration of low-dose leucovorin. On the basis of the results obtained in these individuals, we treated 15 additional patients with this three-drug regimen plus leucovorin rescue. These subjects could tolerate the treatment with lesser degrees of stomatitis and received higher edatrexate doses in subsequent courses as compared with the patients who previously received this regimen without leucovorin rescue. This approach is expected to improve the therapeutic indices of edatrexate and edatrexate-containing chemotherapy regimens by modifying the dose-limiting toxicity of this antineoplastic agent.

Introduction

Edatrexate (10-ethyl-10-deaza-aminopterin; CGP 30 694) is a methotrexate (MTX) analogue that functions like MTX by competing avidly for the folate-binding site of the enzyme dihydrofolate reductase (DHFR) [3, 24, 25]. Preclinical studies have shown it to be superior to MTX in antitumor efficacy [21, 22, 25], the improved therapeutic index apparently being related to increased specific transport and enhanced polyglutamation within tumor cells [18, 26]. Phase I and II clinical trials have since revealed that edatrexate shows promising antitumor activity in non-small-cell lung cancer (NSCLC) and other solid tumors [11–13, 23]. The most common and dose-limiting toxicity in those trials was stomatitis. However, edatrexate only infrequently caused myelosuppression, a characteristic that enables it to be combined with myelosuppressive agents without significantly compromising their dose schedules.

Recently, we initiated a phase II study evaluating the efficacy and toxicity of an edatrexate-cyclophosphamide-cisplatin regimen in NSCLC patients who had not previously received chemotherapy. This regimen, which lacks overlapping toxicities, was formulated to exploit the potential synergistic antitumor effects suggested by the report of Schmid et al. [22]. Edatrexate was initially given i.v. at 80 mg/m^2 on days 1 and 8; cyclophosphamide, at 800 mg/m^2 on day 1; and cisplatin, at 80 mg/m^2 on day 1. Courses were repeated every 3 weeks with dose modifications according to toxicity (schedule A) [13]. The initial dose schedule yielded 7 objective responses (47%) in 15 evaluable patients (among 16 subjects) [13]. However, the schedule was associated with significant side effects, mainly stomatitis, myelosuppression, and neutropenic fever, and substantial reductions in the edatrexate dose were required. Therefore, we reduced the starting dose of each agent by 12.5% and allowed further edatrexate dose modification on day 8 of a given course (schedule B). Although toxicity then became tolerable for most patients, stomatitis remained a major dose-limiting side effect, and objective tumor responses were observed in only 4 (27%) of 15 evaluable patients (among 16) [13]. To determine whether leucovorin (citrovorum factor; Lederle, Wayne, N.J.) given its use as a rescue agent in MTX therapy, would ameliorate the dose-limiting stomatitis caused by edatrexate, we revised the protocol so as to give leucovorin following edatrexate administration. In this interim report, we describe clinical data indicating that leucovorin can ameliorate, if not prevent, stomatitis associated with edatrexate treatment. Complete results of our protocol incorporating leucovorin rescue, including other toxicities and response, will be described at a later date.

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Table 1. Edatrexate dose-modification schedule for stomatitis

Schedule	Protocol revision	Dose on day 1 (mg/m²)	Dose on day 8 for stomatitis grade		Dose for next course for stomatitis grade			
			I	II	0	I	II	III, IV
A	Original,	80	100%	50%	Increase	Increase	Same	Reduce
	2/8/89	80	50%	25%	Increase	Increase	Same	Reduce
B	3/10/89	70	50%	25%	Increase	Same	Reduce	Reduce
	4/21/89	70	50%	0	Increase	Same	Reduce	Reduce
C	11/13/89	80	50%	0	Increase[a]	Same	Reduce	Reduce

[a] To be increased only if myelotoxicity is grade II or less

Patients and methods

Between November 1988 and August 1989, as previously reported [13], 32 patients with a histologically or cytologically confirmed diagnosis of NSCLC clinically staged as IIIB or IV [19], a Zubrod preformance status of ≤ 2 and a life expectancy of ≥ 12 weeks were enrolled for treatment with this three-drug regimen (16 began on schedule A and then 16 began on schedule B as described above). No patient had previously received systemic chemotherapy. The nature and purpose of the study were fully discussed with each subject, and written informed consent was obtained before enrollment.

All patients enrolled were required to have adequate WBC ($\geq 4{,}000/mm^3$), granulocyte ($\geq 1{,}500/mm^3$), and platelet ($\geq 100{,}000/mm^3$) counts and adequate renal (serum creatinine, <1.5 mg/dl) and hepatic (Serum bilirubin, <1.5 mg/dl; alanine aminotransferase, ≤ 1.5 times the upper limit of normal) function. Subjects with brain metastasis were eligible if the brain lesion or lesions were adequately controlled and other measurable disease was present. However, individuals with multiple bony metastases were excluded from the study, as were those with clinically significant third-space fluid (peripheral edema, pleural effusion, or ascites), unstable angina or clinical evidence of congestive heart failure, or serious concomitant infection, including postobstructive pneumonia.

Four patients who remained in the study in September 1989 and exhibited significant stomatitis were given 15 mg leucovorin orally every 6 h for 4 doses starting at 24 h after the administration of each edatrexate dose. Because there was significant alleviation of stomatitis in all four patients so treated (see below), a third schedule, schedule C, was designed. This incorporated leucovorin rescue, as given to the four above-mentioned patients, into schedule A, i.e., 80 mg/m² i.v. edatrexate given on days 1 and 8, 800 mg/m² i.v. cyclophosphamide given on day 1, and 80 mg/m² i.v. cisplatin given on day 1. The present report deals with the effects of leucovorin on stomatitis in the first 15 patients who received the protocol treatment according to schedule C and who were enrolled according to the criteria described above.

For all schedules, response and toxicity in the protocol were assessed using the modified World Health Organization criteria [17]. Specifically stomatitis was graded as follows: grade I – soreness and erythema; grade II – erythema, patchy ulceration, ingestion of solid food; grade III – confluent ulceration, ingestion of a liquid diet only; and grade IV – hemorrhagic ulceration, necrosis, alimentation not possible.

Within the 3 schedules, edatrexate dose revisions according to toxicity were undertaken as follows. Initially, the edatrexate dose was reduced by half on day 8 for patients who exhibited grade II or greater stomatitis on that day (schedule A). Subsequently, the dose was reduced by half on day 8 for grade I stomatitis and was withheld on day 8 for grade II or greater stomatitis. In chemotherapy courses given thereafter, the edatrexate dose was modified so as to avoid grade III or IV stomatitis (schedule A) and was later revised again to prevent the occurrence of stomatitis of grade II or greater (schedules B and C; Table 1). Chemotherapy courses were repeated every 3 weeks as toxicity permitted, with chemotherapy doses being modified in attempts to achieve a nadir granulocyte count of 500–1,000/mm³ and a nadir platelet count of 50,000–100,000/mm³ and to avoid infectious or bleeding complications. For schedule C, however, dose escalation in subsequent courses was allowed for edatrexate but not for cyclophosphamide or cisplatin. For statistical analysis, the chi-square test without Yates' correction was used.

Results

Schedule A/B enrollees

In each of the first four patients who received leucovorin on these protocols, stomatitis improved markedly after folinic acid administration. Stomatitis completely disappeared in two cases (both of whom had grade II and III stomatitis and was reduced in severity in the other two (from grade II to grade I stomatitis). The subjects also experienced weight gain and an improved sense of well-being. All four individuals managed to tolerate higher doses of edatrexate in subsequent courses without developing any severe complications. We present the two cases in which stomatitis disappeared after the addition of leucovorin to the regimen and the tumor response continued.

Case 1. This 59-year-old white woman was diagnosed in February 1989 as having $T_1N_2M_1$ adenocarcinoma of the right upper lobe of the lung. Three courses of edatrexate-cyclophosphamide-cisplatin (according to schedule A) yielded a partial remission. However, the patient consistently lost weight because of grade II and III stomatitis that occurred despite edatrexate dose reduction (Table 2). After the fifth course of chemotherapy, she weighed only 70.5 kg, 12.5 kg less than before the study. She was given leucovorin for the first time on day 9 of course 7 in accordance with the revised protocol; the edatrexate dose had been reduced by half, to 25 mg/m², on day 8 because of grade I stomatitis. The stomatitis resolved within 2 days, and the patient started to regain weight and had an improved sense of well-being. She tolerated the next 3 courses of chemotherapy without experiencing stomatitis, even with the edatrexate dose being escalated to 70 mg/m² on both day 1 and 8 of course 10.

Case 2. This 49-year-old Hispanic man received chest irradiation in February 1989 for $T_2N_2M_0$ squamous-cell carcinoma of the right lung. In May 1989, chest radiography revealed bilateral lung lesions consistent with metastases, and the patient was enrolled in this three-drug protocol at

Table 2. Effects of leucovorin rescue on stomatitis and weight: case 1

	Course number									
	1	2	3	4	5	6	7	8	9	10
Chemotherapy dose (mg/m^2)[a]:										
Edatrexate	80/80	80/20	70/35	60/30	60/60	50/50	50/25	50/50	60/60	70/70
Cyclophosphamide	800	800	800	800	700	600	600	600	700	800
Cisplatin	80	80	80	80	70	60	60	0	0	0
Leucovorin given	No	No	No	No	No	No	Yes	Yes	Yes	Yes
Stomatitis grade:										
Day 8	0	II	I	I	0	0	I	0	0	0
Day 15	II	III	II	0	III	II	0	0	0	0
Stomatitis duration (days)	14	16	20	9	9	14	3	0	0	0
Weight (kg):										
Day 1	83	81.2	78.4	73.5	72.3	70.5	71.6	72.4	73.1	74.1
Day 21	80.7	78.8	74.4	72.3	70.5	–	72.4	73.1	74.1	74.5
Course interval (days)	27	26	66[b]	21	21	56	21	21	24	–

[a] Edatrexate was given on day 1/day 8; cyclophosphamide and cisplatin, on day 1
[b] Chemotherapy delayed because of cholecystectomy

Table 3. Effects of leucovorin rescue on stomatitis and weight: case 2

	Course number								
	1	2	3	4	5	6	7	8	9
Chemotherapy dose (mg/m^2)[a]:									
Edatrexate	70/70	60/60	50/50	50/25	50/25	50/25	50/50	50/25	50/50
Cyclophosphamide	700	800	900	900	800	700	700	700	600
Cisplatin	70	80	90	90	80	70	70	70	60
Leucovorin given	No	No	No	No	No	Yes	Yes	Yes	Yes
Stomatitis grade:									
Day 8	0	0	0	I	I	I	0	I	0
Day 15	II	II	II	II	III	0	0	I	0
Stomatitis duration (days)	3	3	5	12	33	6	0	9	0
Weight (kg):									
Day 1	72.1	70.7	71.7	71.9	71.5	70.1	70.8	70	69.9
Day 21	70.7	71.7	71.3	70.1	67.6	70	70.3	68.4	70
Course interval (days)	25	24	28	33	36	28	28	28	–

[a] Edatrexate was given on day 1/day 8; cyclophosphamide and cisplatin, on day 1

the reduced dose level (schedule B). The first course of chemotherapy yielded a partial remission. After each course, however, the patient experienced grade II or III stomatitis despite reductions in the edatrexate dose (Table 3). Leucovorin was given during courses 6 through 9, which were tolerated much better than the previous cycles. The patient experienced no stomatitis during course 7 despite an increase in the edatrexate dose. In addition, his weight, which had dropped to 67.6 kg as of day 21 of course 5, rose to 70.8 kg by the time course 7 was started.

Schedule C enrollees

Leucovorin rescue enabled the patients to tolerate the treatment better with lesser degrees of stomatitis, even as compared with the subjects who received the reduced doses on schedule B. Following the first dose of edatrexate during the first course of chemotherapy, for instance, 4 of 15 patients on schedule A (26.7%) and 5 of 16 subjects on schedule B (31.3%) had experienced grade II or III stomatitis by day 8 of the treatment; an additional 3 individuals each on schedules A and B experienced grade I stomatitis. In comparison, on schedule C with leucovorin rescue, by day 8 of the first course of treatment, only 1 of 15 patients (6.7%) had developed grade II stomatitis (occurring as a small, painless mucosal ulcer) and 4 had developed grade I stomatitis. Two fo these four subjects could later tolerate further treatment with higher doses of edatrexate, demonstrating that we were overcautious in modifying the doses of this agent.

Table 4. Stomatitis of grade II or greater on schedules A, B, and C

Schedule	Number of patients (%) with stomatitis of grade		
	II	III	IV
Course 1			
A ($n = 15$)	8 (53.3)	3 (20)	1 (6.7)
B ($n = 16$)	5 (31.2)	4 (25)	1 (6.2)
C ($n = 15$)	5 (33.3)	3 (20)	0 (0)
Course 2:			
A ($n = 13$)	5 (38.5)	4 (30.8)	0 (0)
B ($n = 12$)	9 (75)	1 (8.3)	0 (0)
C ($n = 10$)	3 (30)	0 (0)	0 (0)
Course 3:			
A ($n = 11$)	6 (54.5)	2 (18.2)	1 (9.1)
B ($n = 8$)	5 (62.5)	1 (12.5)	0 (0)
C ($n = 7$)	1 (14.3)	0 (0)	0 (0)

Table 5. Chemotherapy doses given on schedules A, B, and C

Schedule	Dose delivered (mg/m^2)		
	Edatrexate	Cyclophos-phamide	Cisplatin
Course 1:			
A ($n = 15$)	143 ± 27	800 ± 0	80 ± 0
B ($n = 16$)	113 ± 31	700 ± 0	70 ± 0
C ($n = 15$)	139 ± 30	800 ± 0	80 ± 0
Course 2:			
A ($n = 13$)	132 ± 25	754 ± 78	75 ± 8
B ($n = 12$)	99 ± 42	758 ± 79	76 ± 8
C ($n = 10$)	136 ± 38	750 ± 53	75 ± 5
Course 3:			
A ($n = 11$)	107 ± 33	791 ± 130	79 ± 13
B ($n = 8$)	81 ± 44	813 ± 83	81 ± 8
C ($n = 7$)	151 ± 25	714 ± 90	71 ± 9
Course 4:			
A ($n = 11$)	88 ± 27	755 ± 151	75 ± 15
B ($n = 6$)	68 ± 28	800 ± 89	80 ± 10
C ($n = 4$)	165 ± 44	700 ± 141	70 ± 14
Course 5:			
A ($n = 8$)	86 ± 25	757 ± 207	68 ± 18
B ($n = 4$)	50 ± 14	700 ± 50	75 ± 6
C ($n = 1$)	160 ± 0	700 ± 0	70 ± 0

Data represent mean values ± SD

This protective effect of leucovorin against stomatitis became much more obvious as the treatment continued, as shown in Table 4. Overall, during the first 3 courses of chemotherapy, grade III stomatitis occurred in only 3 of 32 schedule C treatment courses (9.4%, all during the first course), whereas grade III or IV stomatitis occurred in 11 of 39 schedule A cyles (28.2%) and in 7 of 36 schedule B courses (19.4%; schedule C vs schedule A, $P = 0.048$; schedule C vs schedule B, $P = 0.242$).

Although the dose-modification scheme for stomatitis was more generous on schedule C than on schedule A (Table 1), the amount of edatrexate given to patients on schedule C was comparable with that given on schedule A for the first two courses of chemotherapy and exceeded schedule A levels in subsequent courses (Table 5). No clinically significant differences in myelotoxicity were found between treatment schedules A and C after the first course of treatment (data not shown).

Discussion

Leucovorin rescue [9] has been firmly established for high-dose methotexate (MTX) treatment [1, 2, 7]. At sufficient doses, the agent effectively ameliorates the toxic effects of MTX in the bone marrow and in the intestinal and oral mucosa. Leucovorin is also expected to ameliorate edatrexate toxicity, since the latter drug is an MTX analogue that exhibits the same mechanism of action as the parent drug [24]. The present report is the first demonstration of such protective effects. More importantly, on the addition of leucovorin to the regimen, the patients could tolerate edatrexate doses that were higher than even those used on the initial schedule (schedule A). In terms of leucovorin's effects on myelosuppression, however, we could not draw any firm conclusions because of the concurrent administration of cyclophosphamide and cisplatin.

Considering that a seemingly important drop in the objective response rate, from 47% to 27%, occurred when the starting dose level was reduced by 12.5% between schedules A and B [13], we hoped that leucovorin rescue using the nonreduced dose would enable us to maximize the antitumor effects of edatrexate, particularly in this combination with two antitumor agents that were potentially synergistic with this MTX analogue. In fact, an interim analysis of the response to schedule C showed an objective response rate of 43%. Although this response rate is statistically no better than our previous results, it is reassuring to know that leucovorin did not compromise the efficacy of this regimen.

The original premise of leucovorin rescue was that the provision of reduced folate to normal cells would circumvent the metabolic block produced by MTX and enable the resumption of purine and pyrimidine synthesis. However, this does not explain the presumed selectivity of leucovorin rescue in normal tissues; an alternative explanation is needed, and the recent experimental observations by Matherly and associates [14, 15] seem to account for it. These investigators have shown that leucovorin can competitively displace MTX from dihydrofolate reductase (DHFR), enabling reactivation of the enzyme, but that such competitive displacement does not occur in the presence of MTX polyglutamates (and, thus, DHFR inhibition is sustained).

In normal gut epithelium and bone marrow precursors, relatively little MTX polyglutamate synthesis occurs [5, 8] as compared with that in sensitive tumor cells. As a result, in normal cells that contain few MTX polyglutamates, leucovorin administration would promote dissociation of MTX from DHFR and reactivation of the enzyme, thereby enabling the reactivation of purine and thymidylate biosynthesis. In contrast, accumulation of MTX polymglutamates in tumor cells would resist competitive displacement by leucovorin, and enzyme inhibition would be sustained.

Cellular levels of dihydrofolate polyglutamates would thus remain high and directly inhibit purine biosynthesis, thereby preventing the entry of leucovorin into the reduced folate pool. At the same time, the accumultion of MTX polyglutamates would result in persistent inhibition of thymidylate synthesis [16].

Edatrexate has also been shown to be extensively polyglutamated and retained in target tumor cells, with a relatively small portion of the drug given to animals being found as polyglutamates in the small intestine, a sensitive host tissue [24, 26]. In a patient with metastatic breast cancer to the skin, Kris et al. [11] showed that there was a substantial increase in polyglutamate forms of edatrexate in the breast cancer tissue, whereas the drug content in normal skin from the same patient directly paralleled the plasma concentration without showing detectable conversion to polyglutamate forms. This similarity of cellular pharmacology between edatrexate and MTX seems to provide a theoretical basis for our clinical findings and forms a strong rationale for leucovorin rescue following edatrexate treatment in future clinical trials.

The optimal dose schedule for leucovorin rescue after edatrexate treatment, however, needs to be further studies. Even for the most widely accepted high-dose MTX regimens, there is considerable variation in the literature concerning the optimal dose, schedule, and method of administration of leucovorin following MTX treatment. Nevertheless, the appropriate dose and duration of leucovorin given as rescue therapy are largely dependent on the clinical pharmacokinetics of MTX in individual patients, and the same would hold true for edatrexate. In general, when patients exhibit plasma MTX levels of $10^{-8}-10^{-6}$ mol/l, low doses of leucovorin appear to be effective in preventing MTX toxicity [27].

The size and the number of leucovorin doses given following high-dose MTX treatment do not seem to be critical in patients showing normal MTX clearance. In fact, three or four doses of 15 mg/m^2 leucovorin given 6 h apart have been shown to be adequate [4, 6, 10, 20], and some data suggest that even this amount often exceeds that required to achieve adequate rescue after high-dose MTX treatment [27]. Accordingly, the schedule used in the present study – four 15-mg doses of leucovorin given orally starting at 24 h after edatrexate administration – seems to represent a reasonable starting point for future trials using edatrexate. We hope that this approach will ultimately improve the therapeutic indices of edatrexate and edatrexate-containing combination chemotherapy regimens by modifying the agent's dose-limiting toxicity.

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