Phase I and Pharmacological Studies of Pentamethylmelamine Administered by 24-Hour Intravenous Infusion


ABSTRACT

A Phase I study of pentamethylmelamine (PMM) was conducted, administering the drug as a 24-hr i.v. infusion once weekly for 3 weeks. Doses ranged from 80 to 3000 mg/sq m/week. Twenty-six evaluable patients received a total of 30 courses of PMM. The median performance status of the patients was 60% (range, 40 to 90%), and the median age was 58 years (range, 43 to 72 years). The highest tolerated dose was 2000 mg/sq m/week. Nausea and vomiting were the dose-limiting toxicities; myelosuppression was neither consistent nor severe.

One objective response lasting 10 months was noted in a patient with renal cancer. Pharmacokinetic studies using [ring-14C]PMM demonstrated a postinfusion half-life of 14C of approximately 12 hr, with the majority of the radiolabel excreted in the urine. PMM was introduced as a parenteral form of hexamethylmelamine. The present schedule does not permit administration of PMM in a dose greater than the tolerated dose of hexamethylmelamine and does not appear to offer an advantage over the p.o. use of the parent compound.

INTRODUCTION

PMM, NCS 118742, is the monodemethylated derivative of HMM, NSC 13875 (Chart 1), an s-triazene compound with antineoplastic properties. HMM has been a subject of clinical investigation for over 15 years. During that time, it has demonstrated clinically useful antitumor activity against a variety of human cancers, including carcinomas of the ovary, breast, and uterine cervix; small-cell carcinoma of the lung; and malignant lymphomas (3, 13). Since HMM is minimally soluble in physiologically compatible fluids, it is given p.o. but does produce dose-limiting nausea and vomiting (22, 23); it would be desirable, therefore, to have a form of this drug suitable for parenteral administration.

HMM is metabolized by sequential N-demethylation, and PMM is the first intermediate in that process (24). Both compounds demonstrate only marginal activity against most of the rodent tumors usually used by the National Cancer Institute in the screening of antitumor compounds (18). However, HMM and PMM possess similar antitumor activity against Sarcoma 180 and Lewis lung carcinoma in C57BL X DBA/2 F, mice (12) and against the P-246 human lung xenograft in immunodeprived mice (7). In the subrenal capsule assay, HMM but not PMM is active against the CX-1 human colon tumor xenograft, but both agents are active against the MX-1 and MX-2 mammary tumor xenografts (6). PMM is chemically stable and is 24 times more soluble than is HMM (7). It was therefore selected for study as an i.v. form of HMM.

In sensitive murine tumor systems, PMM is active whether given on a daily or an intermittent schedule, but with either schedule the therapeutic index is narrow (18). In large animal toxicology studies, acute toxicity was prominent with vomiting, diarrhea, anorexia, and neurological dysfunction consisting of seizures, hyperexcitability, disorientation, and lethargy. The acute neurotoxicity was found to be related to the rate of drug administration. A dose which was lethal when administered as a rapid injection could be given safely when divided into 2 to 4 equal aliquots given at 1-hr intervals. Acute neurological toxicity was not associated with histological changes in the central nervous system. Other toxicities included mild to moderate myelosuppression, increased serum levels of hepatic enzymes, elevations of the blood urea nitrogen and creatinine, hypoglycemia, and local inflammation at the site of injection (18).

The mechanism of action of HMM and PMM is not clearly understood. Unlike other triazene compounds (2), HMM does not inhibit leukemia P-388 dihydrofolate reductase in vitro (18). Structurally, HMM resembles triethylenemelamine, a known alkylating agent (20), but neither HMM nor its metabolites exhibit alkylating activity in the 4-( p-nitrobenzyl)pyridine test (24). This in vitro assay does not, however, exclude alkylating activity as a mechanism of action, in that microsomal enzymes are required for the formation of formaldehyde, a weak alkylator, from the methylmelamines (12). HMM and PMM are capable of producing prolonged inhibition of DNA synthesis in i.p. implanted Lieberman plasmacytoma cells (17).

In the present study, we investigated the biological effects of 24-hr i.v. infusions of PMM given once weekly for 3 weeks. This schedule was chosen in an attempt to minimize acute side effects while administering as much of the drug as possible over the period of treatment. Pharmacokinetic studies were conducted using [ring-14C]PMM.

MATERIALS AND METHODS

Patient Selection and Evaluation. All patients treated had a histologically or cytologically confirmed diagnosis of cancer refractory to conventional modalities of treatment or for which no effective therapy is known. Patients had not received radiotherapy or chemotherapy during the 4 weeks prior to starting treatment with PMM. A performance status of 40% (Karnofsky Received October 3, 1980; accepted January 9, 1981.

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2 Recipient of American Cancer Society Junior Faculty Fellowship.
3 To whom requests for reprints should be addressed, at Memorial SloanKettering Cancer Center, 1275 York Avenue, New York, N. Y. 10021.
4 Landrum/Karnofsky Fellow.
5 The abbreviations used are: PMM, pentamethylmelamine; HMM, hexamethylmelamine; CBC, complete blood count.

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Phase I Study of PMM

As a base line, the following laboratory tests were obtained for each patient prior to initiation of treatment.

1. CBC with differential, platelet count, reticulocyte count, electrolytes, creatinine clearance, and prothrombin time; and a biochemical profile which included serum glucose, urea nitrogen, calcium, phosphorus, total protein, albumin, total bilirubin, alkaline phosphatase, lactic dehydrogenase, and serum aspartate aminotransferase. Radionuclide scans of liver and bone were performed only when clinically indicated. Although this was a Phase I study, an attempt was made to identify evaluable parameters of disease in all patients; the absence of measurable lesions, however, did not preclude entry into this study.

All patients were hospitalized at the time of drug administration, although frequently patients were discharged between individual doses of PMM. Initially, patients were followed with daily CBC and platelet counts; a biochemical profile and a prothrombin time were obtained 3 times weekly. After the 3-week course of PMM at a low dose level could be treated at a higher dosage after a 2-week rest period.

A course of treatment was interrupted if the WBC fell below 3500/cu mm or if the platelet count fell below 100,000/cu mm. The next dose of PMM was withheld until hematological recovery occurred. A minimum of 3 weekly doses was required for a patient to be considered as having received an adequate trial of PMM.

Pharmacological Studies. PMM labeled with 14C in the amine ring was supplied by Dr. Robert Engle, Clinical Resources Section, Pharmaceutical Resources Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md. The compound had a specific activity of 54 μCi/mg, and chemical purity was >98% by autoradiography.

For the radiochemical studies, approximately 4 mg of 14C-labeled drug were weighed out under laboratory-clean conditions from its original container into a sterile vial. This was combined with 110% of the calculated dose of unlabeled PMM for the first patients studied and then filtered through a sterile 0.22-μm filter into a sterile vial. The solution was tested for sterility by the Microbiology Laboratory of Memorial Hospital; pyrogenicity testing was conducted by Leberco Laboratories, Roselle Park, N. J., by USP and NF standards. After the initial specimen was shown to be sterile and nonpyrogenic, the same technique was used to prepare the dose of [14C]PMM for the second patient in whom PMM kinetics was evaluated. Ten percent of that dose was stored frozen in the laboratory for sterility and pyrogenicity testing in the event of a febrile reaction.

For each patient studied, plasma, urine, and respiratory gases were evaluated for 14C content. Samples of venous blood were drawn into 10-ml heparinized tubes (Becton-Dickinson Vacutainer 3200 A; Becton-Dickinson and Co., Rutherford, N. J.) prior to initiation of the infusion at 30 min and at 1, 2, 4, 6, and 24 hr into the infusion and at 30 min and 2, 4, 8, 24, 48, and 72 hr after completion of the infusion. Urine specimens were obtained prior to treatment and in 6-hr collections for 5 days from the time the infusion was started. Respiratory gases were collected into a 100-liter gas bag via a nonrebreathing valve mouthpiece for 5 min at 1, 4, and 24 hr after initiation of the infusion.

Plasma was prepared by centrifugation of whole blood at 2000 rpm for 10 min in Model PR-2 centrifuge (International Equipment Co., Boston, Mass.). Aliquots of plasma were combusted in a Model 306 sample oxidizer (Packard Instruments Co., Downers Grove, Ill.), and 14CO2 was collected in a combination of Carbo-sorb and Permafluor V (Packard Instruments Co.). Respiratory 14CO2 was recovered by bubbling gas from the bag through 2 gas-washing bottles in series, each containing 75 ml of anhydrous ethanolamine; the bag was evacuated over the course of 1 hr using low suction. Contents of the 2 gas-washing bottles were combined for analysis. Aliquots of urine and of the ethanolamine were added to Hydrofluor (National Diagnostics, Somerville, N. J.). All specimens were counted in a Packard Tri-Carb Model 3380 liquid scintillation counter (Packard Instruments Co.).

RESULTS

Twenty-seven patients were entered into this study over 7 months. One patient was considered unevaluable after receiving only one dose of PMM; this patient’s treatment was discon-
continued because of progressive neurological deterioration related to cerebral metastases. Twenty-six patients received a total of 30 evaluable courses of PMM. The characteristics of these patients are indicated in Table 1.

Toxicity. As shown in Table 2, occasional myelosuppression was seen, but it was neither clearly dose dependent nor consistent. Nausea and vomiting were the dose-limiting toxicities. At doses <900 mg/sq m, nausea and vomiting occurred rarely. However, as the dose was escalated to 900 mg/sq m and greater, nausea and vomiting became more frequent and severe. At 1350 mg/sq m, it was universal but tolerable. At 2000 mg/sq m, it was severe, and at 3000 mg/sq m, it was clearly dose limiting. One-half of the patients at the highest level refused to finish the course of 3 doses. Vomiting generally began 3 to 6 hr after initiation of the PMM infusion and lasted for 12 to 48 hr. Four patients who had previously been treated with cis-platinum-diamminedichloride (cisplatin), 60 to 120 mg/sq m, felt that the nausea and vomiting with PMM, 3000 mg/sq m, was at least as severe as that which they had experienced with cisplatin.

One patient treated with 160 mg/sq m and another patient treated at 2000 mg/sq m developed mild maculopapular cutaneous eruptions between treatments with PMM. A skin biopsy from the first patient showed a nonspecific reaction compatible with a drug-induced eruption. Both patients, however, were also receiving antibiotics p.o. at the time that their rashes appeared. The relationship of the eruptions to PMM, therefore, is not clear.

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<th>PMM treatment: characteristics of 26 patients</th>
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<td><strong>Age</strong></td>
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<td><strong>Performance status</strong></td>
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During an infusion at 3000 mg/sq m, one patient experienced unusual agitation which was resolved shortly after the infusion was completed. Another patient at this dose level experienced progressive slowing of mentation without focal neurological signs following the second of 2 doses of PMM. This patient had a 3-year history of metastatic adenocarcinoma of the lung and had been treated for the previous 18 months with a combination of cyclophosphamide, Adriamycin, and cisplatin. The patient died 6 weeks after this last dose of PMM. At autopsy, there was a marked diffusion leukoencephalopathy with gliosis and spongiosis of the central white matter of the brain. The association tracts, paraventricular white matter, and spinal cord were spared, and there was no evidence of metastatic tumor involving the central nervous system. The findings resembled those seen in patients treated with intrathecal methotrexate, but the leukoencephalopathy in our patient was more extensive. Peripheral neuropathy was not observed in any patient.

No patient experienced hepatic, renal, cardiopulmonary, or endocrine dysfunction related to PMM administration. Many patients treated had preexisting alopecia, but no new alopecia occurred in patients treated in this study. Five patients received courses at 2 dose levels; cumulative toxicity was not observed. With the severe and prolonged nausea and vomiting seen with the 24-hr infusion of 2000 to 3000 mg/sq m, it became obvious that such a schedule was impractical. Since the acute gastrointestinal toxicity observed at 900 to 1350 mg/sq m given by a 24-hr infusion was acceptable, we decided to investigate a schedule of 2-hr infusions of 1000 to 1500 mg/sq m given twice weekly. All 3 patients treated in this manner experienced unacceptable nausea. Two of these patients received 24-hr infusions at the same dose as their 2-hr infusions; these patients reported that the longer infusion produced less nausea and vomiting.

**Pharmacological Studies.** The kinetics of 14C in the plasma of 2 patients who received PMM, 3000 mg/sq m, labeled with [14C]PMM is shown in Chart 2. Postinfusion plasma half-lives of total radioactivity in these 2 patients were 11.9 and 12.4 hr. Chart 3 shows the cumulative urinary recovery of 14C as a fraction of the administered 14C dose. In these 2 patients, 67.4 and 69.2% of the total radioactivity was recovered in the urine by 24 hr postinfusion. Less than 1% of the administered 14C could be detected in respiratory gases.

**Table 2**

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<th>Hematological toxicity of PMM</th>
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<td><strong>Dose (mg/sq m)</strong></td>
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**Chart 2.** Plasma kinetics of 14C expressed as µg equivalents of PMM during and following 24-hr infusions of [ring-14C]PMM, 3000 mg/sq m.

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of PMM, 3000 mg/sq m. It has been anticipated that, by use of a parenteral dosage form, the amount of drug that could be administered would not be limited by the severe gastrointestinal toxicity seen with HMM. In addition, after p.o. administration the absorption of HMM varies widely among patients (8), and the drug is exposed to degradative enzymes in the liver during its first pass through the circulation. The use of a parenteral form provides the potential advantage of achieving adequate, consistent levels of active drug in all patients.

Lake et al. (12) demonstrated that, as HMM is sequentially demethylated, there is a progressive reduction in the antitumor potency of the methylmelamine metabolites. PMM, N2,N2,N4,N4-tetramethylmelamine, N2,N4,N4-trimethylmelamine, and N2,N2,N4-trimethylmelamine all possess antitumor activity and account for the majority of circulating metabolites during infusions of PMM (11). Thus, during the i.v. infusion of [ring-14C]PMM, most of the 14C is associated with active methylated melamines than does p.o. HMM (1, 8), the evaluation of continuous infusions of lower doses of PMM over many days may be worthwhile. A dose of 300 to 500 mg/sq m/day is a reasonable starting dose. Although prolonged i.v. administration of any drug is associated with logistic problems, the approach is particularly well suited to certain situations. Persistent nausea and intermittent bowel obstruction are frequent complications of advanced carcinomas of the ovary and cervix, 2 tumors that are sensitive to HMM. Patients with these tumors are frequently dependent on i.v. fluids and require hospitalization. Study of continuous i.v. infusions of PMM may be warranted in this group of patients.

ACKNOWLEDGMENTS

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REFERENCES

4. Bonomi, P. D., Mladineo, J., Morrin, B., Wilbanks, G., Jr., and Staley, R. E. Phase II trial of hexamethylenimine in ovarian carcinoma resistant to activity against human tumors; both are active on a daily schedule of administration.

The dose of HMM used in early clinical studies was 12 mg/kg daily for 21 days (23). This is approximately equivalent to 450 mg/sq m daily or 3000 mg/sq m weekly. In more recent trials, HMM has been given at doses of 8 mg/kg or 250 mg/sq m daily indefinitely as tolerated (4, 21); this is roughly equivalent to a weekly dose of 2000 mg/sq m. Both gastrointestinal and hematological toxicities were seen in these trials.

The present study demonstrates that, when PMM is given as a 24-hr i.v. infusion, the dose-limiting toxicity is severe nausea and vomiting; myelosuppression is neither dose dependent nor consistent. A weekly dose of 3000 mg/sq m was found to be intolerable on this schedule, and severe gastrointestinal toxicity was frequently seen at 2000 mg/sq m/week. Administration of the drug over 2 hr did not shorten the duration or severity of nausea. Indeed, as had been anticipated from preclinical studies, the drug was better tolerated dose for dose when given over 24 hr than when given over 2 hr. Other investigators have recently reported preliminary results of Phase I trials of PMM given in a variety of doses and schedules (9, 10, 14, 19). Nausea and vomiting were found to be the most prominent toxicities, although, at doses of 750 mg/sq m daily for 10 days, central nervous system toxicity was severe (9). A schedule which permits the administration of PMM in a dose greater than the tolerated dose of HMM has not been identified in this or other studies. Because it is unlikely that PMM has broader or more potent therapeutic activity than does HMM and since high doses of the drug are poorly tolerated by patients, further testing of intermittent high-dose, 24-hr infusions of PMM cannot be recommended.

Nonetheless, there may still be a place for PMM in clinical practice. Since daily administration of HMM is the schedule which has been shown to be active against human tumors and since i.v. PMM produces more consistent plasma levels of active methylated melamines than does p.o. HMM (1, 8), the evaluation of continuous infusions of lower doses of PMM over many days may be worthwhile. A dose of 300 to 500 mg/sq m/day is a reasonable starting dose. Although prolonged i.v. administration of any drug is associated with logistic problems, the approach is particularly well suited to certain situations. Persistent nausea and intermittent bowel obstruction are frequent complications of advanced carcinomas of the ovary and cervix, 2 tumors that are sensitive to HMM. Patients with these tumors are frequently dependent on i.v. fluids and require hospitalization. Study of continuous i.v. infusions of PMM may be warranted in this group of patients.

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