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Phase I toxicity and pharmacology study of trimethylcolchicinic acid in patients with advanced malignancies*

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Summary. A phase I study of trimethylcolchicinic acid (TMCA) given orally once daily for 5 days every 3rd week was performed in 19 patients with advanced malignancies. Myelosuppression and mucositis were the major toxicities observed. Serum TMCA levels were monitored and appear to be useful in predicting toxicities. A partial response was seen in one lymphoma patient and stabilization of disease was noted in one patient each with prostatic and ovarian cancer.

Introduction

Colchicine and closely related derivatives are powerful mitotic poisons with significant antitumor activity in experimental systems, which have been useful in the treatment of chronic myelogenous leukemia [6]. Unfortunately, at the doses required for antitumor activity, colchicine produces severe toxicities, which prevents it from being a useful chemotherapeutic agent [1]. Trimethylcolchicinic acid (TMCA), also known as *N*-desacetylcolchicine, is a colchicine derivative that retains the antitumor effects of colchicine, but at greatly reduced toxicity, having a therapeutic index 25 times greater than that of colchicine [3] in sarcoma 180-bearing mice. Previous human studies [6, 8] conducted in the 1960s using low doses of daily oral TMCA demonstrated clinical activity in several malignancies, including lymphomas, chronic myelogenous leukemia, melanoma, and breast cancer. The side effects seen in these trials consisted mainly of mucositis, diarrhea, nausea, and myelosuppression. Of significance in these trials was the antitumor activity, observed even in heavily pretreated patients, and the apparent lack of cross-resistance with other standard chemotherapeutic agents. These data suggest that TMCA may have significant potential as an antitumor agent and that this class of antitumor drugs should be reassessed. Moreover, a dosing schedule different from those previously used (intermittent high doses) could prove to be more suitable against aggressive lymphomas and other malignancies, and its safety might be enhanced by monitoring of drug serum levels. We therefore initiated a new phase I trial to determine an alternative dose/schedule for TMCA. Detailed quantitation of serum levels of TMCA were performed to determine the pharmacokinetic profile after oral dosing and blood levels achieved at various doses.

Patients and materials

Drug. TMCA is a synthetic derivative of colchicine. The drug is supplied as a capsule containing 1 mg TMCA by the National Cancer Institute.

Clinical study. Patients eligible for this study had to have histologically proven malignancies that were refractory to conventional modes of treatment. They were also required to have adequate renal and liver function, as well as normal blood counts. Other eligibility criteria included a Karnofsky performance status of 70 or higher, no gastrointestinal abnormalities, and no signs suggestive of CNS involvement with tumor.

Eligible patients were treated daily with TMCA given orally for 5 days every 3 weeks. Based on previous clinical trials in which 4 mg/m^2 TMCA was given on a daily $\times 5$ schedule in combination with other drugs without producing major toxicity [8], a starting dose of 5 mg/m^2 per day for 5 days was chosen. Patients were instructed to take the drug in the morning, before breakfast and after an overnight fast. Dose levels were to be escalated by increments of 2 mg/m^2 daily, with a minimum of three patients entered at each dose level. After the development of toxicity of grade 3 or greater in two patients, three additional subjects were to be placed on this dose level. On observation of the development of grade 3 or 4 toxicity in four of six patients, the dose immediately below this level was to be defined as the maximal tolerated dose (MTD). Toxicity was graded according to the common toxicity scale of the Southwest Oncology Group [7].

Study parameters included the collection of serum and urine specimens for pharmacokinetic assessment in addition to usual tumor measurements and toxicity evaluation. Blood samples for TMCA levels were taken predose (baseline) and at 1, 2, 4, 6, 8, 12, and 24 h after the first dose on day 1; this was repeated on day 5 of the treatment cycle.

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Urine samples were also collected on the same days in combined 6-h aliquots for quantitation of renal excretion.

TMCA assay. TMCA was analyzed in serum or urine using a selective, sensitive, high-performance liquid chromatographic (HPLC) method [5]. To 0.5 ml serum or 0.1 ml urine diluted to 0.5 ml was added 50 µl demecolcine (2 µg/ml) as internal standard. The sample was extracted using a C-2 reverse-phase solid-extraction column. TMCA and the internal standard were eluted from the column using methanol. The combined eluates were evaporated to dryness and the residue was reconstituted with water. The reconstituted sample was injected into a C-18 reverse-phase column and then eluted using a mobile phase consisting of 0.1 *M* potassium dihydrogen phosphate and 5 m*M* 1-pentanesulfonic acid in methanol and acetonitrile (final pH, 6.0) at a flow rate of 1.5 ml/min. TMCA and the internal standard were detected using a variable-wavelength UV detector at 254 nm. The minimal assay sensitivity was 0.4 ng/ml, and the standard curve was linear over the concentration range of 1–200 ng/ml.

Pharmacokinetic analysis. Plasma concentration-time data were analyzed using PCNONLIN (Statistical Consultants, Inc.). Data that could not be described by a model were analyzed using model-independent methods. Area under the concentration-time curve (AUC) were calculated using the LAGRAN program. The extent of accumulation of the drug was calculated by determining the ratio of the AUC of dose 5 (from 0 to 24 h) to that of dose 1 (from 0 to infinity). Since no bioavailability data was available, all pharmacokinetic parameters dependent on the extent of absorption were expressed in terms of bioavailability (F). Total body clearance was calculated using a model-independent method. Correlation between selected pharmacokinetic parameters and toxicity index were analyzed using linear regression.

Results

Patient characteristics

A total of 19 patients were entered in the study. The clinical characteristics of the patients are shown in Table 1. The mean age of the patients was 54 years, and all subjects had a Karnofsky performance status of at least 70. Overall, seven patients had breast cancer, three had lung cancer, two had ovarian cancer, and one each had melanoma, lymphoma, Kaposi's sarcoma, and renal cell, prostate, colon, and fallopian tube carcinomas. Most subjects had received extensive prior therapy, especially patients 7 and 8, both of whom had failed multiple combination chemotherapy.

Toxicity

The major toxicity related to TMCA was myelosuppression and gastrointestinal (GI) toxicity (Table 2). The GI symptoms consisted of mucositis and diarrhea; nausea and vomiting was rare and mild in nature. The severity of mucositis and diarrhea paralleled the degree of myelosuppression, with the onset usually occurring 1–2 days after the last dose of TMCA and preceding a sudden dip in WBC by 2 days. Recovery of WBC usually required 1–2 weeks. The decline and recovery in the platelet count lagged behind those of the WBC by several days.

The trial was started at a dose of 5 mg/m^2 daily ×5. Patients 1–4 were initially accured. Patient 2 was deemed ineligible for evaluation due to the development of considerable elevations in liver-function values, whereas patients 1, 3, and 4 had no significant (grade 3 or 4) toxicity. Next, subjects 12 and 13 were accrued at a dose of 7 mg/m^2 daily ×5. Both of these patients developed severe toxicities. Patient 12 developed fever on day 5 and was treated with sulfamethoxazole-trimethoprim; diffuse skin erythema, hypotension disseminated intravascular coagulation, renal and liver insufficiency developed 2 days later, followed by the onset of mucositis and neutropenia. This subject died shortly thereafter of multi-organ failure. Patient 13, with an extensively pretreated malignant lymphoma, developed

Table 1. Patient characteristics

Patient number	TMCA dose	Diagnosis	Age/Sex	PS	Prior treatment	Disease sites
1	5 mg/m^2 daily ×5	Ovarian	48/F	90	S,R,C	Ln
2	"	Breast	57/F	80	S,R,C	Lu,B,Li
3	"	Breast	51/F	80	S,R,C	Chest wall
4	"	Melanoma	52/F	90	C,IL2	Skin
5	"	Lung	60/M	70	R,C	Ln,Li,Lu
6	"	Lung	52/F	80	R,C	Lu,Ln
7	"	Breast	37/F	80	ABMT,S,R,C	Lu
8	"	Breast	70/F	70	S,R,C	Lu, skin, chest wall
9	"	Breast	45/F	80	S,R,C	Ln,Lu,B
10	"	Fallopian	74/F	90	S,R,C	Ln
11	"	Ovarian	45/F	90	S,R,C	Ln
12	7 mg/m^2 daily ×5	Colon	64/M	80	S,C	Li
13	"	Lymphoma	67/M	80	R,C	Ln,B
14	6 mg/m^2 daily ×5	Breast	36/F	80	S,C	Ln, chest wall
15	"	Breast	60/F	90	S,R,C	
16	"	Lung	65/M	80	C	Lu,B, skin
17	"	Prostate	67/M	90	H	B
18	"	KS	35/M	90	-	Skin
19	"	Renal	34/M	90	S,IFN	Lu, kidney

PS, performance status; KS, Kaposi's sarcoma; S, surgery; R, radiation; C, chemotherapy; H, hormonal; ABMT, autologous bone marrow transplantation; IFN, interferon; Ln, lymph node; Li, liver; B, bone; Lu, lung

Table 2. Toxicity

Patient number	TMCA dose	Nadir WBC ($\times 10^3/mm^3$)	Plt ($\times 10^3/mm^3$)	GI (grade)	Other	Cycles (*n*)
1	5 mg/m² daily ×5	4.1	435	0		3
2[a]	"	<0.3	29	4		1
3	"	2.4	249	1		3
4	"	5.1	337	0		1
5[b]	"	–	–	–		–
6	"	1.4	184	1		3
7[c]	"	0.4	41	2		1
8[c]	"	<0.5	–	2		1
9	"	1.9	312	0		2
10	"	2.7	213	0		1
11	"	6.8	233	0		2
12	7 mg/m² daily ×5	<0.3	<20	3	DIC, sepsis, erythema multiforme	1
13	"	0.6	21	3	Pulmonary	1
14	6 mg/m² daily ×5	3.8	191	0		1
15[d]	"	3.4	328	0		1
16	"	<0.2	16	3		1
17	"	2.6	167	1		8
18	"	2.3	277	2		1
19	"	1.2	275	2		1

[a] Ineligible due to elevated liver values
[b] Treatment discontinued after day 1 due to elevated liver values
[c] Extensive prior chemotherapy
[d] Received only 4 days of TMCA
Plt = platelets; DIC = disseminated intravascular coagulation

Table 3. TMCA pharmacokinetic parameters on days 1 and 5 in patients with a WBC of ≤1.2 or ≥1.9

	Patient number	Dose (mg/m²)	Vd (l/kg)	AUC (μg h/l⁻¹)	Cl (ml/min⁻¹/kg⁻¹)	$t_{1/2}$ (h)	C_{max} (μg/l)
Day 1:							
WBC <1.2	7	5	4.80	320	6.90	8	26.70
	19	6	6.80	511	5.80	13.50	21
	16	6	6	399	6.50	10.70	19.80
	13	7	6.50	285	12	8.20	17
	Mean		6.03	378.75	7.80	10.10	21.13
	SD		0.88	100.23	2.84	2.58	4.08
WBC >1.9	9	5	10.10	239	9.80	11.90	13.50
	17	6	6.60	199	12.30	6.20	11.30
	15	6	6.90	404	6.30	12.70	16.80
	14	6	6.30	289	7.50	9.70	15.80
	13	7	6.50	285	12	8.20	17
	10	5	6.80	293	8.00	9.80	17.90
	Mean		7.20	284.83	9.32	9.75	15.38
	SD		1.44	68.92	2.47	2.38	2.50
Day 5:							
WBC<1.2	7	5					
	19	6	5	616	4.80	23.30	34.70
	16	6	3.80	418	6.20	7.80	30.70
	13	7	4.50	490	7	9.50	30
	Mean		4.43	508	6	13.53	31.80
	SD		0.60	100.22	1.11	8.50	2.54
WBC >1.9	9	5					
	17	6	7	190	12.90	6.90	20.20
	15	6					
	14	6	4.60	344	6.30	11	20.40
	13	7	4.50	490	7	9.50	30
	10	5					
	Mean		5.37	341.33	8.73	9.13	23.53
	SD		1.42	150.02	3.63	2.07	5.60

grade 4 neutropenia and subsequent diffuse pulmonary infiltrates. The subject became extremely dyspneic, showing p02 values in the 40s on room air. Bronchoscopy and biopsy revealed nonspecific interstitial inflammation believed to be consistent with acute drug-induced injury. No evidence for either fungal, mycobacterial, viral, or bacterial infection was found on the special stains and cultures of the lung biopsy specimen. Steroid therapy resulted in rapid reversal of all pulmonary symptoms and chest X-ray abnormalities.

The next group of patients (subjects 14–19) were enrolled at the dose level of 6 mg/m^2 daily ×5. Significant myelosuppression (grade 3 or 4) was seen in two of the six patients; because of the toxicity seen in this trial, additional patients were accrued at 5 mg/m^2 daily ×5. The data of patients 5–11 confirmed that 5 mg/m^2 daily is a suitable starting dose for a phase II trial, except perhaps for poor-risk patients (i.e., those with refractory disease after extensive prior therapy). From these subjects we also obtained additional pharmacokinetic information during the development of an HPLC assay. Patient 5 was noted to have rapidly deteriorating liver status, and TMCA was terminated after only 1 day of therapy. Four patients had only mild toxicity, if any, whereas two poor-risk cases (patients 7, 8) developed grade 4 myelosuppression but only grade 2 mucositis. The discrepancy in the severity of bone marrow vs GI toxicities in these two patients probably reflects the extent of stem-cell damage from prior chemotherapy and radiotherapy.

Pharmacokinetics

Plasma samples from 11 patients were available. Subjects 5 and 15 received less than the planned 5 days of treatment. The pharmacokinetic data, correlating the nadir WBC in ten patients, are summarized in Table 3. The mean half-life of TMCA was 10.5 h on day 1, with a total body clearance of 8.25 ml/min^{-1}/kg^{-1}/F^{-1}. The mean volume of distribution on day 1 was 6.52 l/kg.

The average plasma concentration vs time profile for the three doses is shown in Fig. 1. The following interesting points are suggested by the results:

1. Toxicity appeared to correlate with the peak concentration (C_p) of TMCA on day 1 and the C_p and AUC on day 5.

2. Drug accumulation on the current dosing schedule resulted in an increased in AUC and C_p values on day 5 as compared with day 1. When TMCA was given at a fixed daily dose over a 5-day period, the accumulation index was 1.1 at a dose of 6 mg/m^2 and 1.7 at 7 mg/m^2.

3. Metabolic clearance of the drug by the liver was suggested by the abnormally long half-life in patient 5, who had a rapidly deteriorating liver function. The $t_{1/2}$ in this subject was >15 h compared with a mean $t_{1/2}$ of 10.9 h in the other patients. The 24-h urine sampling showed that 4.6%/F of TMCA was excreted unchanged in the urine. HPLC analysis of the urine showed a peak in the chromatogram whose retention time was identical to that of colchicine; however, the identity of the peak could not be confirmed.

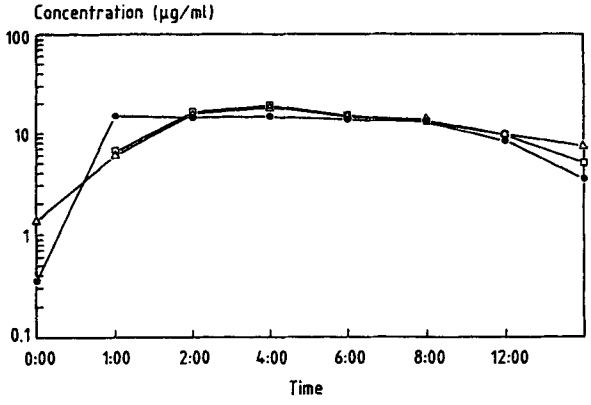

Fig. 1. Average plasma concentration-time profile for TMCA given on day 1 at 5 mg/m^2 (—■—), 6 mg/m^2 (—●—), and 7 mg/m^2 (—□—)

4. Alteration in the absorption of TMCA due to previous high-dose chemotherapy was suggested in patient 7, who had received high-dose chemotherapy in a phase I trial along with autologous bone marrow transplantation 2 months prior to entry into this study. The time to achieve peak plasma concentration was shortest in this patient and the peak plasma level was highest, indicating that absorption of TMCA was rapid, possibly due to alteration in the GI tract secondary to previous high-dose chemotherapy.

5. Significant variability in drug absorption was seen among different patients on identical doses, with the AUC following dose 1 ranging between 167–436 and 199–510 µg/h/l^{-1} for daily doses of 5 and 6 mg/m^2, respectively.

Tumor response

Patient 13, who had lymphoma, achieved a partial response in the size of the lymph nodes. Stabilization of disease was achieved in subject 17, who had prostate cancer and showed a 50% reduction in the level of prostatic acid phosphatase, with stable symptoms and bone scan; patient 2 (ovarian cancer) had stable disease in lymph nodes for 3 months. All other patients experienced disease progression.

Discussion

Colchicine is a powerful mitotic poison with significant antitumor activity as well as severe toxicities [1]. TMCA is a derivative of colchicine that has shown a 25-fold increase in therapeutic index in sarcoma 180-bearing mice [3]. Previous trials using TMCA were conducted at low oral doses, with responses being documented predominantly in subjects with hematological malignancies but also occasionally in those with solid tumors (see Table 4). In these prior studies, toxicities, which included mucositis and neutropenia, were seen in only a portion of patients treated with the same dose. No significant relationship was found between toxicity and the patient's age, pretreatment blood counts, or prior radiotherapy or chemotherapy.

Table 4. Literature review of prior trials using TMCA

Reference	Disease	Patients (*n*)	Response	Dose
Stolinsky et al. [10]	Hodgkin's disease	37	11 PRs	0.025–0.075 mg/kg daily
	NHL-mixed	2	1 PR	
	Histocytic	9	1 CR, 3 PRs	
	Lymphocytic	22	2 PRs	
Stolinsky et al. [11]	CML	9	9	0.05 mg/kg daily
Gomez et al. [4]	CML-blast crises	33	4 CRs, 15 PRs	6.12 mg/day
	Accelerated	13	2 CRs, 3 PRs	
Stolinsky et al. [9]	Malignant melanoma	44	4 PRs	0.05 mg/kg daily
Stolinsky et al. [8]	Various tumors	153	19[a]	0.05 mg/kg daily

[a] Responses seen in patients with Hodgkin's disease, melanoma, chronic myelogenous leukemia (CML), and, occasionally, in patients with adenocarcinoma of the cervix, lung, or breast

NHL, non-Hodgkin's lymphoma; PR, partial response; CR, complete response

These previous trials demonstrated that TMCA had potential as an antitumor agent but that the toxicity was unpredictable. The present phase I trial, in which TMCA was given at intermittent high doses, was initiated to maximize the antitumor activity and to minimize the unpredictable toxicity. In addition, analytical methods for determining serum drug levels were developed and the analysis of TMCA pharmacokinetics following its oral administration was studied as a possible method of predicting toxicity.

A total of 19 patients were entered in this study. As expected, the predominant toxicities were mucositis, diarrhea, and myelosuppression. Although the toxicities appeared to be dose-related, there was significant variability in the degree of myelosuppression at each dose level, reflected by the marked variability in drug absorption among different patients. For TMCA, the C_p on day 1 along with the C_p and AUC on day 5 appears to be a reliable predictor of toxicity. For example, a C_p of >20 μg/ml on day 1, calculated by linear regression, or an AUC value of $\geqslant 500$ μg/h/l^{-1} would be predictive of significant myelosuppression if therapy were allowed to continue for a full course.

Like colchicine, TMCA appears to be metabolized in the liver. In patient 5, who showed rapidly deteriorating liver function, the $t_{1/2}$ of TMCA was one of the longest obtained among the patients studied. Similarly, subject 2 was ineligible because of liver dysfunction and had marked toxicity after receiving TMCA; unfortunately, serum samples from this patient were not available for analysis. Urine specimens obtained from two patients showed an HPLC chromatographic peak with a retention time similar to that of colchicine. However, the identity of this peak could not be positively confirmed without additional analysis. The acetylation of TMCA to colchicine at the primary amino group may possibly explain why most of the delivered dose could not be accounted for in the urine.

A new toxicity probably related to TMCA was also noted in this study. Patient 13 developed diffuse pulmonary infiltrates, which on biopsy were consistent with drug-induced changes. The rapid resolution achieved with steroids also indicates that the pneumonitis was drug-related.

TMCA appears to have a steep dose-toxicity curve. Although only two of six patients developed significant toxicity at the daily dose of 6 mg/m^2, considering the severity of myelosuppression seen in this study, a conservative phase II dose schedule would be 5 mg/m^2 p.o. $\times 5$ days. However, marked variability in myelosuppression was seen even at this dose level, not unlike that previously reported using low daily doses, and was noted especially in patients with liver dysfunction. The cause of this variability is likely to be multifactorial, with oral absorption, hepatic clearance, and extent of prior therapy being major variables. The plasma level profile appears to be predictive of excessive toxicity and could be used as a safeguard. Although responses were unusual in the present trial, based on results of prior studies of TMCA, phase II trials in hematological malignancies and selected solid tumors should be undertaken, with pharmacokinetic analysis incorporated.

Future phase II studies of TMCA are of additional interest, as the drug is a prototype of a class of anticancer drugs that have thus far been inadequately studied. This is surprising in view of the long history of colchicine and its derivatives in medicine [2]. The availability of analytical methodology and the pharmacokinetic findings described herein may facilitate such trials since the unpredictability that has plagued these compounds to date would be minimized. Moreover, the toxicities described consist primarily of myelosuppression and mucositis. In this respect, colchicine-derived drugs are potentially advantageous over other mitotic inhibitors such as the vinca alkaloids, which are neurotoxic. We hope that the stimulus generated by new trials using TMCA will facilitate the development and study of other derivatives with even greater potential [2].

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