Phase I Clinical and Pharmacological Study of Merbarone


ABSTRACT

Merbarone, a nonnarcotic derivative of thiobarbituric acid, has demonstrated excellent activity against certain murine tumors, including L1210 and P388 leukemias, B16 melanoma, and M5076 sarcoma. Preclinical studies suggested that the antitumor effects of this drug were schedule dependent, since repeated dosing increased killing of tumor cells when compared to intermittent injections. We have completed a Phase I clinical and pharmacological study of merbarone in which the drug was administered both as a 2-h infusion and as a continuous i.v. infusion over 24 h. In view of the increased toxicity observed in animals following bolus injections and the possibility of schedule-dependent anticancer activity, a schedule of drug administration daily for 5 days was selected.

Fifty patients with advanced cancer were treated at dose levels that ranged from 100 to 1500 mg/m²/day. When the drug was administered by peripheral vein, phlebitis was observed at the infusion site at daily doses ≥150 mg/m². Therefore, all patients who received drug doses ≥200 mg/m² were treated by continuous i.v. infusion using central venous catheters. Renal insufficiency, initially observed at a dose of 1000 mg/m²/day, was the dose-limiting toxic reaction at 1500 mg/m²/day. Three of five patients treated at the highest dose level were unable to complete the infusion due to this effect. Marked hypouricemia was observed in all patients. Other toxic effects were mild and included nausea, fatigue, leukopenia, thrombocytopenia, and anorexia. Alopecia was noted in several patients who received doses ≥1000 mg/m²/day. No major antitumor effects were observed.

Dose-dependent, steady-state plasma concentrations of merbarone were reached within 24–48 h after beginning the continuous i.v. infusion. Elimination of drug from plasma followed a two-compartment model, with a t½ of 4.2 h and a t½ of 15.3 h. Renal excretion of merbarone and its major metabolites accounted for less than 30% of the administered dose.

We conclude that merbarone is relatively well tolerated with few constitutional symptoms. The current formulation of the drug causes phlebitis when administered by peripheral vein, and renal insufficiency is commonly observed at daily doses which exceed 1250 mg/m². The recommended dose for extended Phase II evaluation is 1000 mg/m²/day daily for 5 days administered by central venous catheter.

INTRODUCTION

Merbarone, 5-(N-phenylcarboxamido)-2-thiobarbituric acid, is a nonnarcotic derivative of thiobarbituric acid that has shown broad anticancer activity in experimental systems (1). Merbarone was active against P388 and L1210 leukemias, B16 melanoma, and the M5076 sarcoma (2, 3); however, no effect was observed against CD8F1 mammary tumor, colon 38, or human mammary xenograft MX-1 (1). The drug was also active when administered orally (1). The antitumor effect of merbarone appeared to be schedule dependent. In L1210 leukemia, the drug was most effective when administered daily for 5–9 days, whereas intermittent i.p. or single-dose i.v. schedules were inactive.

Although the mechanism of drug cytotoxicity is not firmly established, several effects have been observed in model systems. Cytopharmacologic analysis of drug-treated L1210 cells have revealed accumulation in late S-phase at a concentration of 5 μM (4). When L1210 cells were exposed to merbarone (10–50 μM) for 16–24 h, increased numbers of single-strand breaks in DNA were demonstrated (4). Such an effect would be consistent with activity as an inhibitor of topoisomerase II. This mechanism of action was also supported by the observations of Drake et al. (5), who found that merbarone inhibited topoisomerase II but had no effect on topoisomerase I. In these experiments, merbarone caused unknotted of P4 phase DNA and relaxation of supercoiled plasmid DNA (5). Merbarone was approximately as potent as etoposide in this system; however, these effects were not associated with DNA-protein cross-links. The drug did not bind to DNA, and substantial >50% inhibition of DNA, RNA, and protein synthesis was recorded only when concentrations >10⁻⁴ M were exceeded (3). Cooney et al. (6) demonstrated drug-associated free radical generation in liver microsomal preparations incubated with NADPH. In those experiments, merbarone exhibited oxidizing activity slightly greater in magnitude than doxorubicin (6).

Rats treated with single doses of merbarone developed drug-related toxicity to hematological, cardiovascular, renal, hepatic, nervous, and lymphatic systems. Dogs were particularly sensitive to central system effects—the apparent dose-limiting toxic reaction in that species. A substantially greater amount of drug was tolerated if the dose was delivered over a 5-day period. In this paper, we report our experience in a Phase I trial of merbarone administered daily for 5 days.

MATERIALS AND METHODS

Patient Eligibility. Patients were eligible for this study if they had histologically documented cancer and met the following criteria: age ≥ 18 years, performance status ≥ 50 (Karnofsky scale), no history of CNS disease, leukocyte count ≥ 3,500/mm³, platelet count ≥ 100,000/mm³, serum creatinine ≤ 1.5 mg/dl, and serum bilirubin ≤ 1.5 mg/dl.

Cytotoxic chemotherapy and radiation were proscribed both during the trial and within 4 weeks preceding entry. Written informed consent was required, and the study was approved in advance by this center's Institutional Review Board.

Clinical Study Design. Merbarone was supplied as a lyophilized powder containing N-methylglucamine by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. Two schedules were initially evaluated: a 2-h infusion administered daily for 5 days by peripheral vein, and a 24-h infusion administered daily for 5 days. The 24-h infusion schedule was abandoned after intolerable phlebitis was observed in the first group of patients (see below). For the 24-h infusion, daily drug doses were reconstituted with sterile water, diluted to a total volume of 1 liter of 5% dextrose solution, and infused over 24 h.

The trial was initiated at 100 mg/m²/day × 5, a level which corresponded to approximately one-third of the highest nontoxic dose in beagle dogs. At least three patients were treated at each dose level before patient accrual at higher levels was undertaken. Patients who were stable or who showed an antitumor response were eligible for advanced therapy.
retreatment at the same dose level, and treatment courses were repeated approximately every 3 weeks. Dose escalation within individual patients was not permitted.

Prior to the start of therapy, all patients underwent a physical examination, complete blood count, screening biochemical profile, serum creatinine, and creatinine clearance. A complete blood count, biochemical profile, and serum creatinine were obtained twice during the treatment period and approximately on a weekly basis thereafter.

Pharmacokinetic Samples. Plasma pharmacokinetic studies were performed on heparinized blood samples collected as follows: prior to therapy; at 1, 2, 3, 4, 5, and 6 h on Day 1; at approximately 8 a.m. and 4 p.m. on Days 2–5; at the end of the infusion; at 10, 20, and 30 min and 1, 2, 3, 4, 12, 24, 36, 48, and 72 h thereafter. Plasma was separated by centrifugation at 1500 x g for 10 min at room temperature and then stored at -70°C until assayed. Throughout the 5 days of treatment, the patient’s 24-h urine volume was collected, and aliquots were stored at -70°C.

Reagents. Merbarone, 4'-OH-merbarone, 2-oxo-2-desethylmerba
rone, and 3'-F-merbarone were supplied by the Drug Development Branch, National Cancer Institute, Bethesda, MD. 4'-OH-2-oxo-2-desethylmerbarone was prepared by reacting 4'-OH-merbarone (50 µM) with H2O2 (10 mM) for 1 h at room temperature in borate buffer (0.1 M, pH 8.5). All other chemicals were of analytical grade and obtained from Baker Chemical Co. (Phillipsburg, NJ).

Analytical Methods. Plasma levels of merbarone were analyzed by HPLC with UV detection using a method originally developed by Malspeis et al. (7–9) and modified by Stevens et al. (10). Briefly, plasma samples were spiked with sodium thiopental (0.46 µg) as an internal standard, and proteins were precipitated by the addition of methanol-dimethyl sulfoxide mixture (85:15 v/v). After centrifugation at 15,600 x g for 5 min, the supernatant was assayed for merbarone using a C18 Nova-Pak reverse-phase column (15 cm x 3.9 mm, 4-µm particle size; Waters Associates, Milford, MA). Separation of both merbarone and thiopental was achieved using a convex gradient consisting of 40% methanol (in 0.3 M ammonium acetate, 0.06 M MgCl2, and 7.2 mM triethylamine) to 80% methanol (in 0.1 M ammonium acetate, 0.06 M MgCl2, and 7.2 mM triethylamine) over 15 min at a flow rate of 1 ml/min. Merbarone and the internal standard were monitored by UV at 300 nm.

Urinary metabolites of merbarone were assayed using a precolumn derivatization and subsequent chromatographic technique developed by Malspeis et al. (7–9). Briefly, urinary samples were spiked with 3'-F-merbarone (15 µg) as an internal standard, alkalized with potassium carbonate (final concentration, 0.1 M), and derivatized with 8% (v/v) acetic anhydride for 10 min at room temperature. The derivatized samples were then diluted with the HPLC mobile phase and centrifuged at 15,600 x g for 5 min, and the supernatant was assayed by HPLC. Merbarone and its derivatized metabolites were separated by HPLC using an isocratic elution with 30% methanol (in 60 mM ammonium acetate, 32 mM acetic acid, 42 mM magnesium sulfate, 1 mM sodium dodecyl sulfate, and triethylamine, pH 7.1), and a flow rate of 1 ml/min was used. The eluate was monitored by UV at 293 nm.

Pharmacokinetic Calculations. Merbarone and its metabolites were quantitated by either area or peak-height ratio of the internal standard versus compound. Standard curves for parent drug and each metabolite were constructed for each patient using pretreatment urine samples. Plasma concentration versus time was fitted to the equation

\[ C_t = \frac{\sum_{i=1}^{N} \theta_i e^{-\lambda_i t}}{\sum_{i=1}^{N} \theta_i} \]

using NONLIN84 (Statistical Consultants, Inc., Lexington, KY), and pharmacokinetic parameters were calculated by standard methods (11).

RESULTS

Fifty patients were treated with 62 infusions of merbarone. The clinical characteristics of the patients are shown in Table 1. Nine patients at the 100–200 mg/m² levels received infusions via peripheral veins for 5 days. Three patients at the 150-mg/
Table 2  Hematological and renal effects of merbarone

<table>
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<th>Dose (mg/m²/day)</th>
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<th>No. of courses</th>
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</table>

* Patients treated by 2-h infusion.
* Only two of five patients completed the 5-day course by continuous infusion at this level due to renal toxicity.

Fig. 1. Relation of changes in serum creatinine concentration to administered daily dose of merbarone.

Fig. 2. Plasma drug concentration versus time in a patient who received merbarone at a dose of 1250 mg/m²/day. A, steady-state plasma concentrations; B, decay phase following termination of the infusion.

resulted in formation of a hardened “cord” along the peripheral vein, which persisted in some patients for several months after discontinuing drug therapy. Mild to moderate nausea occurred in a 15 of the 50 patients in this study, and in 11 of 21 individuals who received doses ≥1000 mg/m². Partial alopecia was observed in several patients who received doses ≥1000 mg/m²/day but was not seen at lower doses. Mild lethargy (doubtfully drug related) was noted in three patients. This effect resolved in each case within 48 h after completion of treatment.

Pharmacokinetics. Detailed pharmacokinetic studies were performed on 14 of the 50 patients. The plasma concentration × time profile for a patient receiving 1250 mg/m²/day is shown in Fig. 2. A and B. Steady-state plasma concentrations were achieved within 24–48 h after starting the infusion and were maintained throughout the 5-day period with no evidence of accumulation. At the end of the infusion, merbarone was eliminated from plasma in a bi-exponential fashion with median t½ and t½ of 4.2 ± 3.4 and 15.3 ± 6.9 h, respectively. No metabolites were detected in plasma.

Derived pharmacokinetic parameters at low-dose (100–300 mg/m²/day) and at high-dose (1000–1500 mg/m²/day) levels are summarized in Table 4. Merbarone plasma steady-state levels and area under the curve correlated well with the dose of drug administered (r = 0.97 for each). The mean residence time of 12.1 ± 4.3 h was consistent with the relatively long t½. The mean total-body clearance was 12.9 ± 4.1 ml/min/m² and was not dose dependent over the dose range studied. The mean central volume of distribution of merbarone was 5.6 ± 3.2 liters/m², while the mean steady-state volume of distribution was 8.9 ± 2.8 liters/m², suggesting that merbarone was principally distributed in the intravascular fluid compartment.

Less than 1% of the parent drug was excreted unchanged in urine (Table 5); however, consistent with other reports (7–9), three metabolites of the drug were observed in urine, namely...
effect indicates that the minimal effective dose was not identified; however, this dose would be lower than the starting dose evaluated in this Phase I study (100 mg/m²/day). Although merbarone inhibits xanthine oxidase in vitro (19, 20), we previously showed that administration of merbarone causes only a minor increase in urinary oxypurine excretion and that the magnitude of this effect is substantially less than that observed with allopurinol (12). Hypouricemia was primarily related to a drug-induced increase in uric acid excretion (12, 21, 22).

Our observations regarding the pattern and rate of drug disappearance from plasma and the urinary excretion of parent drug and metabolites are in accord with results reported by others (16). The observations of a correlation between the t½ and total plasma protein (r = 0.72), combined with the relatively small total-body clearance and volume of distribution, suggest that merbarone is extensively bound to plasma protein in vivo. Merbarone binds to plasma proteins in vitro; however, the extent of binding has been difficult to quantify since the drug also binds to partition membranes.⁴

Renal excretion accounts for approximately 25% of administered drug. These results are consistent with murine studies using [¹⁴C]merbarone which demonstrated prolonged retention of radioactive material (drug and/or metabolites) in the liver and gastrointestinal tract as well as kidney (15). However, the median steady-state plasma concentration (80 µg/ml) reported to be lethal in mice and dogs. Pronounced species differences in metabolism may in part account for the substantial species differences in toxic reactions. In dogs, glucuronide conjugation of merbarone is observed (14). Our clinical data indicate that glucuronidation is not a major metabolic pathway in humans. Myelosuppression was observed only sporadically in this study with only a possible indication of a relationship to total dose (Table 1). Conceivably, if some means were available to ameliorate the nephrotoxicity, myelosuppres-

⁴ L. Malspeis, personal communication.
sion would become more evident (as has been observed in other species).

The pronounced increase in urinary excretion of β2-microglobulin indicates that high doses of merbarone can cause renal tubular damage (22). The etiology of the renal toxicity is unclear, but it did not correlate with uricosuria. Merbarone is known to be metabolized to oxidized radicals, and incubation of merbarone with hepatic microsomes plus NADPH can generate oxidized radicals (4, 6). Moreover, reaction of merbarone with hydrogen peroxide generates at least one of the metabolites observed in vivo, 2-desthio-2-oxomerbarone.5 Thus, renal metabolism of merbarone may also be associated with generation of reactive oxygen species which could be nephrotoxic.

Merbarone has shown substantial anticancer activity in preclinical model systems. Although no major antitumor effects were noted in this trial, the drug was generally well tolerated. Disease-oriented studies at doses of 1000 mg/m2/day for 5 consecutive days by continuous i.v. infusion using central venous catheters should be considered. In view of the potent hypouricemic effects of merbarone, further study of merbarone as a treatment for hyperuricemic disorders is also indicated.

REFERENCES

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