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Substitution of l-leucovorin for d,l-leucovorin in the rescue from high-dose methotrexate treatment in patients with osteosarcoma

Norman Jaffe, Keith Jorgensen, Resa Robertson, Martine George,[1] Laurie Letvak[1] and Greg Barrett[1]

University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030, USA.
Tel: (+1) 713 792 2121.
[1]Lederle Laboratories, Division of American Cyanamid, Pearl River, NY 10965, USA.

Studies in which high-dose methotrexate (HDMTX) is used for the treatment of osteosarcoma have utilized commercial formulations of d,l-leucovorin (leucovorin calcium) for rescue from potential methotrexate (MTX) toxicity. These formulations are racemic mixtures containing equal amounts of d and l isomers of leucovorin. All of the available data indicate that the l isomer is the pharmacologically active diastereomer. A clinical study was conducted to determine if l-leucovorin was as safe and efficacious as d,l-leucovorin in the rescue of patients with osteosarcoma who were treated with HDMTX (12.5 g/m^2 over 6 h). Because d,l-leucovorin consists of equal proportions of d and l isomers, l-leucovorin was administered at half the usual dose of d,l-leucovorin. In patients with delayed methotrexate excretion, l-leucovorin doses were escalated from 7.5 to 50 mg every 3 h until the MTX level was 0.3 μmol/l or less. Due to the low incidence of osteosarcoma, a control group of patients previously treated with d,l-leucovorin was utilized for comparison. Efficacy of l-leucovorin was determined by its ability to prevent HDMTX-associated toxicity. Demographic and clinical toxicity data from three patients who received 22 courses of MTX rescued with l-leucovorin were compared with data from six patients who had received 42 MTX courses rescued with d,l-leucovorin. Some liver function abnormalities and leukocyte elevations were found in both groups and were attributed to MTX administration. No clinical toxicity attributable to l-leucovorin was observed. l-Leucovorin in half the d,l-leucovorin dose was (equally) effective as a rescue treatment.

Key words: Clinical, leucovorin, osteosarcoma.

Introduction

Leucovorin is used clinically to diminish the toxicity of methotrexate (MTX) and other antifolates. The administration of leucovorin after MTX can selectively 'rescue' normal cells from toxicity, thus allowing the use of high-dose methotrexate (HDMTX) (8–12.5 g/m^2) with the potential for achieving greater antitumor effects.[1–3] High-dose MTX with leucovorin rescue (HDMTX-LCV) is included in most combination chemotherapy regimens used to treat metastatic osteosarcoma. As such, it may be administered alone or in combination with doxorubicin, cisplatin, bleomycin, cyclophosphamide and dactinomycin. It is also utilized as adjuvant therapy to prevent, or if unsuccessful, possibly delay metastases after surgery in patients with non-metastatic osteosarcoma.[4–6] In neoadjuvant chemotherapy, it is administered preoperatively, allowing for histologic assessment of the response of the primary tumor to chemotherapy after amputation or limb salvage.[7] This strategy has been utilized as a prognostic factor and a way to devise more specific oncolytic treatment.

The currently marketed preparations of leucovorin used for MTX rescue contain equal amounts of two diastereoisomers (d and l). l-Leucovorin is converted to 5,10-methylenetetrahydrofolate and then to other tetrahydrofolates, thereby replenishing the pools of reduced folate cofactors, which are depleted in cells exposed to MTX. In contrast, the d-isomer of leucovorin is not metabolized to any significant degree by humans and is not taken up in tissues.[8,9] All the available data indicate that the l isomer of leucovorin accounts for virtually all of the efficacy of d,l-leucovorin in MTX rescue.[10–12] Because d,l-leucovorin consists of equal proportions of the d and l isomers and based upon the similarity of important pharmacokinetic parameters of l-leucovorin at half the usual dose of d,l-leucovorin,[13] l-leucovorin (at one half the d,l-leucovorin dose) was expected to have clinical effectiveness equal to that of d,l-leucovorin in MTX rescue.

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Correspondence to N Jaffe

The purpose of this study was to compare the efficacy and safety of l-leucovorin (at one half the d,l-leucovorin dose) in MTX rescue for the treatment of patients with osteosarcoma with that of d,l-leucovorin, as evaluated from data obtained retrospectively.

Patients and methods

Patients

Patients with an established diagnosis of high-grade osteosarcoma who relapsed following treatment with intraarterial cisplatin and doxorubicin, and, in some cases, dactinomycin and cyclophosphamide (administered in addition to the previous agents for small cell osteosarcoma) were eligible for treatment. In these circumstances, MTX was administered as definitive therapy in an effort to eradicate overt disease; alternatively, it was administered as postoperative adjuvant therapy after surgical extirpation of recurrent (metastatic) disease. Patients undergoing current treatment with HDMTX and d,l-leucovorin or patients with previous exposure to this regimen who required retreatment were also eligible. One patient referred from another institution who failed to achieve a satisfactory response to preoperative chemotherapy with cisplatin and doxorubicin (under 60% tumor destruction) was also considered eligible for adjuvant postoperative HDMTX-LCV utilizing l-leucovorin for rescue. Informed consent was obtained from the legal guardians of all patients. This study was approved by the institutional review board of University of Texas MD Anderson Cancer Center.

Patients who were over 16 years of age and those who were pregnant were excluded from treatment. Contraindications to the administration of HDMTX-LCV included the following: dehydration; severe infection; ascites or pleural effusion; a serum MTX concentration $\geqslant 0.3$ μmol/l; suspected, anticipated or overt MTX toxicity; a serum bilirubin >1.2 mg/dl, a serum lactate dehydrogenase (LDH) or a serum aspartate aminotransferase (SGOT) elevated three times the upper limit of normal; a white blood cell (WBC) count $<1000/\mu l$; a platelet count $<50\,000/\mu l$; and a creatinine clearance <60 ml/min/m^2, or a creatinine clearance between 50 and 60 ml/min/m^2 with a serum creatinine >1.3 mg/dl.

Drug administration schedules

Following admission, a maintenance infusion of 5% dextrose in 0.2% sodium chloride infusion at 3 l/m^2/24 h was initiated for 6–12 h. MTX was then administered in a dose of 12.5 g/m^2 over 6 h. At 12 h after the completion of the MTX infusion, rescue was implemented. In the historical control series, this consisted of d,l-leucovorin, 15 mg, administered intravenously every 3 h for 18 doses. In contrast, in patients treated with l-leucovorin, 7.5 mg was administered every 3 h for 18 doses. In patients with delayed MTX excretion, the dose of d,l-leucovorin in the historical control series was increased to 100 mg, and in the current study, the dose of l-leucovorin was increased to 50 mg. Both forms of leucovorin were administered intravenously every 3 h until a serum MTX concentration below 0.3 μmol/l was attained.

Serum MTX concentrations were determined at 24, 48 and 72 h after initiation of the MTX infusion. If the MTX concentration was in excess of 30 μmol/l at 48 h or in excess of 0.3 μmol/l at 72 h, the following measures were instituted: (i) the rate of the maintenance 0.2% sodium chloride infusion was increased to 4 l/m^2 per 24 h; (ii) escalated doses of leucovorin (50 mg of l-leucovorin or 100 mg of d,l-leucovorin) were administered every 3 h until the serum MTX concentration was below 0.3 μmol/l as outlined earlier. The escalated doses of leucovorin were retained in these respective patients in all subsequent courses of HDMTX-LV. They were administered every 3 h intravenously until the serum MTX concentration fell to 0.3 μmol/l. This generally occurred by 72 h.

Results

The first patient was enrolled and treated with l-leucovorin in November 1989 and data on the last l-leucovorin doses presented in these results were obtained in March 1990. The first historical control (d,l-leucovorin) patient was treated in July 1987 and the last observations for the d,l-leucovorin group were made in September 1989.

Three patients were treated with l-leucovorin: one had metastatic disease and two were receiving postoperative adjuvant therapy. They received six, seven and nine courses of therapy, respectively. Thus, data were available for a total of 22 courses of HDMTX-LV. The patients ranged in age from 4 to 15 years. The time from initial diagnosis ranged

from 9 to 21 months. All three l-leucovorin patients had received previous chemotherapy that included cisplatin and had undergone limb salvage surgery.

One patient in the l-leucovorin group was given 50 mg doses of l-leucovorin during all courses because of a history of previous delayed MTX excretion while receiving d,l-leucovorin rescue. One patient required an increase in five doses of l-leucovorin from 7.5 to 50 mg during the fourth course of HDMTX-LCV (given for five doses) and in subsequent courses because of delayed MTX excretion. The third patient, because of a small body surface area, was given 25 mg doses of l-leucovorin in view of a previous experience of delayed MTX excretion.

Data were obtained from six matched historical control patients treated with d,l-leucovorin; each course occupied the same relative position in the treatment sequence as the l-leucovorin courses. Five patients received treatment for metastatic disease and one received postoperative adjuvant therapy. Two of the six patients in the control series each received 12 courses of therapy; data from each of their first nine courses were included in the presentation of results. The other four matched d,l-leucovorin control patients each received six courses of therapy. Thus, data were available for a total of 42 courses of d,l-leucovorin. This represented an approximate course ratio of 2:1 for historical control to study patients. The age range in the control patients was 10–17 years. The time from diagnosis ranged from 8 to 20 months. Five of the d,l-leucovorin patients had received previous chemotherapy that included cisplatin. All had undergone previous surgery (resection or amputation). In the control group, two patients received 15 mg of d,l-leucovorin for all courses, one received 100 mg for all courses and three had their doses increased to 100 mg (during course 1 for one patient, during course 4 for another patient, and to 50 mg for the third patient during course 3 and then to 100 mg during course 4 because of delayed MTX excretion).

Efficacy

Efficacy of l-leucovorin was determined by its ability to prevent toxicity associated with HDMTX therapy. Patients were monitored for adverse experiences during and for 1 week after administration of HDMTX-LCV. If any toxicity was observed, assessments were continued until the toxicity had resolved. Hematology and clinical laboratory tests were performed to identify any other adverse effects.

General toxicity

In the l-leucovorin group, only one clinical adverse experience was reported. This occurred in one of the 22 courses of therapy: mild neuropathy (fixed gaze) lasting for 30 min. This occurred before the administration of l-leucovorin and was probably due to HDMTX.

Five of the six patients in the control d,l-leucovorin group had clinical adverse experiences. None of these were considered related to d,l-leucovorin and all were probably a consequence of HDMTX. One patient had severe sepsis associated with a PORT-A-CATH® (Pharmacia Deltec, St Paul, MN). This condition was treated and resolved. All other adverse experiences were of a mild or moderate severity: four patients had one or more gastrointestinal adverse experiences (including abdominal pain, diarrhea, nausea, ulcerative stomatitis and/or vomiting), three had fever and two had sepsis. The following adverse experiences were also reported for one patient each: rash, dehydration, pneumothorax, leukocytosis and urinary tract infection. In total, adverse experiences were recorded for 31 of 42 courses of HDMTX and d,l-leucovorin therapy in five of the six patients in the control group.

Hematologic toxicity

No severe abnormality was observed in the l-leucovorin group. The lowest WBC count in this group was 2200/ μl and was observed in a patient with delayed MTX excretion (see below). In the d,l-leucovorin group, one patient had severe sepsis (as noted above in association with a PORT-A-CATH®) and had several instances of WBC counts below 2500/μl (the lowest value was 1200/μl). Another patient had WBC counts below 2500/μl at baseline and transiently during the first course (the lowest value was 2100/μl). With the exception of one patient in the d,l-leucovorin group who had a transient drop to 54 000/μl, platelet counts in both the l- and d,l-leucovorin groups were above 100 000/μl.

Liver function abnormalities

All patients in the l- and d,l-leucovorin groups had elevations in serum alanine aminotransferase (SGPT) and LDH levels and transient elevations in bilirubin. In most patients with elevated SGPT levels, the maximum values were two to five times the upper limit of normal. However, one patient (in the l-leucovorin group during course 3) had a maximum SGPT test value that was 26 times the upper limit of normal. This test result returned to the normal range during the next course and remained slightly elevated during the remainder of the study. The maximum LDH value observed was five times the upper limit of normal in a patient in the d,l-leucovorin group during course 1; it returned to the normal range during the same course. The highest total serum bilirubin value was somewhat less than three times the upper limit of normal and occurred in a patient in the d,l-leucovorin group. This value returned to the normal range in the same course. These abnormalities are commonly seen with HDMTX therapy, all returned to values that were somewhat above or within the normal range and none was regarded severe enough to delay chemotherapy.

Renal abnormalities

Elevated serum creatinine and blood urea nitrogen (BUN) values occurred sporadically in both groups and may have been indicative of transient renal impairment caused by previous treatment with cisplatin. The highest BUN value of 49 mg/dl (somewhat over twice the upper limit of normal) was observed in a patient in the l-leucovorin group and was transient; it was accompanied by a mildly and transiently elevated serum creatinine of 2.0 mg/dl.

MTX excretion

This paper has focused on an initial analysis of data encompassing a total of 22 courses of HDMTX-LCV therapy in the l-leucovorin group. Subsequent to this analysis, additional courses of therapy were administered to one patient in the l-leucovorin group. Information for this patient is discussed because it provides additional support in confirming the efficacy of l-leucovorin in rescue from HDMTX toxicity. A delayed excretion of MTX was observed in this patient during course 22. The previous 21 courses had been administered without difficulty. Courses 1–3 had been rescued with 7.5 mg of l-leucovorin. At the end of course 4, some delayed excretion was noted and the l-leucovorin dose was increased to 50 mg and maintained at that dose for courses 4–21. Following the prolonged delay of MTX excretion in course 22, the escalated l-leucovorin doses utilized in courses 4–22 were retained for courses 23–31 and rescue was successfully accomplished in each instance. The MTX concentrations and hematologic and clinical chemistry laboratory test values associated with course 22 and the prolonged MTX decay are outlined in Table 1 and Figure 1, respectively. No clinical toxicity during course 22 was detected.

Table 1. Serum MTX concentrations, liver and renal function test values, and hemogram findings associated with delayed MTX excretion in a patient receiving l-leucovorin rescue

Day of course 22	MTX concentration (μmol/l)	Hemoglobin (g/dl)	Hemocrit (%)	WBC count ($\times 10^3/\mu l$)	Platelet count ($\times 10^3/\mu l$)	Serum bilirubin (mg/dl)	SGPT (μ/l)	SGOT (μ/l)	BUN (mg/dl)	Serum creatine (mg/dl)
1	—[a]	13.0	39	6.9	328	0.2	16	—	21	0.6
2	1280	13.0	38	30.0	278	2.8	388	—	19	1.5
	332[a]									
3	22.8	12.4	36	13.3	273	1.8	273	139	10	1.5
4	6.2	11.2	37	11.2	264	1.6	285	124	9	1.5
	3.6[b]									
5	2.5	—	—	—	—	1.3	193	77	7	1.1
6	1.0	11.9	34	7.7	231	1.0	167	69	8	1.1
7	0.7	11.4	34	5.6	234	1.3	170	84	7	1.1
8	0.5	11.3	32	5.8	197	—	—	—	7	1.0
9	0.3[b]									

[a] —, not determined.
[b] Only MTX concentrations determined.

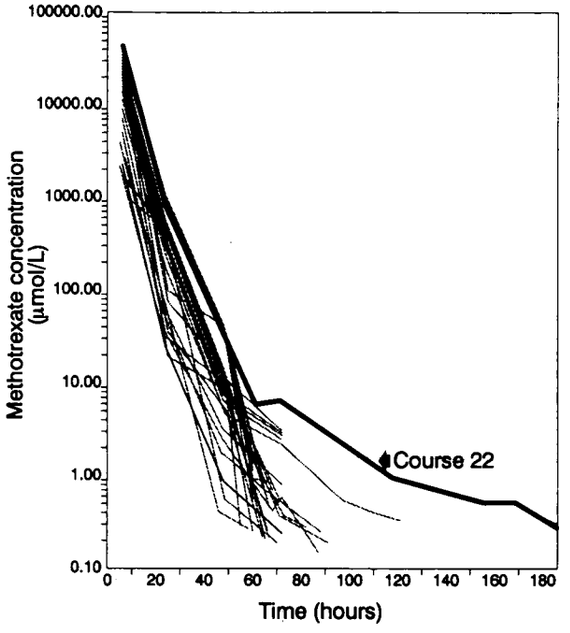

Figure 1. MTX concentrations (courses 1–31) observed in a patient with delayed excretion during course 22.

Discussion

Historically, severe toxicity has been observed in approximately 10% of courses of HDMTX-LCV;[14–16] However, in this study, essentially no clinical toxicity was observed in the group receiving l-leucovorin. This lack of toxicity may be attributed to careful monitoring of serum MTX concentrations and the increased administration of l-leucovorin in instances where a delayed MTX excretion was detected. This is exemplified by a patient where delayed excretion was detected during course 22. Following the return of the clinical status and laboratory values to normal parameters, the patient was able to tolerate an additional nine courses without incident (Figure 1). The only abnormalities recorded were biochemical and hematological. These were similar to those observed in the other patients, and with the administration of additional doses of l-leucovorin, no clinical toxicity was encountered. The liver and hemogram abnormalities returned to normal when the MTX concentration reached an acceptable non-toxic level (0.3 μmol/l).

All patients in the group receiving l-leucovorin had been previously treated with cisplatin. Cumulative doses of this agent are known to be associated with impaired renal function, which can also cause a delay in MTX excretion. This increases the potential for toxicity; however, only hematologic and biochemical abnormalities were observed. There was no clinical toxicity in patients treated with l-leucovorin. This demonstrates that l-leucovorin was an effective rescue mechanism.

The data regarding effectiveness of rescue from this study are consistent with a recently published report of a comparison of d,l-leucovorin and l-leucovorin (at half the dose of d,l-leucovorin) used in the rescue from HDMTX therapy in children with acute lymphocytic leukemia.[17] Data analysis from this study showed no statistically significant differences in the incidence of toxicity between the two treatment groups; in addition, all toxic effects were rapidly reversible and no treatment delays were needed.

The efficacy of l-leucovorin was also confirmed by comparing appropriate data from the patients receiving l-leucovorin with clinical, hematologic and biochemical profiles of historical control patients rescued with d,l-leucovorin. The procedure used to match control to test patients (approximately two d,l-leucovorin patients to one l-leucovorin patient), where possible, selected data in which the same time sequence of treatment was used, to insure comparable circumstances. In this initial analysis, 22 courses of MTX rescued with l-leucovorin in three patients were compared with 42 MTX courses in six patients rescued with d,l-leucovorin. l-Leucovorin at half the dose of d,l-leucovorin was comparable with d,l-leucovorin in its ability to rescue patients with pediatric osteosarcoma who were treated with HDMTX.

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