Prolonged Infusion of Hexamethylene Bisacetamide: A Phase I and Pharmacological Study

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ABSTRACT

Hexamethylene bisacetamide (HMBA, NSC 95580), a potent polar-planar differentiating agent in vitro, was studied in a phase I trial as a 10-day continuous infusion administered every 4 weeks. Since preclinical evidence had demonstrated that the duration of HMBA exposure was an important variable in the induction of differentiation, and HMBA steady-state concentrations (Css) achieved during 5-day infusions were minimally effective at inducing in vitro differentiation, this study was conducted to evaluate the feasibility of maintaining HMBA Css for 10 days similar to levels achieved and maintained for 5 days. Twelve patients received 17 evaluable courses at three dose levels, 12.15.8, and 20 g/m²/day. Platelet toxicity limited further dose escalation. Mean nadir and percentage of decrement in platelet counts were 175,000/µl and 66%, and 43,500/µl and 83%, at 15.8 and 20 g/m²/day, respectively. In this 10-day study, the percentage of decrement of platelet counts was linearly related to mean HMBA Css and to the area under the plasma concentration versus time curve (AUC). However, when combined platelet and pharmacological data from both 5- and 10-day studies were analyzed, it was apparent that the duration of HMBA exposure was an additional significant variable in predicting the magnitude of thrombocytopenia.

Renal and metabolic toxicities that precluded dose escalation in previous 5-day trials of HMBA were mild and insignificant in this study. Mean HMBA Css were 0.65 ± 0.09 mmol/liter at 15.8 g/m²/day, and 0.99 ± 0.22 mmol/liter at 20 g/m²/day. Depletion of intracellular polyamines in peripheral blood mononuclear leukocytes was noted during several courses that were associated with profound myelosuppression, but no clear relationships were apparent between the magnitude of polyamine changes and toxicity or between fluctuations in polyamines and the magnitude of mean HMBA Css values.

Based on this study, the maximum tolerated and recommended phase II doses for HMBA administered on this schedule were 20 and 15.8 g/m²/day, respectively. The administration of HMBA by a ten-day infusion at the maximum tolerated dose resulted in the delivery of lower daily doses and lower HMBA Css than on the 5-day schedule. The major toxicities differed on these schedules with thrombocytopenia being most prominent on the 10-day schedule and metabolic and CNS toxicities on the 5-day schedule. The administration of HMBA for prolonged periods may be disadvantageous in the treatment of myelodysplastic syndromes since thrombocytopenia and marrow failure are common at presentation; however, the efficacy of the two schedules cannot be assessed from these phase I studies.

INTRODUCTION

The limitations of conventional cytotoxic chemotherapy and drug screening programs in selecting antineoplastic that possess novel mechanisms of action have provided the impetus to formulate new drug screening procedures and to develop agents that may modulate the basic cellular mechanisms responsible for the emergence and persistence of neoplasia (1-5). One such mechanism is based on the presumption that cancer is a disease of faulty maturation, which prevents the normal differentiation of an expansive stem cell compartment (5-9). The ability of pharmacological agents to overcome these maturational defects and to induce both morphological and functional differentiation has already been demonstrated in a variety of tumor systems in vitro (10-12).

Low molecular weight polar-planar compounds such as DMSO3 (NSC 763), NMF (NSC 3051), and HMBA (NSC95580) constitute a major class of agents that induce the morphological and functional differentiation of malignant cells in vitro (10). Once differentiated, malignant cells lose their capacity to proliferate efficiently; they tend to lose their clonogenicity in vitro as well as their ability to be transplanted into animals (9-13). However, to date, the biological activity of these agents has been demonstrated only in cell culture and attempts to demonstrate any clinical utility have been disappointing (14-20). For NMF, this may reflect the inability to reach and sustain concentrations that are effective at inducing terminal differentiation in model cell culture systems (20, 21).

Hexamethylene bisacetamide (Fig. 1), the prototype of the polyethylene bisacetamides with six methylene linking its functional acetamide groups, shares structural and physicochemical properties with other polar-planar agents. However, HMBA is a more potent inducer of differentiation in cultured murine and human leukemic cell lines (22-28). For example, at a concentration of 5 mmol/liter for 5 days, HMBA induces more than 99% of the Friend murine erythroleukemic cell line to differentiate, manifested by the synthesis of hemoglobin, whereas concentrations of DMSO of 280 mmol/liter or NMF of 250 mmol/liter result in the differentiation of 70 and 28% of Friend murine erythroleukemic cells, respectively (22-26). At a concentration of 2 mmol/liter for 6 days, HMBA induces 95% of human HL-60 promyelocytic cells to form mature myeloid cells in vitro as compared to 75% induced by 180 mmol/liter of DMSO (25-27). In addition, HMBA is capable of inducing changes indicative of a more differentiated phenotype in a variety of solid tumor cell lines (29-32).

In contrast to other putative differentiating agents such as low-dose cytosine arabinoside, the differentiating activity of HMBA occurs at concentrations (1-5 mmol/liter) that have not been demonstrated to be cytotoxic in tissue culture. An evaluation in the National Cancer Institute Division of Cancer Treatment tumor screen demonstrated that HMBA offered no therapeutic benefit to animals bearing several murine leukemic and solid tumors and possessed no cytotoxic activity against human mammary, colon, and lung xenografts in nude mice (32). However, drug administration in these in vivo systems did not closely duplicate the continuous exposure used in vitro. Therefore, although HMBA did not demonstrate any conven...
PHASE I TRIAL OF HMBA

![Structure of HMBA](image)

**MATERIALS AND METHODS**

Patients with histologically documented solid tumors refractory to conventional therapy or for which no effective therapy was known were candidates for entry onto the study. Eligibility criteria included: (a) age between 20 and 75 years; (b) ECOG performance status of 3 or better; (c) life expectancy of at least 4 months; (d) to seek preliminary evidence of therapeutic activity in patients with advanced solid tumors; and (e) to investigate potential drug-induced changes in intracellular polyanine concentrations that could aid in understanding mechanisms responsible for HMBA's toxicity and differentiating activity.

**Dosage and Formulation.** The starting dose was 15.8 g/m²/day administered as a continuous 10-day infusion every 28 days. This dose was selected because it was associated with minimal toxicities when HMBA was administered continuously for 5 consecutive days in previous phase I studies (33, 34). Escalations to 20 g/m²/day, and then to 24 g/m²/day, the recommended phase II dose for HMBA administered as a continuous 5-day infusion, were planned.

HMBA was supplied by the Division of Cancer Treatment, National Cancer Institute (Bethesda, MD), as 50 mg HMBA/ml (5%) in 0.9% sodium chloride. The volume of fluid administered ranged from 316 ml/m²/day in patients treated at 15.8 g/m²/day to 400 ml/m²/day in patients receiving 20 g/m²/day of HMBA. The drug was provided in glass infusion bottles and administered undiluted with a Flo-Guard 8000 infusion pump (Travenol Laboratories, Deerfield, IL).

Follow-up. Patients were seen weekly while on study, with history and physical examinations performed along with the recording of weight and performance status. Blood and urine studies noted previously were also obtained on each visit. Tumor measurements were performed every 28 days. Toxicity was graded weekly according to ECOG criteria (36).

Minimal status exams were administered to all patients before each course of drug, daily during the HMBA infusion, and following drug administration. This exam, as described by Folstein et al., consists of 11 questions answered over 5 to 10 min and scored from 0 to 30, and has been validated as a screen for dementia and delirium in hospitalized patients (37). An EEG was repeated on the tenth day of the infusion.

**Pharmacological Studies.** Blood samples were collected before each course and twice daily during infusions to assess steady-state concentrations of HMBA (Cₚ) and its major metabolite, 6-acetoamidohexanoic acid. Urine was collected continuously throughout the infusion. HMBA concentrations in plasma and urine were measured by gas chromatography using octamethylene bisacetamide (OMBA) as an internal standard.

**Pharmacodynamic Relationships.** Mean HMBA Cₚ was calculated as the sum of the HMBA concentrations obtained during steady-state divided by the number of samples assayed. Since HMBA was administered by a continuous infusion over a period of time that was much greater than its half-life, AUC was estimated as the mean HMBA Cₚ multiplied by the duration of the infusion.

Three models were used to describe the relationships between changes in platelets and parameters that reflect HMBA exposure, AUC and infusion duration. The first model was a modification of the Hill equation, commonly used to describe relationships in biological systems (38):

\[
\% \text{ Change in platelets} = \frac{(100\%)(\text{AUC})}{(\text{AUC}₀) + (\text{AUC})}
\]

This equation was fit to our data using nonlinear regression analysis based upon the simplex algorithm of Nelder and Mead (PCNONLIN, Statistical Consultants, Lexington, KY). The data were also modeled using a linear model:

\[
\% \text{ Change in platelets} = a₀ + a₁(\text{AUC}) + a₂(\text{duration of infusion})
\]

and a proportional model:

\[
\% \text{ Change in platelets} = b₀(\text{AUC})^{\text{(duration of infusion)}}
\]

These models were fit using stepwise multiple linear regression analysis (Statpak, Northwest Analytical, Portland, OR).
PHASE I TRIAL OF HMBA

Lymphocyte Polyamines. Blood samples were collected before the infusion, then on weekday mornings during the early (days 2–3), middle (days 4–6), and late (days 9–10) phases of the infusion. Lymphocytes were separated immediately by a Ficoll-Hypaque density gradient (Pharmacia, Piscataway, NJ), washed twice in phosphate buffered saline, extracted with perchloric acid (0.6 mol/liter), and diluted to a concentration of 2 × 10⁶ cells/ml in perchloric acid. Next, the sample was placed in an ice bath for 30 min, spun in a microcentrifuge for three min, and assayed for putrescine, spermidine, and spermine by modifications of a previously described procedure (39). Briefly, to 50 μl of the acid-extracted sample, 50 μl of the 1.7-diaminohexane internal standard (50 mmol/l in 0.1 N HCl), 200 μl of saturated sodium carbonate, and 200 μl of dansyl chloride (10 mg/ml diluted 1:10 with acetonitrile) were added, mixed by vortexing for 15 s, and then placed in a water bath at 70°C for 10 min. Then, 100 μl of proline (250 mg/ml) was added and the mixture was briefly vortexed. For further clean-up, the mixture was transferred to a Bond-Elut CIS column (Analytical Biochemistry, Harbor City, CA) that was previously activated by washings with two column volumes each of methanol and 25% acetonitrile. The column was then washed with two column volumes of 25% acetonitrile and the dansylated polyamines were eluted with 500 μl of acetonitrile and the dansylated polyamines were eluted with 500 μl of acetonitrile for 3 min to remove contaminants. Under these conditions, the dansylated polyamines were eluted with 500 μl of methanol.

The high-performance liquid chromatographic reversed-phase system included a 250-× 4.6-mm ODS C18 column (Beckman, San Ramon, CA) maintained by a column oven at 50°C and solvent delivery by two Waters Model 510 pumps. The system was equipped with a Spectroflow Model 980 (Kratos, Ramsey, NJ) fluorescence detector. Samples were injected in 50 μl aliquots with a Waters Model 710 Intelligent Sample Processor (WISP) and separation was achieved at a flow rate of 2 ml/min with a linear gradient beginning with 45% acetonitrile/55% water that increased linearly over 20 min to 90% acetonitrile (curve No. 6 on Waters Model 680 Automated Gradient Controller). After each run, the column was flushed with 90% acetonitrile for 3 min to remove contaminants. Under these conditions, the retention times for putrescine, spermidine, spermine, and the internal standard were 8.8, 14.0, 16.9, and 11.4 min, respectively. Peaks were traced and integrated with a Perkin-Elmer Model ML61-100 Laboratory Computing Integrator (Perkin-Elmer, Norwalk, CT).

RESULTS

Twelve patients were entered on this study. Patient characteristics are displayed in Table 1. Patient ages ranged from 47 to 69 years with a median of 61 years. All had received prior radiation and/or chemotherapy.

Seventeen evaluable courses of HMBA were administered at three dose levels, 12, 15.8, and 20 g/m²/day (Table 2). Five patients received more than one course; one patient received HMBA at more than one dose level. No antitumor responses were observed.

Hematological Toxicity. In contrast to previous 5-day studies demonstrating only mild myelosuppression, thrombocytopenia was the dose-limiting toxicity in this 10-day study. The effects of HMBA on individual platelet counts are depicted in Table 3. All seventeen courses were associated with absolute decreases in platelet counts. Mean and median platelet count nadirs were 175,000 and 137,000/μl, respectively, at 15.8 g/m²/day and 43,500/μl and 21,000/μl, respectively, at 20 g/m²/day. At the starting dose, 15.8 g/m²/day, three of 10 courses were associated with grades 1 and 2 thrombocytopenia (platelets between 50,000 and 90,000/μl and grade 4 thrombocytopenia (platelets ≤ 25,000/μl) was observed during one course in a 62-year-old male with diffuse histiocytic lymphoma. His past history was significant for more extensive prior chemotherapy and radiotherapy than any other patient in the study. A second course of HMBA administered at 12 g/m²/day had to be discontinued prematurely (day 8) due to a decrease in his platelet count to 55,000/μl; his subsequent platelet nadir was 46,000/μl. At 20 g/m²/day, HMBA induced remarkable platelet toxicity in all six courses; grade 3 and 4 thrombocytopenia (platelets < 50,000/μl) developed in four courses, and the remaining two courses were associated with milder grades 1 and 2 thrombocytopenia. Platelet nadirs occurred on days 13 to 18, and platelet toxicity resolved between days 22 and 29. In addition, the most severe episodes of thrombocytopenia were observed in patients whose platelet counts had decreased to less than 100,000/μl during the actual HMBA infusion. No significant bleeding occurred. Bone marrow examinations were performed during two thrombocytopenic courses and revealed decreased numbers of megakaryocytes, however, myeloid and erythroid precursors were present in normal quantities and normal maturation ratios. Megaloblastic changes were not observed.

Significant treatment-related leukopenia and anemia were observed less frequently than thrombocytopenia. Mean white blood count nadirs were 2800/μl at 12 g/m²/day (1 course), 6640/μl at 15.8 g/m²/day, and 3450/μl at 20 g/m²/day. Two

Table 1 Patient characteristics

<table>
<thead>
<tr>
<th>Table 1 Patient characteristics</th>
<th>Number of patients</th>
<th>Number of courses</th>
<th>Sex ratio (M:F)</th>
<th>Median age (range)</th>
<th>Performance status</th>
<th>Prior therapy</th>
<th>Chemotherapy</th>
<th>Radiotherapy</th>
<th>Both</th>
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</thead>
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<tr>
<td>Number of patients</td>
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<td>17</td>
<td>6:6</td>
<td>61 (47–69)</td>
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Table 2 Dose escalation

<table>
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<th>No. of patients</th>
<th>No. of courses</th>
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<td>12.00</td>
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<tr>
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</tr>
<tr>
<td>20.00</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>

* One patient treated at both dose levels.

Table 3 Effect of HMBA on platelet counts

<table>
<thead>
<tr>
<th>Table 3 Effect of HMBA on platelet counts</th>
<th>Dose (g/m²/day)</th>
<th>Pretreatment platelet count*</th>
<th>Nadir platelet count (×10⁴)</th>
<th>Nadir/pre-treatment (%)</th>
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<td>12</td>
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<td>46 (10)</td>
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<td>15.8</td>
<td>130</td>
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<td>15.8</td>
<td>487</td>
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<td>239</td>
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<td>26</td>
<td></td>
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<td>108</td>
<td>32 (15)</td>
<td>29</td>
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<td>320</td>
<td>19 (18)</td>
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<td>260</td>
<td>13 (15)</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>332</td>
<td>21 (18)</td>
<td>6</td>
<td></td>
</tr>
</tbody>
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* Platelet count × 1,000/μl.

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of 10 courses at 15.8 g/m²/day and four of six courses at 20 g/m²/day were associated with grades 1 and 2 leukopenia (WBC between 2000 and 4000/µl). One patient treated at the 20 g/m²/day dose level developed grade 4 leukopenia; the WBC nadir was 500/µl on day 15 and the development of granulocytopenic fever necessitated the use of systemic antibiotics. Leukopenia and anemia generally developed in patients who concomitantly developed the most severe episodes of thrombocytopenia. Myelosuppression was usually more pronounced in patients who had received the most prior myelotoxic chemotherapy and radiotherapy. For heavily pretreated patients, mean nadir and percentage of decrement in platelet counts were 113,000/µl and 62% at 15.8 g/m²/day, and 17,600/µl and 94% at 20 g/m²/day, respectively. For patients who received less prior myelotoxic treatment, mean nadir and percent decrement in platelets were 251,000/µl and 58% at 15.8 g/m²/day and 69,300/µl and 72% at 20 g/m²/day, respectively. Interestingly, seven courses were also associated with a modest leukocytosis (WBC between 11,800/µl and 21,500/µl) that occurred both during and following the HMBA infusion.

Renal and Central Nervous System Toxicities. Although renal and central nervous system toxicities limited dose escalation of HMBA administered as a 5-day continuous infusion, these toxicities were mild and subclinical at doses of HMBA up to 20 g/m²/day administered for 10 consecutive days. An absolute decrease in creatinine clearance rates were noted during 15 of 17 course; however, grades 1 and 2 renal toxicity (serum Cr > 2.1 mg/dl) occurred in only three of 10 courses administered at 15.8 g/m²/day and one of six courses at the 20 g/m²/day dose level. The most profound alteration of renal function was observed in a 61-year-old male who had a pretreatment serum creatinine concentration of 1.5 mg/dl that modestly increased to 2.2 mg/dl during the infusion. A lowering of serum bicarbonate ion concentrations occurred during 16 of 17 courses, but metabolic acidemia was mild and asymptomatic. A decrease in the serum bicarbonate concentration to 20 Meq/l or less was observed in only two of 10 courses at 15.8 g/m²/day and one of six courses at 20 g/m²/day. The magnitude of the decrement in serum bicarbonate concentrations was related to the magnitude of drug-induced renal dysfunction as measured by elevations in serum creatinine and urea nitrogen concentrations. Patients with lower pretreatment creatinine clearance rates were generally more susceptible to both renal and metabolic toxicities. Modest hyponatremia additionally developed in one patient treated at the 15.8 g/m²/day dose level; her serum sodium concentration progressively declined from 139 Meq/liter to 130 Meq/liter during the HMBA infusion. Simultaneous measurements showed that her urine osmolality and sodium concentrations were inappropriately high compared to her plasma osmolality and sodium concentrations (Uälle = 83 Meq/liter, Uosm = 479 mOsm/kg, P osm = 265 mOsm/kg). In her addition, sodium excretion remained substantially elevated in response to an oral free water challenge test. These findings, in addition to her euclidean state and normal thyroid, renal, and adrenal functions, suggested the syndrome of inappropriate antidiuretic hormone secretion induced by HMBA. All drug-induced renal and metabolic abnormalities were most pronounced towards the end of the HMBA infusion and resolved rapidly following the discontinuation of drug.

Symptomatic central nervous system toxicity was not observed during this study. Daily minimental examination scores remained unchanged throughout the infusions in all patients. However, five of 14 EEGs done on the 10th day of HMBA demonstrated new abnormalities as compared to pretreatment studies. These changes [excessive diffuse slowing (2), slow runs of delta activity (1), and slowing of posterior medium basic rhythm (2)] were suggestive of mild diffuse disturbances of cerebral function and have been seen in association with many other toxic and metabolic conditions. Patients who exhibited EEG changes remained on the identical medications as they were taking at the time of their pretreatment EEG.

Other Toxicities. Nausea, vomiting, and mucositis were mild and characteristically occurred towards the end of the 10-day infusion. Nausea and vomiting were never intractable and occurred during one course at 15.8 g/m²/day and 3 courses at 20 g/m²/day. One patient treated at 15.8 g/m²/day complained of oral and pharyngeal discomfort on infusion days eight to 10. Examination of the oropharynx was unremarkable and symptoms resolved rapidly following the end of the infusion. Other toxicities observed included moderate phlebitis and nonulcerative cellulitis that occurred at sites of drug extravasation and resolved within 1 week (two courses), anorexia (one course), and malaise (three courses). Hepatotoxicity was characterized by very mild and transient elevations of SGOT and SGPT in the absence of neoplastic liver involvement and occurred during two courses of HMBA administered at the 20 g/m²/day dose level. While one patient had an isolated elevation of SGOT from 19 to 45 IU/liter (n = 0–30), the second had elevations of SGOT and SGPT from 20 and 21 IU/liter to 58 and 36 IU/liter, respectively, without changes in either serum bilirubin or alkaline phosphatase concentrations. Elevations in hepatic transaminases occurred between days 7 and 15, and returned to baseline by day 26.

Dermatological toxicity in the form of cutaneous vasculitis was observed during two courses of HMBA administered to a patient at 15.8 g/m²/day (40). Toxicity was not noted during the patient's first infusion however, a short-lived macular erythematous periorbital and malar rash appeared immediately following drug administration. On day 3 of her second course, erythematous papules appeared on both ankles, soon after coalesced, and were identified as palpable purpura. Histopathological examination of a skin biopsy revealed focal epidermal necrosis, an extensive inflammatory infiltrate, endothelial swelling with hemorrhage, and peri- and intravascular fibrin deposition in the dermis. In addition, direct immunofluorescent studies demonstrated granular deposits of IgA and C3 in the dermal vasculature. These histopathological findings were consistent with a drug-induced leukocytoclastic vasculitis mediated by IgA immune complexes. However, circulating immune complexes were not detected by a Clq binding assay and circulating levels of C3 and C4 were normal. There was no physical nor laboratory evidence of systemic vasculitic involvement. The purpuric lesions subsequently became confluent and progressed to her mid thighs by day 8, at which time she also redeveloped her malar rash. HMBA was discontinued on day 8, and all skin lesions resolved by day 22.

Pharmacology. The pharmacokinetics of HMBA have been previously described (34, 35). In our previous 5-day study, HMBA plasma concentrations were found to be adequately fit by a single-compartment pharmacokinetic model. Steady-state conditions were achieved rapidly (usually within 12–24 h), and the half-life of elimination ranged from 8 to 301 min (mean, 144 min). Since extending the duration of the infusion from 5 to 10 days was not expected to affect HMBA's pharmacokinetic behavior, an extensive reanalysis of HMBA pharmacokinetics was not undertaken. Instead, in an attempt to discern if relevant in vitro differentiating concentrations could be maintained in the plasma for 10 days, only mean HMBA Cmax and clearance
rates were determined (Table 4). As expected, the steady-state was achieved rapidly (usually within 12 h) and drug accumulation was observed only in those patients who developed concomitant drug-induced renal toxicity that resulted in decreased creatinine as well as HMBA clearance rates. Mean HMBA \( C_{\text{ss}} \) values were 0.65 ± 0.09 mmol/liter at 15.8 g/m²/day and 0.99 ± 0.22 mmol/liter at 20 g/m²/day. Total systemic clearance rates of HMBA ranged from 50 to 246 ml/min/m² (mean, 110 ± 20 ml/min/m²) at 15.8 g/m²/day and from 55 to 229 ml/min/m² (mean, 101 ± 27 ml/min/m²) at 20 g/m²/day. At both dose levels, there was an association between the magnitude of mean HMBA \( C_{\text{ss}} \) and the development of drug-induced renal toxicity as measured by reductions in the creatinine clearance rate. Large variations in renal clearance rates of the parent compound were observed among patients, 2 to 105 ml/min/m² (mean, 48 ± 12 ml/min/m²) at 15.8 g/m²/day and 4 to 60 ml/min/m² (mean, 24 ± 9 ml/min/m²) at 20 g/m²/day. Daily urinary excretion of HMBA accounted for 4 to 77% (mean, 33 ± 6%) of the disposition of the parent compound.

Pharmacodynamic Relationships. There was a strong association between mean HMBA \( C_{\text{ss}} \) and the magnitude of the dose-limiting toxicity, thrombocytopenia. The percentage of change in platelet counts (100 × [pretreatment platelet count − nadir platelet count]/[pretreatment platelet count]) was greater for those patients with higher mean HMBA AUC values. For all patients who were treated with a continuous infusion of HMBA for 10 consecutive days, the relationship between the percentage of change in platelet counts and AUC was linear with a correlation coefficient of 0.66 (\( p < 0.01 \)); however, when the values of the only patient with malignancy-associated thrombocytopenia (pretreatment platelet count, 960,000/nl) were excluded from the analysis, the correlation improved with the correlation coefficient increasing to 0.84 (\( p < 0.001 \)). This relationship is depicted in Fig. 2. For the 10-day study, the relationship between the percentage of change in platelet counts and AUC was simply and well described by the equation:

\[
\% \text{ Change in platelets} = 2.28 \times (\text{AUC}) + 55.79 \quad (D)
\]

The relationships between AUC and platelet counts were described less well when analyses were performed using absolute changes in platelet counts or absolute platelet nadirs instead of the percentage of change in platelet counts.

When a similar analysis was performed using data from 28 courses of HMBA administered for 5 consecutive days in a previous phase I study at The Johns Hopkins Oncology Center, a linear relationship between AUC and the percentage of decrease in platelet counts was less evident (\( r = 0.29, p < 0.20 \)). These data are displayed in Fig. 3. Because of the failure of a simple linear model to adequately describe the association between AUC and platelet counts using combined 5/10-day study data, the Hill equation was used to model the combined data. The relationship was best described by the equation:

\[
\text{Decrease in platelets} = \frac{(100)(\text{AUC}^{0.66})}{2.48 + (\text{AUC}^{0.66})} \quad (E)
\]

This model fit the combined data from both 5- and 10-day studies moderately well (\( r = 0.5 \)). However, visual inspection of the data revealed that for comparable AUCs, disproportionally greater decreases in the platelet count occurred in the 10-day study compared to the 5-day study. This suggested that the magnitude of the reduction in platelet counts was not dependent only upon AUC, but also upon the duration of the infusion. To discern the contribution of both AUC and exposure duration as independent variables in the induction of thrombocytopenia, stepwise multiple linear regression was performed. The relationships among percentage of decrease in platelet count, AUC, and exposure duration for all patients treated with HMBA was well described (\( r = 0.75 \)) by the equation:

\[
\% \text{ Decrease in platelets} = -1.34 + 1.86 \times (\text{AUC}) + 5.97 \times (\text{exposure days}) \quad (F)
\]

Both AUC and exposure duration were significant independent variables (coefficient for AUC, \( t = 2.74 \); coefficient for exposure days, \( t = 4.87 \)).

Polyamines. The effects of HMBA on intracellular concentrations of putrescine, spermidine, and spermine in peripheral blood mononuclear leukocytes are demonstrated in Table 5. There were no specific patterns of polyamine fluctuations during HMBA infusions nor did there appear to be any relationship between HMBA \( C_{\text{ss}} \) and the magnitude of polyamine changes.

Interestingly, intracellular concentrations of spermine and sper-

<table>
<thead>
<tr>
<th>Dose (g/m²/day)</th>
<th>Mean steady-state HMBA concentration (range, mmol/liter)</th>
<th>Mean systemic clearance (range, ml/min/m²)</th>
<th>Mean renal clearance (range, ml/min/m²)</th>
<th>Mean systemic/membran renal clearance</th>
<th>Platelet nadir* (° decrement)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.0³</td>
<td>0.47 (0.30-0.87)</td>
<td>99 (48-139)</td>
<td>58 (29-107)</td>
<td>0.50</td>
<td>46 (58)</td>
</tr>
<tr>
<td>15.8³</td>
<td>0.63 (0.40-0.70)</td>
<td>89 (78-106)</td>
<td>44 (27-58)</td>
<td>0.50</td>
<td>63 (71)</td>
</tr>
<tr>
<td>15.8³</td>
<td>0.35 (0.26-0.48)</td>
<td>164 (114-211)</td>
<td>79 (46-109)</td>
<td>0.47</td>
<td>207 (55)</td>
</tr>
<tr>
<td>15.8³</td>
<td>0.23 (0.15-0.27)</td>
<td>246 (203-274)</td>
<td>65 (39-104)</td>
<td>0.26</td>
<td>141 (65)</td>
</tr>
<tr>
<td>15.8³</td>
<td>0.45 (0.33-0.73)</td>
<td>126 (75-166)</td>
<td>90 (53-123)</td>
<td>0.77</td>
<td>137 (63)</td>
</tr>
<tr>
<td>15.8³</td>
<td>0.48 (0.36-0.43)</td>
<td>140 (128-152)</td>
<td>105 (71-130)</td>
<td>0.75</td>
<td>214 (62)</td>
</tr>
<tr>
<td>15.8³</td>
<td>0.96 (0.51-2.10)</td>
<td>67 (26-107)</td>
<td>6 (5-7)</td>
<td>0.12</td>
<td>590 (39)</td>
</tr>
<tr>
<td>15.8³</td>
<td>0.77 (0.64-0.92)</td>
<td>72 (60-86)</td>
<td>30 (21-40)</td>
<td>0.42</td>
<td>232 (59)</td>
</tr>
<tr>
<td>15.8³</td>
<td>1.68 (0.99-1.98)</td>
<td>35 (21-55)</td>
<td>2 (1-2)</td>
<td>0.06</td>
<td>53 (89)</td>
</tr>
<tr>
<td>15.8³</td>
<td>1.26 (0.79-2.70)</td>
<td>50 (20-69)</td>
<td>2 (2-3)</td>
<td>0.04</td>
<td>90 (79)</td>
</tr>
<tr>
<td>15.8³</td>
<td>0.66 (0.26-1.28)</td>
<td>107 (63-211)</td>
<td>61 (7-97)</td>
<td>0.42</td>
<td>24 (81)</td>
</tr>
<tr>
<td>15.0³</td>
<td>0.65 (0.29-0.99)</td>
<td>110 (84-20)</td>
<td>48 ± 12</td>
<td>0.38 ± 0.08</td>
<td>172 (66)</td>
</tr>
<tr>
<td>15.0³</td>
<td>0.31 (0.25-0.34)</td>
<td>229 (193-256)</td>
<td>60 (44-81)</td>
<td>0.26</td>
<td>63 (73)</td>
</tr>
<tr>
<td>20.0</td>
<td>0.66 (0.53-0.84)</td>
<td>107 (89-131)</td>
<td>28 (6-47)</td>
<td>0.26</td>
<td>32 (70)</td>
</tr>
<tr>
<td>20.0</td>
<td>1.72 (1.08-2.28)</td>
<td>43 (30-64)</td>
<td>4 (3-7)</td>
<td>0.10</td>
<td>19 (94)</td>
</tr>
<tr>
<td>20.0</td>
<td>1.50 (0.95-3.27)</td>
<td>55 (20-69)</td>
<td>3.5 (2-5)</td>
<td>0.06</td>
<td>13 (95)</td>
</tr>
<tr>
<td>20.0</td>
<td>1.11 (0.79-1.70)</td>
<td>66 (53-88)</td>
<td>14 (3-28)</td>
<td>0.18</td>
<td>21 (93)</td>
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<tr>
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<td>107 (93-125)</td>
<td>36 (18-62)</td>
<td>0.37</td>
<td>113 (73)</td>
</tr>
</tbody>
</table>

* Platelet count × 1,000/µl.
\³Treated at 12 g/m²/day after toxicity at 15.8 g/m²/day.
\'Developed renal toxicity by ECOG criteria.
cytology levels of spermidine and spermine decreased to 13 and 28 nmol/10^6 cells, corresponding to 20 and 28% decreases in platelet counts, respectively. These effects of HMBA were most profound. For example, lymphohistiocytic lymphoma who exhibited the earliest and most profound decrement in platelet count after receiving HMBA at 12 g/m^2/day to a 62-year-old male with diffuse extramedullary erythroleukemia. The AUC of HMBA during 5-day continuous infusion study. Points, individual courses of HMBA.

Fig. 3. Relationship between percentage of decrement in platelet count and HMBA AUC during 5-day continuous infusion study. Points, individual courses of HMBA.

Table 5 Effects of HMBA on intracellular polyamines

<table>
<thead>
<tr>
<th>Dose (g/m^2/day)</th>
<th>Putrescine</th>
<th>Spermidine</th>
<th>Spermine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-</td>
<td>Early^a</td>
<td>Late^a</td>
</tr>
<tr>
<td>12.0</td>
<td>0.02</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>15.8</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>15.8</td>
<td>0.03</td>
<td>0.02</td>
<td>0.03</td>
</tr>
<tr>
<td>15.8</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>15.8</td>
<td>0.04</td>
<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>15.8</td>
<td>0.03</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>20.0</td>
<td>&lt;0.01</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>20.0</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>20.0</td>
<td>0.02</td>
<td>0.02</td>
<td>0.01</td>
</tr>
</tbody>
</table>

^a Nanomoles/10^6 cells.  
^b Sample collected on days 2 to 3 of infusion.  
^c Sample collected on days 9 to 10 of infusion.

midamine decreased during four courses in which the myelotoxic effects of HMBA were most profound. For example, lymphocyte levels of spermidine and spermine decreased to 13 and 36% of pretreatment levels, respectively, during HMBA administration at 12 g/m^2/day to a 62-year-old male with diffuse histiocytic lymphoma who exhibited the earliest and most profound sensitivity to drug-induced thrombocytopenia.

DISCUSSION

The implications of identifying potent differentiating agents with broad therapeutic indices are significant. If effective concentrations of these agents can be achieved clinically, the potential to transform rapidly proliferating primitive cells into functionally and morphologically mature cells that lack self-renewing capabilities may have many important implications in cancer prevention and therapeutics. Conceptually, these agents may be used prophylactically in patients who are at high risk to develop malignant disorders, to treat premalignant syndromes (i.e., myelodysplasia, actinic skin lesions, and oral leukoplakia), or used alone or in conjunction with classical cytotoxic drugs to convert malignant tumors to neoplasms that possess less aggressive growth characteristics. The identification of HMBA is important since HMBA is active at concentrations several orders of magnitude lower than other differentiating agents. In addition, previous phase I and pharmacological studies of 5-day infusions have demonstrated that HMBA can be achieved (1–2 mmol/liter) that approach the lower limits necessary for in vitro differentiating effectiveness (1–5 mmol/liter). In contrast, peak clinically achievable NMF concentrations (1–10 mmol/liter), fall significantly short of optimal differentiating concentrations in vitro (>100 mmol/liter) (20, 21).

With preclinical evidence suggesting that the duration of HMBA exposure is an important variable in the induction of differentiation and the finding that HMBA achieved during previous 5-day studies only approach minimally effective in vitro differentiating concentrations, this study was conducted to evaluate the feasibility of maintaining HMBA for 10 days similar to levels achieved during 5-day infusions. Although decreases in the platelet counts occurred in 31 of 35 courses of HMBA administered for 5 consecutive days, thrombocytopenia was mild and nadir platelet counts were rarely less than 100,000/µl. However, thrombocytopenia was the primary dose-limiting toxicity in this study of prolonged drug administration. Severe thrombocytopenia was sporadic at the 15.8 g/m^2/day dose level, but consistently occurred at the 20 g/m^2/day dose level preventing further dose escalation. Of more importance, the magnitude of the decrease in platelets correlated well and in a linear fashion with the mean HMBA C50 and AUC in the 10-day study. In addition, other factors that predisposed to thrombocytopenia included the extent of prior myelotoxic therapy, having received a previous course of HMBA, and the concomitant development of renal toxicity accompanied by a diminished clearance of drug.

An understanding of the relationship between platelet toxicity and pharmacological parameters reflecting HMBA exposure is potentially important in that it may permit the rational selection of optimal HMBA dosing and scheduling if the information is used in conjunction with preclinical data regarding optimal in vitro differentiating schemes. Analysis of the 10-day study data demonstrated that the relationship between HMBA AUC and platelet toxicity was linear. However, a linear relationship was not as apparent when a similar analysis was conducted using only data from our previous 5-day phase I study. The pharmacodynamic relationship between HMBA AUC and drug-induced platelet changes has been described by Egorin et al. using more complex nonlinear models, more specifically by the Hill equation, which is commonly used to describe relationships in biological and receptor systems (34, 38). Our 5-day data satisfactorily fit a similar model, but analysis of the combined data from both 5- and 10-day studies indicated that the magnitude of the percentage of decreases in platelet counts was not strictly...
proportional to HMBA exposure for all delivery schedules and that the HMBA exposure duration was a significant factor as was AUC in determining the magnitude of thrombocytopenia. For example, based on our data, a particular HMBA $C_m$ maintained for 10 consecutive days would be expected to produce a dis-proportionately greater percentage of decrease in the platelet count than an identical HMBA $C_m$ maintained for 5 consecutive days.

The mechanism responsible for HMBA-induced thrombocyto-penia is unclear. Because thrombocytopenia was accompanied by a relative sparing of white blood cells and resembled toxicities associated with DFMO, a potent inhibitor of ornithine decarboxylase, the rate-limiting enzyme involved in polyamine biosynthesis (41, 42), intracellular concentrations of the polyamines, putrescine, spermidine, and spermine were measured in peripheral blood mononuclear leukocytes during several infusions. Measurements of polyamines were also undertaken because of the close structural similarity of HMBA to several naturally occurring polyamines, and because several polyamine metabolites of HMBA have been identified (35). In addition, the modulation of the polyamine biosynthetic pathway has been proposed as a possible mechanism accounting for drug-induced differentiation (43–45). Although intracellular concentrations of spermidine and spermine were noted to decrease during several courses of HMBA that were associated with profound myelotoxicity, polyamine behavior during HMBA administration was quite variable. There did not appear to be any consistent relationship between either polyamine changes and toxicity nor between polyamine fluctuations and the magnitude of mean HMBA $C_m$ values. However, the failure to observe these associations may have been due to the selection of peripheral blood mononuclear leukocytes for the analysis of drug-induced polyamine changes. Although it was realized that this cellular population is heterogenous, it was selected for polyamine monitoring because of its easy availability. Peripheral blood mononuclear leukocytes are also relatively quiescent and may not reflect polyamine changes that may be occurring in more rapidly proliferating cells, which may be more susceptible polyamine modulation by various agents (43–44). Although the red blood cell fraction contains 90% of circulating polyamines, the measurements of red blood cell polyamine levels during DFMO administration in rats did not appear to be an effective means to monitor DFMO-induced platelet toxicity (46, 47).

Nonhematological drug effects such as HMBA-induced metabolic abnormalities, nephrotoxicity, and neurotoxicity, which limited dose escalation during phase I studies of HMBA administered for 5 days, were subclinical at dose levels up to 20 g/m$^2$/day during this 10-day study. Although patients with substantially higher mean HMBA $C_m$ values developed renal toxicity, these patients generally had lower pretreatment creatinine clearance rates. The relationship between renal function and HMBA $C_m$ was characterized by an accelerating spiral as patients with relatively worse pretreatment renal functions developed higher HMBA $C_m$, which eventually led to a further decline in renal function. The contribution of the various HMBA metabolites in the induction of CNS toxicity, nephrotoxicity, and hepatotoxicity have not yet been defined.

Based on this phase I trial, the MTD and recommended phase II doses for HMBA administered as a 10-day continuous infusion are 20 and 15.8 g/m$^2$/day, respectively. However, because of substantial interpatient variability of HMBA $C_m$ at any dose level and the clear relationship between HMBA $C_m$ or AUC and dose-limiting toxicity, thrombocytopenia, our data support the notion proposed by other investigators that pharmacological monitoring be used in future studies of HMBA with the aim of further defining and achieving maximally tolerated or recommended plasma concentrations (34). However, schemes that are developed to optimize HMBA therapeutic index based on studies in patients with solid malignancies should be undertaken with caution if applied to the treatment of patients with abnormal bone marrow function such as those with myelodysplastic syndromes. Since HMBA $C_m$ were generally lower and AUCs were not significantly higher at recommended dose levels of HMBA administered for 10 consecutive days as compared to 5 consecutive days, it is not apparent that the 10-day continuous infusion schedule offers any significant advantages over the 5-day schedule. The administration of HMBA for 10 consecutive days may also be disadvantageous if utilized in therapeutic trials in myelodysplastic syndromes, which are associated with decreased pretreatment platelet counts and often occur in elderly patients with relatively poorer renal function. It is also important to emphasize that although objective responses have not been observed in this or other phase I trials of HMBA in patients with advanced solid malignancies, formal dimensional criteria to access cytotoxic response may be inappropriate in the evaluation of drug-induced differentiating activity. Therefore, it is important that investigators who will be conducting therapeutic trials with differentiating agents evaluate changes indicative of differentiation at the cellular and subcellular levels utilizing innovative and novel technologies such as the analysis of DNA restriction fragments, cellular growth kinetics, oncogene expression, cell surface and cytoplasmic markers, and cellular morphology (48, 49).

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