Investigational New Drugs 5: 365–371 (1987)

Phase I study and pharmacokinetics of caracemide (NSC-253272) administered as a short infusion

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Key words: caracemide, phase I, pharmacokinetics

Summary

A Phase I study of caracemide evaluating a short intravenous infusion repeated every 21 days is presented. Patients were entered at 85 mg/m^2 with subsequent escalation levels of 170, 425, 595, and 795 mg/m^2. Mild to moderate nausea and vomiting occurred at all dose levels. An apparent allergic reaction was observed at the 425 mg/m^2 level. A "burning pain" originating in the mucosal areas of the head and neck, progressing to the chest and abdomen, was noted at the 425 mg/m^2 level. Because of this observation, the infusion time was extended to 4 h. At the 795 mg/m^2, this toxicity precluded completion of the 4 h infusion. Pharmacokinetic evaluation disclosed blood levels of 0.74–2.31 μg/ml at the 425 mg/m^2 during the 0.5 h infusion. At the same dose for a 4 h infusion time, blood levels were 0.15–0.18 μg/ml. At 595 mg/m^2 administered as a 4 h infusion, blood levels increased to 0.33 $\pm$ 0.14 μg/ml. The drug was cleared rapidly from the blood compartment with a half-life of 2.5 min and a total body clearance of 11.5 l/min/m^2. No partial or complete response was observed. However, an advanced colon carcinoma patient experienced subjective pain relief with a decrease in carcinoembryonic antigen. The dose-limiting toxicity of caracemide using the 4 h infusion was an intolerable "burning pain" with a maximum tolerated dose of 795 mg/m^2. Further characterization of this dose-limiting toxicity is required prior to further clinical evaluation of caracemide.

Introduction

Caracemide (NSC 253272) is a novel antineoplastic agent synthesized by the Dow Chemical Company (Fig. 1). This drug is a nonspecific inhibitor of macromolecular synthesis with DNA synthesis being affected greater than RNA and protein synthesis [1]. Caracemide is an active inhibitor of ribonucleotide reductase as evidenced in the Novikoff tumor model [2]. These inhibitory effects are concentration-dependent with 70 percent of DNA synthesis inhibited by a drug concentration of 1 μM with a 4 h incubation. However, RNA and protein synthesis inhibition require a concentration of 50–100 μM. DNA strand breaks were observed only at high *in vivo* concentrations of 100 μM [1].

The antineoplastic activity of caracemide was observed in the P388 murine model and in mammary (MX-1) and colon (CX-1) human tumor xenographs implanted in the subrenal capsules of athymic mice. In the MX-1 mammary tumor, a single daily injection provided greater activity than an intermittent schedule. Caracemide was inactive against murine L1210 leukemia, B16 melanoma, Lewis lung carcinoma, CD8F1 mammary carcinoma and Colon 38 [1].

Preclinical toxicology studies in mice and dogs treated with single dose and daily $\times$ 5 schedule revealed acute central nervous system toxicities and possible cholinergic-mediated peripheral neurologic reactions. Seizures, tremors, ataxia, ptyalism and hypothermia were observed at doses greater than

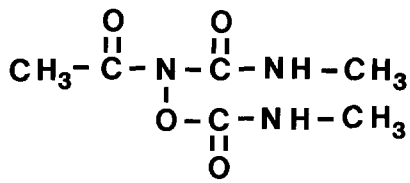

Fig. 1. Chemical structure of caracemide (NSC 253272; N-Acetyl-N-(methylcarbamoyloxy)-N'-methylurea).

the LD_{10}. At lower doses reversible gastrointestinal toxicities of anorexia, diarrhea and vomiting were noted. Myelosuppression was not consistently observed [1].

The present Phase I study was designed to determine the tolerance of patients with advanced carcinomas to escalating doses of caracemide administered as a short intravenous infusion repeated every 21 days. In this paper, we report the toxicities, pharmacokinetics, and antineoplastic activity of caracemide.

Materials and methods

Study design

The study was designed as a Phase I trial starting at a dose "N" and escalating it according to the modified Fibonacci method as outlined by Carter [3]. The Phase I study was initiated at a dose of 85 mg/m^2 (1/10 of murine LD_{10}) administered as a 15 min intravenous infusion every 21 days. The infusion time was increased from 15 min to 30 min, and finally to 4 h because of the observed toxicity described below. Planned escalation scheme was the following: 85, 170, 280, 425, 595, 795 and 1020 mg/m^2. Three patients were to be entered at each level and followed for 20 days prior to dose escalation. Patients completing two non-toxic courses at a given dose level with no evidence of tumor progression were escalated to the higher current dosing level in which 3 patients previously untreated with caracemide were entered.

Patient selection

After informed consent was obtained, patients with histologically-proven advanced carcinomas were entered on the study. All patients were required to have recovered from any acute toxicities of prior therapy. Adequate marrow reserves (white blood counts $> 4000/mm^3$ and platelets $> 100{,}000/mm^3$), and serum creatinine less than 2.0 mg/dl, total bilirubin less than 2.0 mg/dl were required for patient entry. A Karnofsky performance status of 50% or greater was prerequisite. Patients with a history of acute myocardial infarction, congestive heart failure, clinically significant arrhythmias or uncontrolled seizures were ineligible for this study.

Drug preparation and administration

Caracemide was supplied in 250 mg vials of lyophilized white powder with 0.1 M sodium phosphate buffer (Investigational Drug Branch, Division of Cancer Treatment, National Cancer Institute). Solution preparation was accomplished by reconstitution in 4.7 ml of sterile water for injection. The resultant solution contained 50 mg/ml of caracemide in a 0.1 M sodium phosphate buffer, pH 5. The drug was further diluted in 0.9% sodium chloride for injection at a final concentration of 2–5 mg/ml. Caracemide was administered immediately after preparation via a central venous line because of previously reported phlebitis [1], using a constant infusion pump (IVAC Corporation, San Diego, CA). In 0.9% sodium chloride the resultant caracemide solution was stable at room temperature during the 4 h infusion, exhibiting less than 5% decomposition [4, 5].

Treatment evaluation

Pretreatment included a complete history and physical examination, lesion measurements, complete blood counts, urinalysis, multichannel chemistry (serum creatinine, bilirubin, calcium, uric acid, SGOT, LDH and alkaline phosphatase), electrocardiogram and chest X-ray. These studies were re-

peated every 4 weeks or at the time of off-study. Physical examinations, complete blood counts, and multichannel chemistries were performed weekly.

Response criteria

Patients were evaluated for toxicity after receiving a dose of caracemide and manifesting toxic effects, or completing a course of therapy (a drug dose with subsequent follow-up of 20 days). Maximum tolerated dose was defined as that dose which produced predictable and reversible toxicity but did not incapacitate or interfere with the patient's well-being or general function. Complete response required disappearance of all evidence of disease. Patients designated as having a partial response had a 50% or greater decrease in the sum of the product of the two greatest dimensions of measurable lesions accompanied by the stabilization or improvement in performance status for a minimum follow-up period of 1 month. Decrease in tumor size less than 50% was defined as stable disease. Patients deemed as having progressive disease demonstrated increase in the size of measurable lesions or the appearance of new lesions or the appearance of new lesions [6].

Caracemide blood level measurement

Whole blood (0.5 ml), 2 drops of 1 N HCl and 5 ml of methylene chloride were added to a 10 ml glass centrifuge tube and capped. Since caracemide is unstable at physiological pH, this step was performed immediately upon collection. The tubes were shaken in a mechanical shaker for 10 min and the methylene chloride phase was transferred to a 1 ml conical tube. The organic phase was evaporated to dryness under a nitrogen stream at room temperature. The samples were than dissolved in 0.5 ml of the mobile phase and 5 μl of this solution were chromatographed. The high-performance liquid chromatography system consisted of a model M-45 pump, a model 710-B WISP automatic injector, and a model M-730 data module (Waters Assoc., Milford, MA). Separation was accomplished with a C_{18} Nova Pak column, 5 μ, 15 $\times$ 0.39 cm (Waters Assoc.). The mobile phase consisted of 20% methanol in 0.1 M potassium phosphate buffer, pH 4.5. A Kratos 773 variable wavelength UV detector (Anspec Co., Ann Arbor, MI) set at 210 nm was used for detection. The chromatographic system was operated at room temperature with a flow rate of 0.5 ml/min. The retention time of caracemide was approximately 4.5 min. A calibration curve was prepared in freshly withdrawn whole blood from each patient prior to drug administration. The standard curves were linear within the range studied (25 to 5000 ng/ml) and the limit of sensitivity was approximately 25 ng/ml. The drug recovery from blood was about 78%.

Pharmacokinetics

The pharmacokinetic parameters were determined employing standard formulae for the one-compartment model with intravenous infusion [7]. When the infusion time is equal to 7 half-lives, the plasma drug concentration is 99% of the steady-state (C_{ss}). Since the mean half-life was about 2.5 min, the 30 min intravenous infusion corresponds to approximately 12 half-lives, and the 4 hr infusion to 95 half-lives. Consequently, the equation for the steady-state $C_{ss} = k_o/VK$ was used, where k_o is the rate of infusion, V is the volume of distribution, and K is the first-order elimination rate constant. The total body clearance (Cl_T) was calculated as k_o/C_{ss}. The half-life was calculated by dividing 0.693 by K.

Results

Patient characteristics

Twenty patients were entered on the study. Patient characteristics are listed in Table 1. Table 2 provides information regarding patient entry at specific dose levels.

Table 1. Patients characteristics

Number of patients entered	20
Male/female	12/8
Median age	56
Previous therapy:	
Chemotherapy	90%
Radiation therapy	70%
Surgery	70%
Hormonal therapy	5%
Median performance status (Karnofsky)	60
Disease category:	
Bronchogenic carcinoma	5/20
Sarcoma	5/20
Colorectal	8/20
Breast	1/20
Malignant melanoma	1/20

Table 2. Patient accrual and infusion times

Dose level	85	170	425	595	795
Entered	4	1	8	5	2
Eligible	4	1	8	5	2
15 min infusion	4	1			
30 min infusion			3		
4 h infusion			5	5	5

Dose escalation

The dose escalation scheme was modified due to higher doses employed in concurrent Phase I studies; the dose was escalated directly from 170 to 425 mg/m^2. The original infusion time (15 min) was increased to 30 min and finally to 4 h because a "burning pain" toxicity (see below) was related to the rate of infusion.

Toxicities

No hematological toxicities were observed at any dose levels studied. Gastrointestinal toxicity, manifested as mild to moderate nausea and vomiting, was observed in 1 patient at the 85 mg/m^2 level, 4 patients at the 425 mg/m^2 level, and 1 patient at the 595 mg/m^2 level. The nausea and vomiting occurred during the infusion and lasted several hours post-infusion. Conventional anti-emetics (prochlorperazine, metoclopramide) controlled this toxicity.

At the 425 mg/m^2 level, one patient experienced an apparent allergic reaction during the first drug administration. Three hours into the infusion this patient developed generalized hives and audible wheezing which abated after corticosteroid administration and and discontinuation of caracemide.

Starting at the 425 mg/m^2 level, a "burning pain" toxicity was observed. This toxicity began within 15 min to 3 h of the infusion and was associated with excessive lacrimation and mild to moderate burning sensation. The pain frequently was associated with perioral mucosal burning which progressed to the neck, chest and abdomen. At 425 mg/m^2, this toxicity was observed in 4 of the 8 dose administrations. In the 5 patients entered at the 595 mg/m^2 dose level, the reproducibility and severity of this "burning pain" increased and occurred in all patients observed. Patients experiencing this pain would refuse subsequent doses of caracemide. At 795 mg/m^2, this toxicity was of sufficient severity to prevent completion of the infusion. Therefore, further patient entry was curtailed.

The above toxicity consistently abated after termination of the infusion within 10 to 30 minutes. Pretreatment with antihistamines, corticosteroids, and anticholinergics did not prevent this toxicity. No residual sequelae were observed. Cardiac, neurological, gastro-intestinal and pulmonary evaluations failed to disclose the etiology.

Pharmacokinetics

Representative caracemide blood concentration versus time profiles are shown in Fig. 2. For the 0.5 h infusion at the 425 mg/m^2 level, blood levels reached 0.74–2.31 μg/ml (Table 3). When the infusion time was increased to 4 h (425 mg/m^2) the steady-state levels decreased to 0.15–0.18 μg/ml. At the 595 mg/m^2 dose level (4 h infusion), caracemide blood levels reached 0.33 $\pm$ 0.14 μg/ml. The drug disappeared rapidly from the blood compart-

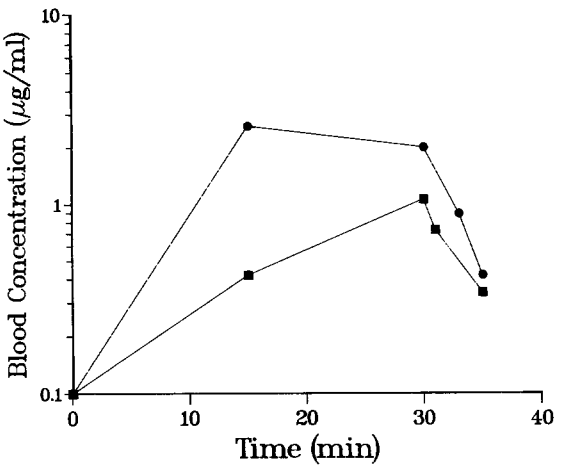

Fig. 2. Representative caracemide blood levels in two patients receiving the drug as a 0.5 hour intravenous infusion (425 mg/m²). Caracemide was assayed by HPLC as described in "Materials and methods".

Table 3. Caracemide pharmacokinetics[a]

Infusion duration (hr)	Dose (mg/m²)	Steady state (μg/ml)	Half-life (min)	V l/m²	Cl_T (l/min/m²)
0.5	425	2.31	2.1	18	6.1
0.5	425	0.74	3.5	68	19.1
4.0	425	0.18	2.9	42	9.8
4.0	425	0.15	1.6	28	12.0
4.0	595	0.59	2.4	14	4.2
4.0	595	0.28	2.3	30	8.8
4.0	595	0.12	2.5	73	20.2
Mean ± SE			2.5 ± 0.2	39 ± 9	11.5 ± 2.3

[a] Caracemide blood levels were determined by HPLC as described under Materials and methods.

ment with a mean half-life of 2.5 min and a total body clearance of 11.5 l/min/m². Caracemide appeared to be distributed in the extracellular fluid with a mean volume of distribution of 39 l/m².

Response

No partial or complete response was observed. However, an advanced colon carcinoma patient experienced subjective pain relief while on caracemide with a decrease in carcinoembryonic antigen from 1940 to 824 ng/ml. The patient had stable disease on bi-dimensional measurable disease parameters. This patient received 4 doses at the 425 mg/m² level and remained on study for 18 weeks.

Discussion

Caracemide, an inhibitor of macromolecular synthesis, was examined in a Phase I study using a short infusion repeated every 21 days. The original infusion time (15 min) was increased to 30 min and finally to 4 h because of a "burning pain" toxicity related to the rate of drug infusion.

The "burning pain" toxicity was characterized as a severe pain originating in the mucosal areas of the head and neck, progressing to the chest and abdomen, associated with excessive lacrimation. This pain began 15 min to 3 h into the infusion and abated within 10–30 min after termination of the infusion. The toxicity was encountered in patients entered at the 425 mg/m² and became progressively more severe at 595 and 795 mg/m². Patients experiencing this toxicity would refuse subsequent doses of caracemide. At the 795 mg/m² level, patients were unwilling to complete the 4 h infusion due to the intensity of the pain; therefore, further patient entry was curtailed. Attempts to elucidate this toxicity were unsuccessful, including neurological, cardiac and gastrointestinal evaluations. Empiric pretreatment with corticosteroids, anti-histamines, or anticholinergic agents failed to alter this toxicity.

A similar toxicity was reported using a daily infusion × 5 days repeated every 21 days [8, 9]. The syndrome, including perioral burning, flushing and chest discomfort, was dose-limiting at 650 mg/m². Central nervous system dysfunction was dose-limiting when the infusion time was lengthened to a continuous infusion × 5 days (800 mg/m² day). Patients experienced depression, lethargy, disorientation and confusion associated with EEG abnormalities [10]. The preclinical studies employing beagle dogs and mice were predictive of acute central nervous system toxicity and possible cholinergic-mediated neurological reactions [1, 10].

In addition to the parent drug toxicity, the acute

toxicities may be attributed to degradation products. Caracemide is unstable under physiological conditions of pH and temperature, generating many degradation products [4, 5, 12]. Investigators from MD Anderson have reported the detection of methylisocynate as a possible caracemide metabolite or degradation product in *in vitro* studies [13]. This toxicity and its rapid resolution at termination of infusion could temporally relate to either the parent compound or toxic degradation products and their rapid elimination. Caracemide is cleared with a short half-life of 2.5 min and a total body clearance of 11.5 l/min/m^2. Degradation products or metabolites also appear to be rapidly cleared from the circulation in dogs [12].

Additional toxicities encountered in this study were mild to moderate nausea and vomiting which occurred at all dose levels. This toxicity was controlled by conventional anti-emetics. An apparent allergic reaction was also noted which abated after corticosteroid administration and termination of the infusion. No evidence of toxicity to rapidly proliferating tissues (e.g. hematological toxicity, alopecia, mucositis) was observed.

Although no partial or complete response was noted, an advanced colon carcinoma patient experienced subjective relief of pain associated with a decrease in carcinoembryonic antigen. This patient remained on study for 18 weeks with satisfactory relief of his cancer pain.

Little information exists demonstrating biological or antiproliferative activity of caracemide in this and other Phase I studies. In preclinical studies, activity was noted in the P388 murine model which frequently predicts for hematological toxicity. However, hematological toxicity was not a reproducible finding in preclinical dog studies and was also not evidenced in this Phase I study. Although gastrointestinal toxicity appeared to be the major target organ of caracemide in dogs [1], we did not observe gastrointestinal toxicity to be dose-limiting. With our clinical schedule, the "burning pain" toxicity may preclude the administration of biologically active doses of the drug.

The intensity, frequency, and reproducibility of this "burning pain" was clearly related to higher doses. Because the severity of this toxicity prevented completion of the 4 h infusion, we report the maximum tolerated dose as 795 mg/m^2. Further characterization of the etiology of these symptoms is required before Phase II testing proceeds.

Acknowledgements

This study was supported in part by Phase I Contract N01-CM-27551 by the National Cancer Institute, Bethesda, Maryland, and by the Ben Kasle Trust for Cancer Research, Detroit, Michigan.

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