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Evaluation of Weekly Escalating Doses of Dichloromethotrexate in Patients with Hepatocellular Carcinoma and Other Solid Tumors

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Summary. *Twenty-one patients with solid tumors were treated with weekly 6-h intravenous infusions of dichloromethotrexate (DCM), with escalating doses every other week. Frequently observed toxicities included leukopenia, thrombocytopenia, and mucositis. Nausea, vomiting, diarrhea, and elevation of hepatic enzymes and bilirubin occurred less often. The toxicity of DCM was dose-dependent; the maximum tolerated dosage excalation plan was 400 mg/m^2 × 2 weeks, 800 mg/m^2 × 2 weeks, and then 1,200 mg/m^2 weekly. Plasma concentrations of DCM were measured during 61 infusions and apparent half-lives determined. The plasma elimination of DCM appears to be similar to that of methotrexate. Three objective tumor responses seen in the seven hepatocellular carcinoma patients treated warrant further investigation.*

Introduction

Dichloromethotrexate (DCM) is a halogenated derivative of methotrexate (MTX) that has not achieved the same clinical importance as the parent drug. Interest in this compound has continued because of superior activity in several experimental chemotherapy systems. In mice with advanced L1210 leukemia and C3H lymphosarcoma, DCM has been shown to have greater antitumor activity than MTX [7, 10]. A human malignant lymphocyte line has been shown to be more sensitive to DCM than MTX in an albumin-free medium [19]. Interestingly, a lymphosarcoma line resistant to the drug methylene dimethane sulfonate was shown to be cross-resistant to MTX but sensitive to DCM [6]. However, previous trials in man have not suggested that the antitumor activity of DCM is greater than that observed for MTX [2, 5, 7, 20]. It is not known whether this apparent lack of superiority of DCM to MTX in clinical studies results from biologic differences between human and animal tumor cells, or from differences between the pharmacokinetics and metabolism of the two drugs.

The pharmacology of DCM has not been extensively investigated. Preliminary studies have shown that in humans up to one-half of an IV dose is eliminated via bile and feces, and as much as one-third may be metabolically inactivated [3]. This contrasts with MTX which, in conventional doses, is predominantly excreted unchanged in the urine. DCM is also more highly protein-bound than MTX [3, 19].

The use of high doses of MTX followed by calcium leucovorin administration is an attempt to improve the therapeutic index of MTX. To our knowledge a study employing high doses of DCM with leucovorin 'rescue' has not been undertaken. We have attempted to establish the maximum tolerable dose of DCM given as a weekly IV infusion without leucovorin before embarking on a trial of high-dose DCM treatment. Escalating doses of DCM were given to solid tumor patients by a 6-h IV infusion. This method of drug administration was chosen since it was concurrently being employed in treatment with high-dose MTX [17]. Drug levels have been measured by a sensitive radioimmunoassay for MTX. The toxicity and pharmacokinetics of DCM given in this manner are described. The antitumor effects of DCM which we observed are also reported. Because of findings [1] that DCM cannot be metabolized by certain transplantable animal hepatomas, we were especially interested in investigating antitumor activ-

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Table 1. Characteristics of 21 patients treated with DCM

Characteristic Median age in years 56 (range 16–72)	No. of patients
Sex	
Males	19
Females	2
Prior therapy	
Chemotherapy only	10
Radiotherapy only	1
Both	9
None	1
Methotrexate	4
Performance status[a]	
0–1	13
2	6
3	2
4	0
Primary tumor site	
Hepatocellular carcinoma	7
Lung	5[b]
Prostate	2
Soft tissue sarcoma	2
Oropharnyx	1
Mycosis fungoides	1
Adenocarcinoma of unknown primary site	1
Esophagus	1
Colon	1

[a] Eastern Cooperative Oncology Group scale: 0, 1, fully ambulatory; 2, ambulatory > 50% of the day; 3, ambulatory < 50% of the day; 4, totally bedridden

[b] Includes two patients with small cell carcinoma

ity of DCM in patients with hepatocellular carcinoma.

Materials and Methods

Twenty-one patients with solid tumors were treated with DCM. Pretreatment characteristics are listed in Table 1. All patients had an evaluable tumor mass, were at least partially ambulatory, and had a life expectancy of at least 1 month. Criteria for protocol entry included leukocyte count (WBC) > 4,000/μl, platelets > 100,000/μl, creatinine < 2.5 mg/dl, and bilirubin < 2.5 mg/dl. Informed consent was obtained from all patients.

DCM was administered by a weekly 6-h IV infusion. No prehydration, alkalinization, or leucovorin was employed. The dose of DCM was escalated every second week as tolerated up to three dose levels with subsequent treatment continued at the third dose. After several patients had been safely treated with a low-dose treatment plan, the next group of patients received higher doses. Three treatment plans were employed (doses in mg/m^2): (a) 100 × 2 weeks, 200 × 2 weeks, and then 400 weekly, (b) 400 × 2 weeks, 800 × 2 weeks, and then 1,200 weekly, and (c) 800 × 2 weeks, 1,200 × 2 weeks, and then 1,600 weekly. Dosage escalation was not permitted in the presence of leukopenia 3,000–4,000/μl, thrombocytopenia 75,000–100,000/μl, or mild stomatitis. Treatment was withheld 1 week for more severe myelosuppression or stomatitis, any increase in serum creatinine or bilirubin, or diarrhea. After recovery from toxicity that mandated treatment delay, DCM was resumed with a 25%–50% dose reduction and then escalated as tolerated. Treatment was continued at the highest tolerated dose level until tumor progression was evident. Four patients who tolerated a low-dose treatment plan without toxicity received doses escalated beyond the upper limit of their plans.

Plasma DCM concentrations was measured from 17 patients at 3, 6, 12, and 24 h after the start of 61 drug infusions. Concentrations were measured by a commercially available radioimmunoassay kit for MTX (Diagnostic Biochemistry, Inc., San Diego, CA, USA). Concentrations of the stock solutions used for DCM standard solutions were verified spectrophotometrically, published molar extinction coefficients being used [12]. The practical limit of sensitivity for this assay in our hands was 0.5 pmol, which allowed the measurement of plasma concentrations of 5 n*M*. The coefficient of variation in this assay procedure was consistently less than 15%.

Although the antibody contained in this kit has been shown to cross-react significantly with metabolites of MTX in which the pteridine position of the molecule is intact [4], 7-hydroxy MTX cross-reacts only to a minor extent. Since the major expected metabolic product of DCM is 7-hydroxy DCM [3, 15], significant interference with assay of the parent compound was not anticipated.

Pretreatment evaluation of patients included physical examination, weight, performance status, complete blood count, biochemical profile, urinalysis, and chest X-ray. Hepatocellular carcinoma patients also underwent radionuclide liver scan, computed tomographic (CT) liver scan, and determination of serum alpha-fetoprotein. Appropriate scans and X-rays necessary to determine response were obtained in patients with other tumors. A complete response was defined as the disappearance of all evidence of tumor. A partial response was the reduction by at least 50% of the product of perpendicular diameters of the most clearly measurable tumor mass, a 30% diminution in the sum of liver measurements below the xiphoid process and each midclavicular line (patients with malignant hepatomegaly), or a substantial decrease in evaluable tumor mass confirmed by two investigators in the absence of increase in any tumor mass or appearance of new areas of tumor. Progressive disease was defined as an increase in any measurable lesion by 25% or more, a substantial increase in an evaluable lesion, or the appearance of new areas of malignant disease. Stable disease was the absence of complete or partial response or progressive disease for a period of 2 months or longer. The duration of a response or stable disease was measured from the first day of treatment until objective evidence of tumor progression.

Results

Table 2 reports the number of treatment cycles given per patient and the frequency of dosage escalation and reduction. Common toxicities in our patients included leukopenia, thrombocytopenia, and mucositis. Nausea, vomiting, diarrhea, and transient elevation of hepatic enzymes and bilirubin were also encountered. The frequency of these toxicities and the number of infusions administered before appearance of the first hematologic toxicity for each treatment plan are reported in Table 3. Fewer infusions were required to produce myelosuppression with the higher dose plans.

Table 2. Number and doses of DCM infusions administered

Treatment plan (mg/m²/week)	Patients	Infusions per patient			% Increased dose cycles[a]	% Modified cycles[b]
		Median	Mean	Range		
100 × 2, 200 × 2, 400 . . .	3	16	23	7–46	39	10
400 × 2, 800 × 2, 1,200 . . .	13	6	12	2–61	3.7	21
800 × 2, 1,200 × 2, 1,600 . . .	5	2	5	1–11	0	65

[a] Percentage of cycles where dose was increased beyond upper limit of plan
[b] Percentage of cycles where dose was decreased or treatment was delayed

Table 3. Clinical toxicities of DCM

Treatment plan (mg/m²/week)	Number of		Number of patients with none/mild/moderate/severe maximum hematologic toxicity[a]		Median number of infusions to first hematologic toxicity (range)[b]		% Toxic infusions[c]		Number of patients with other toxicities		
	Patients	Infusions	WBC	Platelet	WBC	Platelet	WBC	Platelet	Mucositis	Diarrhea	Hepatic[d]
100 × 2, 200 × 2, 400 . . .	3	69	1/2/0/0	2/1/0/0	3,14 (2 patients)	10 (1 patient)	4.3	2.9	2	0	1
400 × 2, 800 × 2, 1,200 . . .	13	162	4/6/2/1	4/6/1/2	3 (1–47)	5 (2–19)	17.3	9.3	7	5	3
800 × 2, 1,200 × 2, 1,600 . . .	5	26	2/0/1/2	0/2/1/2	2 (1–3)	1 (1–6)	15.4	23.0	1	1	2

[a] Nadir WBC and platelet counts (/μl) are scored as follows: none: WBC > 4,000, platelet > 100,000; mild: WBC 2,500–3,900, platelet 50,000–99,000; moderate: WBC 1,000–2,400, platelet 25,000–49,000; severe: WBC < 1,000, platelet < 25,000
[b] In patients with hematologic toxicity
[c] Treatment cycles with WBC < 4,000/μl or platelet < 100,000/μl
[d] Doubling of SGOT or bilirubin

Five cases of infection or neutropenic fever required antibiotic support, and six cases of bleeding or severe thrombocytopenia required platelet transfusion. Of the six cases with elevations of hepatic enzymes or bilirubin, three occurred in hepatocellular carcinoma patients. Uncommon possible side-effects of DCM, each observed once, include renal failure in a septicemic patient, transient azotemia, fever, conjunctivitis, headache, rash, impaired auditory acuity, and pulmonary edema. One probable drug-related death occurred in a patient with hepatocellular carcinoma, who died with progressive hepatic failure, pancytopenia, mucositis, and upper gastrointestinal hemorrhage. Leucovorin rescue was given to only one patient.

With this weekly schedule the maximum tolerated dosage plan was 400 mg/m² × 2 weeks, 800 mg/m² × 2 weeks, and then 1,200 mg/m² weekly. Six of the 13 patients entered onto this plan were treated with the 1,200 mg/m² dose for a median of six (range 1–57) infusions. Of 91 infusions given at this dose level, seven (13%) were associated with some hematologic toxicity (WBC < 4,000/μl or platelets < 100,000/μl), but in only one case was the toxicity

Table 4. DCM pharmacokinetics

Dose (mg/m^2)	Number of infusions	Plasma concentrations[a] (μM)			
		3 h	6 h	12 h	24 h
100	7	7.9 ± 2.1	6.7 ± 3.2	0.56 ± 0.47	0.15 ± 0.17
200	6	112.3 ± 161.7	44.9 ± 48.3	1.3 ± 1.4	0.17 ± 0.16
400	18	455.4 ± 373.3	508.2 ± 432.1	3.5 ± 2.3	0.30 ± 0.22
600	4	535.0 ± 119.0	587.5 ± 217.7	5.3 ± 5.2	0.24 ± 0.14
800	21	722.0 ± 841.9	1,042.3 ± 1,368.5	14.4 ± 13.5	0.84 ± 0.60
1,200	5	676.0 ± 638.1	1,112.0 ± 588.0	13.6 ± 12.9	0.98 ± 1.07

[a] Mean ± 1 SD

severe. The highest dose treatment schedule (800 mg/m^2 × 2 weeks, 1,200 mg/m^2 × 2 weeks, and then 1,600 mg/m^2 weekly) required much more frequent dosage reductions and delays in therapy and induced severe hematologic toxicity in 40% of patients.

Six patients (five treated according to the intermediate and one to the highest dosage escalation plan) tolerated 1,200 mg/m^2 weekly for more than 2 weeks. In these patients, who received a median of 6.5 (range 3–52) consecutive infusions at this dose level, no cumulative myelotoxicity was observed.

The plasma concentrations measured in patients treated at the various dose levels of DCM employed in this study are shown in Table 4. Mean peak plasma concentrations reached at the end of the 6-h infusions ranged from 6.7 μM, at the 100 mg/m^2 dose to greater than 1 mM at both 800 mg/m^2 and 1,200 mg/m^2 doses. Mean plasma concentrations 24 h after the beginning of the infusions were less than 1 μM at all dosage levels. Plasma DCM concentrations were measured during 21 infusions given at the 800 mg/m^2 dose level. Median 24-h drug concentrations for infusions associated with and without hematologic toxicity were 7.0 and 7.2 μM, respectively. Neither elevated peak levels nor elevated 24-h levels were predictive of toxicity. Unfortunately drug concentrations were not measured beyond 24 h.

The limited number of time points examined after the end of the infusions precludes a detailed pharmacokinetic analysis. However, plasma elimination curves at all dose levels appear to be biphasic, as shown in Fig. 1 for the data obtained in patients treated at the 800 mg/m^2 dose. The 'apparent' half-lives of DCM in these patients between 6 and 12 h and between 12 and 24 h were 1.0 ± 0.17 h (mean ± SD) and 3.18 ± 0.62 h, respectively. These mean values were obtained from the slopes of the lines connecting individual patient plasma concentration data at arbitrary time points and therefore cannot be considered true plasma half-lives.

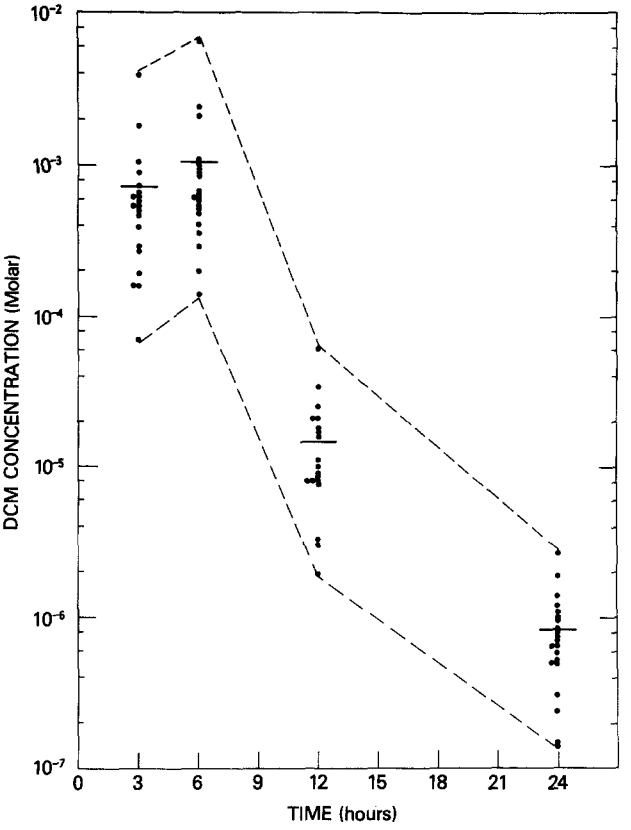

Fig. 1. Plasma DCM concentrations in patients treated with 800 mg/m^2 in a 6-h infusion beginning at time 0. Shown are the individual patient data (•) and the mean values (–) for drug concentrations at each time point for the 21 infusions studied. The *dotted lines* enclose the range of values observed in these patients

Four patients had partial responses (duration 22–67 weeks) and two patients had stable disease for 21 and 26 weeks. Of seven hepatocellular carcinoma patients three achieved partial responses, and a fourth had stable disease. These three responses were documented by improvements in radionuclide and CT liver scans and liver size on physical examination

in one patient, decreased size of multiple pulmonary metastases on chest X-ray in a second, and improvement in radionuclide liver scan and 75% decline in serum alpha-fetoprotein in a third. The fourth partial response occurred in a patient with adenocarcinoma of unknown primary site whose pulmonary metastases decreased in size. The survival from initiation of DCM in the three responding hepatocellular carcinoma patients was 40, 71, and 101 weeks. The median (range) survival of all 21 patients treated was 25 (2–101) weeks. None of the four patients who were previously treated with MTX responded to DCM. Only one of these four had responded to MTX.

Discussion

Previously reported toxicities of DCM include leukopenia, thrombocytopenia, nausea, vomiting, diarrhea, oral ulceration, stomatitis, and increased serum creatinine, bilirubin, and hepatic enzymes [2, 5, 7, 20]. We found the same toxicities when an IV infusion of DCM was given according to a weekly schedule. DCM can safely be administered without leucovorin rescue in this manner. Weekly infusions with dose escalation as tolerated from 400 mg/m^2 to 1,200 mg/m^2 result in acceptable toxicity. Fernbach et al. [5] reported that doses of DCM as high as 2,500 mg/m^2 could be safely given every 3 weeks.

DCM is protein bound in the range of 83%–94% both in vitro and in vivo [3, 8, 19]. Fernbach et al. [5] proposed that the greater protein binding of DCM might explain the relative lack of toxicity of DCM compared with MTX. In a subsequent report [14] eight patients treated with IV bolus DCM every 6 h were found to have hematologic toxicity only when the serum albumin level was less than 4.0 g/dl. We could not confirm this correlation between albumin level and toxicity. In our study serum albumin levels were known during 169 infusions. During 133 infusions the serum albumin was less than 4.0 g/dl. Some hematologic toxicity (WBC $< 4{,}000/\mu l$ or platelets $< 100{,}000/\mu l$) occured during 20.3% of these low-albumin-level infusions. The remaining 36 infusions given to patients with serum albumin greater than 4.0 were associated with some hematologic toxicity in 27.7%. This lack of association between serum albumin level and toxicity may be explained by our use of a different dose and schedule of drug administration or by the much larger number of infusions evaluated in our patients.

The drug concentrations of DCM were measured by means of a commercial radioimmunoassay for MTX. Others report no difference between drug levels measured by radioimmunoassay and competitive enzyme-binding assay [5]. This suggests that the assay used here did not measure inactive metabolites of DCM. The major metabolite, 7-hydroxy DCM, has been shown to have only 0.1% the antifolate activity of DCM [13].

We found that peak plasma levels of DCM greater than 1 m*M* can be achieved at tolerable doses. Considerable variability in drug concentrations at various time points was noted in patients treated at a single dosage level, as previously observed with high doses of MTX [18]. Plasma disappearance follows a biphasic pattern which is similar to that described for MTX [18]. Metabolic conversion to 7-hydroxy DCM could inactivate as much as one-third of an IV dose and potentially allow treatment of patients with renal insufficiency. Drug concentrations at 24 h in our patients were dose-dependent. At 800 and 1,200 mg/m^2 they were similar to those measured by Fernbach et al. [5] in patients receiving 2,000 mg/m^2 every 3 weeks.

Responses to DCM have been reported in hepatocellular carcinoma [11, 16, 20]. One randomized trial [9] showed that intra-arterial MTX prolonged survival of hepatocellular carcinoma patients, also indicating the possible activity of folate antagonists in this neoplasm. The use of DCM in hepatocellular carcinoma is attractive for several reasons: it is bound to liver tissue [15]; it is metabolized by normal liver cells to an inactive metabolite [3, 15]; and it cannot be metabolized by certain transplantable animal hepatomas [1].

We observed three partial responses and one patient with stable disease for 21 weeks in seven hepatocellular carcinoma cases treated with DCM. None of these seven patients had previously received MTX. This suggested activity of DCM and possibly other antifols in hepatocellular carcinoma patients warrants further investigation.

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