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Approaches to optimal dosing of hexamethylene bisacetamide*

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Summary. HMBA is a potent differentiating agent capable of causing >95% morphological differentiation in cell lines in vitro. The induction of differentiation is dependent on both the concentration of and the duration of exposure to HMBA. However, acute toxicities (neurotoxicity and acidosis) have limited the maximal HMBA c_{ss} value to <2 mM, which is at the lower limit of effective in vitro concentrations. When HMBA c_{ss} values have been maintained at 1–2 mM, thrombocytopenia has limited the duration of HMBA infusion to ≤10 days. The present studies were performed to determine whether exposure to HMBA could be individualized and maximized without resulting in intolerable toxicity to patients and to determine which factors would predispose a patient to the development of acute toxicity during treatment with HMBA. For these investigations, patients were given HMBA at a target c_{ss} using an adaptive-feedback-control method rather than at a set dose. Because HMBA administration produces large anion gaps, a simple maneuver such as alkalinization might enable the escalation of plasma HMBA c_{ss} values to >2 mM. HMBA was given as a 5-day CI to 14 patients (26 courses) at 2 target HMBA c_{ss} levels near the maximal tolerated value in the presence or absence of concurrent alkalinization with sodium bicarbonate. Symptomatic acidosis occurred in one patient who did not receive bicarbonate. Neurotoxicity proved to be dose-limiting at the target HMBA c_{ss} value of 1.5–2.0 mM in the absence of concurrent alkalinization and at a c_{ss} level of >2 mM, regardless of alkalinization. No neurotoxicity was seen at target HMBA c_{ss} values of 1.5–2.0 mM in patients who did receive concurrent alkalinization. Alkalinization was not associated with any detectable changes in plasma HMBA metabolites. With the maximal tolerable 5-day HMBA c_{ss} having thus been defined at 1.5–2.0 mM, we attempted to maximize exposure to HMBA by defining a tolerable duration of infusion. Individualization of the duration of HMBA infusion to a target nadir PLT was performed in patients who had received an initial 5-day CI of HMBA at a c_{ss} 1.5–2.0 mM along with concurrent alkalinization. The AUC achieved and the thrombocytopenia produced during this first course were used to predict the duration of infusion that each patient would subsequently tolerate (at an HMBA c_{ss} of 1–2 mM) to achieve a nadir PLT of 75,000–100,000/μl. The observed percentage changes in PLT matched the predicted percentage change in PLT, with the mean error (ME) being –8.9%. For a better determination as to which factors may contribute to neurotoxicity or acidosis in patients receiving HMBA, 98 courses of HMBA given as 5- to 10-day CIs to 56 patients were analyzed (multifactorial logistic regression). An HMBA AUC value of >7.5 mM × day, the use of any concomitant narcotic analgesics, and a mean plasma HMBA level of >1.5 mM or a peak plasma concentration of ≥1.75 mM correlated significantly with grade 3 neurotoxicity (P <0.001), whereas concomitant alkalinization and a mean plasma HMBA concentration of <1.5 mM were associated with a lack of neurotoxicity (P <0.001). An AUC

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Abbreviations: HMBA, hexamethylene bisacetamide; c_{ss}, plasma steady-state concentration; CI, continuous infusion; ME, mean error; PLT, platelet count; AUC, area under the concentration-time curve; C_{cr}, creatinine clearance; DMSO, dimethylsulfoxide; NMF, *N*-methylformamide; UMCC, University of Maryland Cancer Center; ECOG, Eastern Cooperative Oncology Group; WBC, white-blood-cell count; CBC, complete blood count; EKG, electrocardiogram; AG, anion gap; V_{max}, predicted maximal nonrenal HMBA elimination rate; K_m, predicted concentration of HMBA at which the nonrenal elimination rate is half of the V_{max} value; SLOPE, predicted slope relating the renal clearance of HMBA to C_{cr}; Ra, new infusion rate; Hct, hematocrit; 6AcHA, 6-acetamidohexanoic acid; NADAH, *N*-acetyl-1,6-diaminohexane; DAH, 1,6-diaminohexane; 6AmHA, 6-aminohexanoic acid; MAO, monoamine oxidase; MAP, maximum a priori; MAE, mean absolute error

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value of >7.5 mM × day, a mean or peak plasma HMBA level of >1.5 mM, and an age of >70 years correlated with the likelihood of a large anion gap (P <0.03). With the above factors being accounted for, neither the duration of infusion nor the C_{cr} value showed any correlation with these toxicities in this patient population. These results imply that HMBA may be given for individualized durations at a c_{ss} of 1.5 mM in the presence or absence of concomitant alkalinization and that narcotic analgesics should not be given to patients receiving this agent. However, due to the resultant acute neurotoxicity, it is unlikely that AUCs of >7.5 mM × day will be tolerated during simultaneous maintenance of a c_{ss} value of >1.0 mM.

Introduction

HMBA, a polar-planar compound (mol. wt., 200) capable of inducing differentiation in leukemic [5, 26] and solid-tumor [4, 10, 23, 24, 29] cell lines in vitro, has been studied in phase I trials using both 5-day [7, 11, 27] and 10-day CI schedules [28, 36]. A CI schedule was chosen because of the short half-life of HMBA [4, 18] and because in vitro preclinical studies had indicated that the differentiating activity of this agent was both time- and concentration-dependent [5, 20, 25, 26]. At lower HMBA concentrations, longer durations of exposure are required to achieve comparable differentiation in vitro. Moreover, differentiation of solid-tumor cell lines was reversible on the removal of HMBA [29]. HMBA appeared to be more promising for clinical use than either DMSO or NMF [13, 22, 32] because animal studies had shown that plasma concentrations of HMBA needed to cause differentiation in vitro (2–10 mM) were achievable without unacceptable side effects [4, 18].

Previous phase I trials [7, 11, 27] have demonstrated neurotoxicity (hallucinations, obtundation, agitation, confusion) and anion-gap metabolic acidosis as dose-limiting toxicities in trials using 5-day CI schedules, whereas AUC-related thrombocytopenia limits the length of the infusion [28, 36]. Based on traditional escalating-dose regimens, the recommended phase II doses of HMBA were 24 g/m^2 daily for the 5-day CI schedule [11, 27] and 15.8 and 24 g/m^2 daily for the 10-day CI schedule [28, 36]. However, pharmacodynamics studies at the UMCC demonstrated approximately 30% interpatient variability in HMBA c_{ss} values achieved at any given dose between 4 and 33 g/m^2 daily and showed that acidosis and neurotoxicity were unusual at HMBA c_{ss} values of <2.0 mM [11]. In addition, the relationship between the HMBA AUC and the degree of thrombocytopenia shows considerable interpatient (but minimal intrapatient) variability [15].

Therapeutic responses to HMBA have been suggested even when an HMBA c_{ss} of 1–2 mM is maintained for 5–10 days [7, 36]. However, 1–2 mM HMBA is considered to be the lower limit of the range of HMBA concentrations that cause differentiation in vitro [4, 5, 10, 20, 23–26, 29]. It would be reasonable to expect that escalation of the HMBA c_{ss} to >2 mM and maintenance of exposure to HMBA for as long as possible might improve the clinical response. To maintain the HMBA c_{ss} at the highest levels possible without provoking toxicity, we have developed and validated an adaptive-feedback-control algorithm [7] with which the HMBA c_{ss} can be maintained at a desired concentration. We have also developed and retrospectively validated an adaptive control method by which the duration of HMBA infusion can be individually determined on the basis of a desired maximal percentage change in PLT [15].

To determine whether a simple maneuver such as alkalinization with sodium bicarbonate could prevent the anion-gap metabolic acidosis observed at an HMBA c_{ss} of >2 mM and, possibly, to enable the escalation of HMBA c_{ss} values to higher concentrations, we studied cohorts of patients at escalating target c_{ss} levels of HMBA in the presence or absence of concurrent sodium bicarbonate alkalinization. When a maximal nontoxic c_{ss} was determined, the protocol was amended to validate prospectively the algorithm for the maximal tolerable duration of infusion (the duration needed for each patient to produce a PLT nadir of 75,000–100,000/μl). Because the initial clinical impressions of this drug implied that narcotic use or other individual factors may predispose patients to neurotoxicity or acidosis, we also retrospectively analyzed 98 courses of HMBA given as 5- to 10-day CIs to 56 patients in 4 phase I trials conducted at UMCC to discern those factors that would be likely either to predispose patients to or to protect them from the acidosis and neurotoxicity associated with HMBA administration.

Patients and methods

Patient selection and evaluation

The eligibility criteria included documented malignancy for which no standard treatment was available or effective, an age of ≥18 years, an ECOG performance status of ≤2, a WBC of ≥3500/μl, a PLT of ≥100,000/μl, a bilirubin value of ≤2 mg/dl, a serum creatinine level of <2 mg/dl, a C_{cr} value of ≥40 ml min^{-1} 1.73 m^{-2}, no uncontrolled medical illness, and no history of seizure, brain tumor, or psychosis. Baseline clinical studies included a CBC; a PLT; determinations of serum electrolytes, BUN, creatinine, C_{cr}, urinary pH, serum albumin, bilirubin, transaminases, lactic dehydrogenase, and alkaline phosphatase; a chest radiograph; an EKG; a mini-mental status examination [14]; and a history and physical examination. Measurable disease was not required but was measured serially when present. Determinations of HMBA c_{ss}, C_{cr}, CBC, PLT, serum creatinine, serum electrolytes, and urinary pH and mini-mental status examinations were repeated daily during the infusion. Physical examinations and baseline studies (excluding C_{cr}, EKG, chest radiograph, and mini-mental status examination) were performed weekly. Before receiving HMBA, each patient had the investigational nature of the treatment explained and signed an informed consent approved by the University of Maryland Institutional Review Board and the National Cancer Institute (USA). Toxicity criteria were those of the National Cancer Institute, Division of Cancer Treatment. No accepted grading system is available for metabolic acidosis or diaphoresis. Metabolic acidosis was graded as follows: 0, none; 1, asymptomatic (AG, <20); 2, asymptomatic (20<AG≤24); 3, symptomatic – requires therapy; and 4, symptomatic – requires intensive supportive care. Diaphoresis was graded as follows: 0, none; 1, mild; 2, severe (drenching). Response criteria were the same as those used in our previous studies [7, 11].

Table 1. Studies conducted to achieve optimal HMBA exposure

Study 1 – Maximal tolerable c_{ss}[a]:

Cohort	Target (HMBA)	Number of patients/courses	Alkalinization	Initial infusion rate	Number of patients/courses with grade ≥3 neurotoxicity
1	1.5–2.0 mM	3/8	Yes	24 g m^{-2} day^{-1}	0/8
2	1.5–2.0 mM	7/13	No	24 g m^{-2} day^{-1}	4/4
3	2.2–2.7 mM	3/4	Yes	30 g m^{-2} day^{-1}	2/2
4	2.2–2.7 mM	1/1	No	30 g m^{-2} day^{-1}	1/1

[a] "Dose-escalation trial of HMBA given for 5 days continuously in the presence or absence of concomitant alkalinization. Bicarbonate tablets (four 650-mg tablets q 4 h) or infusion (1–2 ampules sodium bicarbonate in 1 l 5% dextrose in water over 24 h) was given to titrate the urinary pH to at least 7.0 throughout the HMBA infusion. The infusion rate of HMBA was adjusted after 18–24 h to maintain the target c_{ss} using the adaptive-feedback control as described in Materials and methods

Study 2 – Maximal tolerable duration[b]:

Course	HMBA c_{ss}	Target PLT nadir	Duration (days)
1	1.5–2.0 mM	–	5
≥2	1.5–2.0 mM	75,000–100,000	Variable

[b] Adaptive control of HMBA c_{ss} and PLT nadir (duration). All patients initially received an infusion of HMBA of 24 g m^{-2} day^{-1}, adjusted after 18–24 h to maintain the target c_{ss}. All patients were given bicarbonate as described for study 1. After each course, each patient's parameters (AUC_{50}, Hill's constant) were updated and used to predict the duration for the following course that would result in the desired PLT nadir

Drug administration

HMBA was supplied by the Investigational Drug Branch of the National Cancer Institute (Bethesda, Md., USA). Each 500-ml bottle contained 15, 25, or 50 g HMBA as a solution in sterile water or normal saline. HMBA was given by CI through a free-flowing peripheral or central venous catheter. The rate of HMBA infusion was controlled by a Travenol Flo-Gard 8000 volumetric infusion pump (Travenol Laboratories, Inc., Deerfield, Ill., USA).

Clinical trials

Table 1 shows the study designs for clinical trials of HMBA designed to optimize exposure. First courses were initiated at infusion rates that had resulted in plasma concentrations similar to the target c_{ss} in a previous phase I trial [11]. Second and subsequent courses were initiated at the rate that had achieved the target HMBA c_{ss} during the individual patient's previous course, provided that the patient's C_{cr} had not changed significantly. There was no dose escalation within patients or crossover between bicarbonate assignments. Study 1 involved a 5-day CI of HMBA, and study 2 was designed to individualize the duration of HMBA infusion to tolerance. Table 2 shows trials of HMBA conducted at UMCC that were used for analysis of the factors contributing to HMBA toxicity.

Analysis of plasma HMBA and metabolite concentrations

Heparinized blood samples were obtained at 16–24 h after the start of the infusion and daily thereafter during the HMBA infusion. Blood samples were immediately centrifuged at 1000 *g* for 10 min, and the resultant plasma supernatants were immediately removed and analyzed for HMBA concentration. During HMBA infusion, all urine was collected daily for determinations of C_{cr}, and aliquots were frozen for determinations of concentrations of HMBA and its metabolites. Plasma and urinary concentrations of HMBA and its metabolites were assessed using a gas chromatographic method [3, 11, 12, 17]. The quantitative aspects of this method as determined in our laboratory have been published elsewhere [11, 12]. The AUC was calculated by the trapezoidal rule.

Adaptive-feedback-control algorithms

Control of C_{ss}. Dose adjustment was accomplished using adaptive feedback control and a model that was retrospectively derived from our previous phase I trial of HMBA. HMBA clearance was described as the sum of a first-order renal HMBA clearance and a Michaelis-Menten nonrenal HMBA clearance [7]. Briefly, HMBA c_{ss} values were predicted (based on the patient's body surface area, all values of C_{cr}, previous HMBA dosing history, and all previous HMBA c_{ss} values) using Bayesian parameter estimation [30]. The a priori probability distributions were based on previously determined values for population means and variances [7]. An ADAPT [9] computer program was used to determine each patient's parameter values (V_{max}, K_m, SLOPE), and the new infusion rate (Ra) was calculated as follows:

$$\text{Ra (mg 1.73 m}^{-2}\text{ h}^{-1}) = \left[\frac{V_{max}}{K_m + C} + (\text{SLOPE} \times C_{cr})\right] \times C,$$

where C is the desired plasma HMBA c_{ss} expressed in milligrams per liter.

Control of the duration of infusion. After the initial 5-day CI of HMBA, the AUC and the percentage change in PLT were determined for each patient. These data were used to estimate each patient's AUC_{50} and Hill's constant (H) using an MAP-Bayesian estimator as described elsewhere [15]. Again, the data were fit with an ADAPT [9] computer program.

The new infusion duration at a c_{ss} of 1.5–2 mM was determined by a computer prediction of the expected percentage change in PLT for a specified AUC using the following relationship and each patient's derived AUC_{50} value and Hill's constant:

$$\text{Desired \% change in PLT} = \frac{(100)\,(\text{AUC}^H)}{\text{AUC}_{50} + (\text{AUC})^H},$$

where 100 represents the maximal percentage of PLT suppression, H represents Hill's constant, and AUC_{50} represents the AUC at which the effect is half-maximal.

The specified AUCs were computed by multiplication of a desired c_{ss} (1.5–2.0 mM) by 24-h infusion periods (usually 4–10 days). Using adaptive feedback control as described above, the c_{ss} was maintained for the duration necessary to approximate the desired AUC.

Table 2. Summary of HMBA phase I trials used for retrospective correlation of patients' factors and pharmacokinetics with neurotoxicity and acidosis

Trial number	Description of trial	Number of patients/courses	Infusion duration (days)	Mean HMBA C_{ss} (mM)
1	Dose escalation [11]	20/25	5	0.15–3.2
2	Adaptive feedback control [7]	12/29	5	0.76–2.64
3	Adaptive feedback control ±alkalinization	14/26	3–5	1.04–2.77
4	Control of pharmacodynamics	10/18	3–10	1.2 –2.0
Total		56/98		

Factors associated with the acute toxicity of HMBA

A total of 98 courses of HMBA given to 56 patients as 5- to 10-day CIs in 4 phase I trials conducted at the UMCC (see Table 2) were reviewed. The following variables were analyzed using multifactorial logistic regression with backward stepping on SAS software for their contributions to the acute toxicities of acidosis and neurotoxicity: age, course number, ECOG performance status, AUC, concomitant alkalinization with sodium bicarbonate, C_{cr}, concomitant narcotic analgesics, peak plasma HMBA concentration, mean plasma HMBA level, and duration of infusion. For the purpose of this analysis, some of these factors were grouped into discontinuous variables. Metabolic acidosis was considered to be either present or absent on the basis of an AG value of >16. The probability that a toxicity would occur ($y = 1$) given the predictor values (x_1, $x_2 \ldots x_P$) was expressed as:

$$P = \text{prob}\,(y = 1 \mid \times) = \frac{e^k}{1 + e^k},$$

where $k = \beta_1, \beta_2, \beta_3 \ldots \beta_P$ and $\beta_1 \ldots \beta_P$ represent weights or regression coefficients for the predictors [16].

Table 3. Patients' characteristics

	Number of patients
Total number of patients:	24
Men	14
Women	10
Median age (range)	60 (40–75) years
Median performance status:	
0	8
1	15
2	1
Tumor type:	
Colorectal carcinoma	9
Squamous carcinoma, head and neck	5
Non-small-cell lung carcinoma	3
Other	7
Previous therapy:	24
Chemotherapy	8
Radiation	6
Both	10

Results

Patient population

The characteristics of the patients entered in the trials described in Table 1 are presented in Table 3. There were no significant differences in these characteristics between the two trials. The characteristics remained similar to those of patients entered in previous phase I trials of HMBA (trials 1 and 2, Table 2).

HMBA treatment in the presence and absence of alkalinization

In all, 14 patients received 26 courses of HMBA in the presence or absence of alkalinization (Table 1). All patients were evaluable for toxicity and response. The median dose of HMBA for patients with a target c_{ss} of 1.5–2.0 mM was 28.9 (range, 20.1–36.0) g m^{-2} day^{-1}. The median dose of HMBA for patients with a target c_{ss} of 2.2–2.7 mM was 36.1 (range, 28.2–43.5) g m^{-2} day^{-1}.

Control of HMBA pharmacodynamics

Ten patients were entered in the study to control prospectively the degree of thrombocytopenia (duration of infusion) associated with HMBA. Five patients received more than one course of HMBA. The target duration of infusion ranged from 5 to 10 days; due to toxicity, the actual duration of infusion ranged from 3 to 10 days. Because of approximations of infusion duration (24-h increments only), the actual target PLT nadirs ranged from 79,000 to 140,000/μl. Five of ten patients received more than one course of HMBA, and these eight courses in five patients were used to evaluate the adaptive control method. The remaining five patients had progressive disease and did not receive further HMBA.

Hematological toxicity

Thrombocytopenia, leukopenia, and anemia were seen but were mild. Median nadir values for PLT, WBC, and Hct in patients who received 5-day CIs of HMBA were 136 (range, 20–466) $\times$ 10^3/μl, 4.5 (range, 1.7–10.4) $\times$ 10^3/μl, and 31.4% (range, 22.8%–43.6%), respectively. In the adjusted infusion-duration trial, median nadir values for all ten patients for PLT, WBC, and Hct were 123 (range, 46–311) $\times$ 10^3/μl, 4.9 (range, 2.0–7.3) $\times$ 10^3/μl, and 29% (23%–37%), respectively. The median number of days to nadir for PLT, WBC, and Hct were 11 (range, 9–18), 17 (range, 13–29), and 15.5 (range, 9–36), respec-

Table 4. Characteristics of patients experiencing neurotoxicity in the c_{ss}-escalation trial of HMBA in the presence or absence of alkalinization

Target HMBA c_{ss} (mM)	Number of patients/courses	Mean plasma HMBA level (mM)	Mean nadir HCO_3^- (mEq/l)[a]	Mean anion gap[a]	Narcotics grade[b]	Alkalinization
1. 1.5–2.0	4/5	1.73±0.24	19.8±2.6	14.6±1.3	2 (2)	No
2. 2.2–2.7	2/2	2.25, 2.04	21, 24	14, 16	2, 2	Yes
3. 2.2–2.7	1/1	2.84	15	19	1	No

[a] Mean values ± SD are shown for groups numbering >2 patients; otherwise, individual values are given
[b] Median values are shown, with ranges appearing in parentheses

Table 5. Characteristics of patients experiencing no neurotoxicity in the c_{ss}-escalation trial of HMBA in the presence or absence of alkalinization

Target HMBA c_{ss} (mM)	Number of patients/courses	Mean plasma HMBA level (mM)	Mean nadir HCO_3^- (mEq/l)	Mean anion gap[a]	Narcotics grade[b]	Alkalinization
4. 1.5–2.0	3/8	1.64±0.26	23.2±1.7	14.6±2.3	1 (1)	Yes
5. 1.5–2.0	2/7	1.56±0.11	20.7±2.1	14.9±2.0	0 (0–1)	No
6. 2.2–2.7	1/2	2.14, 2.58	22, 23	13, 16	1	Yes

[a] Mean values ± SD are shown for groups numbering >2 patients; otherwise, individual values are given
[b] Median values are shown, with ranges appearing in parentheses

tively, for the 5-day CI trial and 19 (range, 7–22), 16 (range, 2–21), and 16 (range, 7–25), respectively, for the trial using individualized infusion duration. Bicarbonate had no apparent effect on these toxicities, which were similar to those reported previously [7, 11, 27, 28, 36].

Nonhematological toxicity

Metabolic acidosis. In the 5-day CI trial of HMBA in the presence and absence of alkalinization, symptomatic acidosis (grade 1: maximal AG value, 19) was observed in only one patient who received HMBA at a target c_{ss} of 2.2–2.7 mM in the absence of concurrent alkalinization. This occurred at 2 days after the completion of the infusion and at a time at which the patient had developed renal failure secondary to sepsis. The peak plasma HMBA concentration in this patient was 3.1 mM. However, three courses in three patients were associated with asymptomatic AG values of >16 during or shortly after the HMBA infusion. Two of these patients received HMBA at a target c_{ss} of 1.5–2.0 mM; one also received concurrent bicarbonate (maximal AG values, 19 and 18, respectively). One patient received HMBA at a target c_{ss} of 2.2–2.7 mM in the absence of bicarbonate (maximal AG value, 19 at 3 days postinfusion). The mean plasma HMBA concentrations, nadir plasma bicarbonate concentrations, and anion gaps in patients who developed neurotoxicity and those who experienced no neurotoxicity are shown in Tables 4 and 5, respectively.

No significant difference (one-way ANOVA, $P > 0.05$) was found in the mean plasma HMBA concentration or the anion gap between neurotoxic patients and those who did not experience neurotoxicity. However, when groups 1, 4, and 5 were compared using one-way ANOVA, the nadir plasma bicarbonate concentrations were significantly higher ($P = 0.018$) in patients targeted to an HMBA c_{ss} of 1.5–2.0 mM in the presence of alkalinization (group 4). There were too few patients at the higher c_{ss} to enable meaningful comparisons. In the variable duration trial, one patient with bronchioloalveolar carcinoma developed an AG value of >16 during the HMBA infusion and died on day 2 of the infusion; an autopsy revealed progressive disease.

Neurotoxicity. In the 5-day CI trial of HMBA in the presence or absence of concurrent alkalinization, seven patients experienced neurotoxicity (Table 4). In three patients treated at a target c_{ss} of 1.5–2.0 mM in the absence of bicarbonate, this manifested as confusion, lethargy, and somnolence occurring at 1–3 days after the HMBA infusion had ended. Four patients experienced hallucinations. One patient who received HMBA at a c_{ss} of 1.5–2.0 mM in the absence of bicarbonate experienced hallucinations during the last day of the infusion and for several days afterward. Another patient treated at a target c_{ss} of 2.2–2.7 mM in the presence of alkalinization experienced hallucinations for several days after the end of the HMBA infusion. A third patient experienced hallucinations at 71 h into an HMBA infusion targeted for a c_{ss} of 2.2–2.7 mM in the presence of alkalinization; however, at the time of the hallucinations, the plasma HMBA concentration was 2.13 mM. This patient had the HMBA infusion stopped due to the neurotoxicity. The fourth patient, receiving HMBA at a target c_{ss} of 2.2–2.7 mM in the absence of bicarbonate, developed hallucinations at 1 day after the HMBA infusion had ended. All neurotoxicity had cleared by 1 week after the end of the HMBA infusion. None of the three patients treated at a target c_{ss} of 1.5–2.0 mM in the presence of bicarbonate experienced neurotoxicity.

In the variable infusion-duration trial, two patients had their HMBA infusions interrupted due to toxicity. One

patient with squamous-cell carcinoma of the head and neck developed aspiration pneumonia, hypoxia, confusion, and acidosis on day 4 of course 3; at the time of cessation of the infusion, the plasma HMBA concentration was 3.0 mM. One patient developed opisthotonus on day 3 of course 2, which may have been related to the concomitant administration of metoclopramide for nausea; at the time of cessation of the infusion, the plasma HMBA concentration was 1.7 mM and the blood pH was 7.2.

Overall, 7 of 14 patients receiving HMBA in the presence or absence of alkalinization experienced acute or delayed (1–3 days postinfusion) neurotoxicity (hallucinations, somnolence, confusion, agitation), including 3 of 4 patients who received HMBA at a target c_{ss} of 2.2–2.7 mM and another 4 patients who received HMBA at a target c_{ss} of 1.5–2.0 mM in the absence of bicarbonate. One patient in each of these groups developed both neurotoxicity and an AG value of >16. It was striking that 5/7 patients who were neurotoxic (Table 4) were also receiving grade 2 narcotics (defined as shown in Table 7) as compared with 0/7 patients who experienced no neurotoxicity. Unfortunately, the performance of daily mini-mental status examinations was not helpful in predicting neurotoxicity, as the findings remained normal until the sudden development of obvious neurotoxicity; once neurotoxicity had developed, patients were unable to undergo the examination.

Other toxicities. Other toxicities were mild (grade 1 or 2) and reversible. Grade 1–2 nausea and vomiting were experienced by nearly all patients. Hypertension was observed during three courses in two patients. Headache was noted during ten courses in seven patients, and diaphoresis was noted during five courses in three patients. Mild to moderate stomatitis occurred during seven courses in seven patients. One patient receiving HMBA at a target c_{ss} of 2.2–2.7 mM experienced severe herpes simplex oral infection. These toxicities were not dose-related and were seen regardless of sodium bicarbonate administration. One patient may have had an unwitnessed seizure at the time of a duodenal ulcer bleed (without thrombocytopenia), but neither toxicity was thought to be definitely secondary to HMBA and the occurrence of a seizure could not be documented with certainty.

Measurement of HMBA metabolites

HMBA and it metabolites 6AcHA, NADAH, DAH, and 6AmHA were measured to discern whether alkalinization would affect the metabolism of HMBA and to determine whether the metabolic profiles of patients developing neurotoxicity would differ from those of patients experiencing no neurotoxicity. No difference in plasma metabolite/HMBA ratios was found between patients who received HMBA in the presence of concurrent bicarbonate and those who received HMBA alone (one-way ANOVA). Likewise, as shown in Table 6, there was no difference between the plasma metabolite/HMBA ratios of patients who experienced neurotoxicity and those who did not (one-way ANOVA). Moreover, no difference in the mean or peak concentrations of NADAH or of 6AcHA was found between patients who experienced neurotoxicity and those who did not. Although the plasma concentration of 6AcHA as well as the ratio of plasma concentrations of 6AcHA to HMBA were lower in patients who experienced neurotoxicity, these differences did not reach statistical significance at the 5% level (one-way ANOVA).

Table 6. Ratio of the plasma concentration of HMBA metabolite: HMBA in patients developing neurotoxicity and those experiencing no neurotoxicity

Metabolite	Plasma metabolite: HMBA (neurotoxicity)	Plasma metabolite: HMBA (no neurotoxicity)
6-Acetamidohexanoic acid	0.38 ± 0.29 mM	0.55 ± 0.26 mM
N-acetyl-1,6-diaminohexane	0.33 ± 0.18 mM	0.35 ± 0.11 mM
6-Aminohexanoic acid	0.08 ± 0.04 mM	0.10 ± 0.05 mM
1,6-Diaminohexane	0.02 ± 0.01 mM	0.02 ± 0.04 mM

Bias and precision of the adaptive-control algorithms

Control of C_{ss}. The precision of accuracy of the algorithm for adaptively controlling the HMBA c_{ss} was similar to that previously reported [7]. The algorithm performed equally well in controlling the HMBA c_{ss} in patients who received bicarbonate and those who did not. The mean HMBA c_{ss} after initial adjustment of the infusion rate for the 5-day infusion lay within the target range for 18 of 26 courses. At a target c_{ss} of 1.5–2.0 mM, the mean HMBA c_{ss} was <1.5 mM in 6 of 21 courses, differing by a mean of 0.20 (range, 0.05–0.48) mM. At a target c_{ss} of 2.2–2.7 mM, the mean HMBA c_{ss} was <2.2 mM in 2 of 5 courses, differing by 0.06 and 0.16 mM. Control of the c_{ss} for the variable-duration HMBA infusion was of similar accuracy and precision.

Control of HMBA pharmacodynamics. For five patients who received more than one course of HMBA, the percentage change in PLT for eight courses subsequent to a first course was predicted on the basis of the actual AUC achieved, with the ME being –8.9% ± 17.7% and the MAE being 16.0% ± 11.7% (*predicted*-observed). The ME of *target*-observed nadir PLT was $-12.6 \pm 71.5 \times 10^3/\mu l$, with the MAE being $56.4 \pm 45.6 \times 10^3/\mu l$. Table 7 shows the target versus achieved PLT for eight courses in five patients along with the achieved and the desired AUC. Some error in the achieved AUC would be expected, as dose adjustments were made only daily and no attempt was made to correct for previous under- or overtarget AUC values from the previous day. In addition, two patients had their HMBA infusions stopped early due to toxicity. Predictions of the percentage change in PLT using the Bayesian parameter estimator (i.e., obtaining a unique AUC_{50} and H value for each patient) were less biased but were of the same precision as those made using the mean population values for AUC_{50} (1200 mg l^{-1} h) and the Hill constant (1.6; data not shown).

Table 7. Adaptive feedback control of PLT nadir

Patient number	Course number	Achieved AUC (mg l^{-1} day)	Target AUC (mg l^{-1} day)	Actual duration (days)	Achieved % Δ PLT	Target % Δ PLT[a]	Achieved PLT nadir ($\times 10^3$)	Target PLT nadir[a] ($\times 10^3$)
1	2	3192	3500	10	90.0	77.6	46	113
	3	2579	2800	8	83	80.2	71	83
2	2	1778	2100	6	64	73.6	115	84
	3	1537	1625	5	69.7	67.5	74	79
3	2	1270	1500	5	69.4	64.2	76	89
4	2	1578	1950	6	32.4	73.4	213	84
	3[b]	1438	2600	4	84.4	64.3	61	140
5	2[b]	812	2800	3	36.8	68.2	234	118

[a] Target PLT nadir or change in PLT, expected if the target AUC is achieved
[b] Courses terminated early due to toxicity

Factors associated with the acute dose-limiting toxicity of HMBA

Because the individual phase I trials of HMBA described above did not contain enough patients to enable determinations of the association of acidemia or concomitant narcotic use with the acute toxicities of acidosis and neurotoxicity, we retrospectively reviewed 98 courses of HMBA given as 5- to 10-day CIs to 56 patients in 4 UMCC phase I studies (Table 2). In addition, we looked for the association of other factors with the likelihood of the development of HMBA toxicity.

The distribution of the various factors analyzed is shown in Table 8. An AUC value of >7.50 mM × day, the use of *any* concomitant narcotic analgesics, and a mean plasma HMBA concentration of >1.5 mM or a peak plasma HMBA level of ≥1.75 mM correlated significantly with the presence of grade 3 neurotoxicity (P <0.001), whereas concomitant alkalinization with sodium bicarbonate and a mean plasma HMBA concentration of <300 mg/l were associated with a lack of neurotoxicity (P <0.001). An AUC value of >7.5 mM × day, a mean or peak plasma HMBA level of >1.5 mM, and an age of >70 years correlated with the likelihood of an AG value of >16 (P <0.03). With the above factors being accounted for, neither the duration of HMBA infusion nor the C_{cr} value showed any correlation with these toxicities in this patient population.

Responses

No objective tumor regression was observed in this group of patients with advanced, previously treated malignancies. Three patients with metastatic colon carcinoma showed no disease progression for 4, 4, and 3 months, respectively.

Discussion

The mechanisms underlying the acute toxicities of HMBA are unknown. The number of patients who experienced acidemia during the trials of this drug in the presence or absence of bicarbonate administration were too small to enable definite conclusions to be drawn regarding the role of acidemia in the production of neurotoxicity. When neurotoxic patients and those who did not develop neurotoxicity at the target range of 1.5–2.0 mM HMBA were compared, the group who underwent alkalinization (no neurotoxicity) had significantly higher nadir serum bicarbonate concentrations than did the neurotoxic or nonneurotoxic patients who did not receive bicarbonate. This finding was not unexpected. No significant difference in the mean plasma HMBA concentration in patients targeted for an HMBA c_{ss} of 1.5–2.0 mM was found between those who did and those who did not experience neurotoxicity. We were unable to document any significant difference in HMBA metabolism between patients who did and those who did not receive supplemental bicarbonate. In the multifactorial analysis, which included 30 courses given with bicarbonate and 68 courses given without bicarbonate, alkalinization was significantly associated with a lack of neurotoxicity. It therefore remains possible that acidemia may play a role (probably minor) in the neurotoxicity seen during HMBA administration, although the mechanism remains mysterious.

Other trials of HMBA, including our own trials, have not used sodium bicarbonate and have achieved HMBA c_{ss} values of 1.0–2.0 mM without neurotoxicity for up to 10 days [28, 34, 36]. Because many patients received sodium bicarbonate as an infusion, it is possible that additional fluid alone may have had a salutory effect with respect to neurotoxicity. In addition, although anion gaps may be elevated, very few patients become symptomatic enough to prompt assessment of blood gases. The administration of sodium bicarbonate does not seem to enable significant escalation of the HMBA c_{ss} or sufficient amelioration of neurotoxicity to warrant its routine use.

Neurotoxicity, however, remains a dose-limiting toxicity, especially at a c_{ss} of >2 mM. In that NADAH, the first metabolite of HMBA, is metabolized by monoamine oxidase (MAO) [6], NADAH may be acting as a competitive MAO inhibitor. Excessive inhibition of MAO is known to produce neurotoxicity [19, 21]. Alternatively, HMBA

Table 8. Factors tested for their relationship to the acute toxicity of HMBA

Factor	Grade	Number of courses or patients
Age	1 (≤50 years)	15 courses
	2 (51–60 years)	17 courses
	3 (61–70 years)	18 courses
	4 (≥71 years)	6 courses
Number of courses	1	34 patients
	2	13 patients
	3	5 patients
	4	2 patients
	7	1 patient
	8	1 patient
ECOG performance status	0	9 patients
	1	33 patients
	2	14 patients
C_{cr}	1 (≤44 ml min^{-1} 1.73 m^{-2})	6 courses
	2 (45–64 ml min^{-1} 1.73 m^{-2})	24 courses
	3 (65–90 ml min^{-1} 1.73 m^{-2})	31 courses
	4 (≥91 ml min^{-1} 1.73 m^{-2})	33 courses
Duration	3 days	1 course
	4 days	11 courses
	5 days	90 courses
	6 days	11 courses
	8 days	1 course
	10 days	1 course
AUC[a]	0 (≤1500 mg l^{-1} day)	49 courses
	1 (1501–1750 mg l^{-1} day)	28 courses
	2 (1751–2000 mg l^{-1} day)	7 courses
	3 (2001–2250 mg l^{-1} day)	2 courses
	4 (≥2251 mg l^{-1} day)	7 courses
Peak and mean plasma HMBA concentration[a] (same grading)	0 (<200 mg/l)	13 courses
	1 (200–300 mg/l)	32 courses
	2 (301–350 mg/l)	25 courses
	3 (351–400 mg/l)	11 courses
	4 (401–450 mg/l)	1 course
	5 (≥451 mg/l)	6 courses
Narcotics	0 = None	40 courses
	1 = short-acting narcotic taken q 6 h or less	43 courses
	2 = Long-acting narcotic or 1-2 short-acting narcotic doses taken at least q 4 h	12 courses
	3 = Both	3 courses
Bicarbonate	Yes	30 courses
	No	68 courses

[a] To convert to mM, divide value by 200

and/or its metabolites could decrease the clearance of other neurotoxic substances, such as narcotics, by mechanisms other than MAO inhibition. Some narcotics have even been reported to interact with MAO inhibitors [2], and the neurotoxicity of HMBA in our trials was significantly associated with narcotic administration. Although morphine in "usual" doses has not been shown to produce adverse reactions when given with MAO inhibitors, it is not known whether the high and frequent doses of morphine commonly given to cancer patients might cause toxicity. It seems prudent at this point to limit HMBA administration to patients who do not require narcotic analgesics.

Because alkalinization alone does not enable the escalation of plasma HMBA c_{ss} values to >2 mM, other methods of increasing the differentiating activity of HMBA could be considered. In that NADAH is an active metabolite of HMBA [31], the effective differentiating activity (HMBA + NADAH) may be increased by the concomitant administration of an MAO inhibitor, which would prevent the metabolism of NADAH to the inactive metabolite 6AcHA [3, 6]. However, more rather than less neurotoxicity was produced in dogs when an MAO inhibitor (isocarboxazid) was given concurrently with HMBA [8]. Thus, NADAH itself may directly or indirectly induce neurotoxicity.

Because the in vitro activity of HMBA is dependent on the concentration and the duration of exposure, a reasonable way of increasing the clinical activity of HMBA would be to prolong the duration of infusion while controlling the plasma HMBA concentration within a nontoxic range. The dose-limiting toxicity would then be thrombocytopenia. Forrest et al. [7, 15] have developed pharmacokinetics and pharmacodynamics models from which MAP-Bayesian parameter estimators were developed. Using this model and the ADAPT [9] computer program, patients' pharmacokinetic and pharmacodynamic parameter estimates are refined on the basis of patient-specific observations. In eight courses (five patients) for which pharmacodynamic feedback was employed, the Bayesian estimator was not biased and could be used to tailor the duration of HMBA infusion for individual patients to an approximate PLT nadir. It is noteworthy (Table 6) that in patients who received three courses of HMBA, PLT nadirs were closer to target values in the third courses than in the second courses, indicating improved performance of the adaptive control algorithms (control of AUC and PLT) with increasing amounts of patient-specific data. Refinements in Bayesian "prior" parameter values may enable even more precise dosing.

The retrospective analysis of toxicity in phase I trials of HMBA implies that there is a limit to the AUC that can be tolerated before acute neurotoxicity or acidosis is produced. Our results suggest that plasma concentrations at or near effective in vitro HMBA concentrations (>1 mM) would not maintainable for longer than 10–15 days. Despite these limitations, there are applications that could be considered. The use of HMBA for bladder tumors or, possibly, premalignant bladder lesions are examples in which the indefinite maintenance of very low plasma concentrations would produce effective concentrations in the bladder, as HMBA and NADAH are concentrated in the urine [11]. Synergy of HMBA or other polar compounds with DNA-interactive agents has been demonstrated in some preclinical models [35, 37]. Combinations of differentiating agents have shown synergy or activity on distinct differentiation pathways in the HL-60 leukemia model [1, 33]. These preclinical data imply that significant clinical application of HMBA may require its use at a low c_{ss} for prolonged durations or in combination with cytotoxic or

other differentiating agents. For practical considerations, an oral formulation [34] would facilitate such studies.

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