Cancer Chemother Pharmacol (1991) 28: 33–38
034457049100052V

Cancer Chemotherapy and Pharmacology

The effect of the monoamine oxidase inhibitor isocarboxazid on the canine metabolism of the cell-differentiating agent hexamethylene bisacetamide*

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Summary. The acute toxicities of the cellular differentiating agent hexamethylene bisacetamide (HMBA) in humans and animals include CNS toxicity (agitation, somnolence, seizures, hallucinations) and an anion-gap metabolic acidosis. *N*-Acetyl-1,6-diaminohexane (NADAH), the first metabolite of HMBA, is as active as the parent compound in causing differentiation of leukemic cells in vitro, whereas 6-acetamidohexanoic acid (6AcHA), which is formed by the oxidation of NADAH in the presence of monoamine oxidase (MAO) and aldehyde dehydrogenase, is inactive. To test whether the inhibition of MAO blocks the production of an inactive and possibly toxic HMBA metabolite (6AcHA) or increases the amount of active compounds (HMBA + NADAH) in vivo, we investigated the effect of the MAO inhibitor isocarboxazid on the metabolism and toxicity of HMBA in beagle dogs. Two groups of dogs, composed of one male and one female dog per group, were used in the study. One group received isocarboxazid (3.3 mg/kg p.o. q8h $\times 9$) beginning at 24 h before the initiation of a 48-h i.v. infusion of HMBA (40 mg kg^{-1} h^{-1}), whereas the other received placebo in an identical fashion prior to the start of an identical HMBA infusion. The mean plasma steady-state concentration (c_{ss}) of HMBA was 0.91 mM in dogs given HMBA and isocarboxazid as opposed to 0.78 mM in those given HMBA and placebo. As measured spectrophotometrically, plasma MAO activity was inhibited by $86\% \pm 3\%$ in dogs receiving isocarboxazid. Gas chromatography/mass spectrometry detected 6AcHA in the plasma of animals that were given placebo but not in the plasma of dogs that received isocarboxazid. Gas chromatographic analysis of urine samples revealed that the total amount of 6AcHA and of NADAH excreted in urine was 8 times less and 3 times greater, respectively, in isocarboxazid-treated dogs than in animals that received HMBA and placebo. One dog was excitable after the initial two doses of isocarboxazid and developed seizures at the end of the HMBA infusion. Another dog was agitated during treatment with HMBA and isocarboxazid. No CNS toxicity occurred in animals that were treated with HMBA and placebo. We conclude that isocarboxazid inhibits the production of 6AcHA in vivo, thus supporting the involvement of MAO in HMBA metabolism. Because the combination of HMBA and isocarboxazid produces CNS toxicity, 6AcHA is probably not the neurotoxic agent in dogs.

* Supported by ACS Career Development Award 89-127 (to B. A. C.) and grants N-01-CN-57734 and NO1-CM-17365 from the National Cancer institute, Department of Health and Human Services

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Abbreviations: HMBA, Hexamethylene bisacetamide; NADAH, *N*-acetyl-1,6-diaminohexane; 6AcHA, 6-acetamidohexanoic acid; MAO, monoamine oxidase; c_{ss}, plasma steady-state concentration; CNS, central nervous system; AUC, area under the plasma drug concentration vs time curve; DAH, 1,6-diaminohexane; 6AmHA, 6-aminohexanoic acid; PMBA, pentamethylene bisacetamide; WBC, total white blood cell count; Hb, hemoglobin; PLT, platelet count; PMN, polymorphonuclear cell count

Introduction

Hexamethylene bisacetamide (HMBA, NSC 95580) is a polar-planar differentiating agent (Fig. 1) that is undergoing clinical trials in patients with advanced cancer [2, 5, 8, 18, 20, 21, 25]. In vitro, HMBA causes differentiation in a number of human and animal solid-tumor and leukemia cell lines [3, 7, 14, 16, 18]. Data from in vivo studies and clinical trials have not been as promising as the in vitro data [2, 5, 8, 20, 21, 25], although some tumor responses have been reported [5, 25]. The reasons for the lack of clinical or in vivo activity may involve inadequate exposure to active differentiating agents. Differentiating activity in vitro is dependent on both the drug concentration and

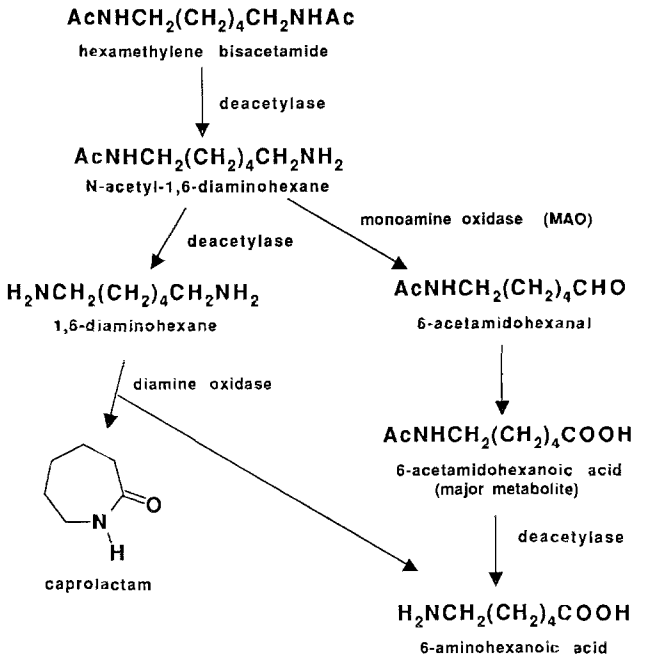

Fig. 1. Metabolic scheme of HMBA in humans

the duration of exposure [10]. Optimal differentiation in vitro usually requires exposure to 2–10 mM HMBA for at least 12 h [10, 17–19]. At low HMBA concentrations, a longer duration of exposure is required to produce the same degree of differentiation induced by a briefer exposure to higher HMBA concentrations. The maximum tolerable plasma steady-state concentration (c_{ss}) in human clinical trials using 5-day (120 h) continuous-infusion schedules is 1.5–2 mM and is limited by acute CNS toxicity (confusion, agitation, somnolence, or hallucinations) and/or anion-gap metabolic acidosis [5, 8, 20, 21, 25]. The duration of infusion at a C_{ss} of 1.5–2 mM is limited to approximately 10 days by thrombocytopenia, which is related to the HMBA AUC [5, 8, 20, 21, 25]. The mechanisms by which HMBA produces these toxicities are not understood.

Our laboratory has shown [1, 9] that HMBA is deacetylated to *N*-acetyl-1,6-diaminohexane (NADAH), which is further metabolized to 6-acetamidohexanoic acid (6AcHA) through sequential oxidations by monoamine oxidase (MAO) and aldehyde dehydrogenase [4] (Fig. 1). In vitro studies have demonstrated that NADAH is at least as active as HMBA in causing differentiation of HL-60 cells, whereas 6AcHA is inactive [22]. Furthermore, HL-60 cells exposed to HMBA and an MAO inhibitor showed greater differentiation than did cells exposed to HMBA alone [23]. Thus, it seemed likely that the inhibition of MAO could increase exposure to active differentiating agents (HMBA and NADAH) and could decrease the formation of the inactive and possibly toxic metabolite 6AcHA. We chose to investigate this hypothesis in dogs, a species in which HMBA pharmacokinetics and toxicity have been well characterized [13], and to use isocarboxazid, an irreversible MAO inhibitor on which considerable canine data have been gathered [11].

Materials and methods

Drug administration. Both isocarboxazid and HMBA were stored at room temperature and remained stable throughout the duration of the study. HMBA was supplied by the National Cancer Institute as a 10% (w/v) solution in sterile water for injection. Aliquots were diluted to 12.1 mg/ml in 0.9% sodium chloride for injection. Isocarboxazid (Marplan) was supplied as individual gelatin capsules for oral dosing (Roche, Nutley, N.J.). Isocarboxazid (3.3 mg/kg) or placebo (in empty gelatin capsules) was given p.o. every 8 h beginning at 24 h prior to the start of HMBA infusion (three doses) and during the infusion (six doses). Doses of all drugs were based on day-1 body weights. HMBA (40 mg kg^{-1} h^{-1}) was given to each dog as a continuous 48-h i.v. infusion through an indwelling catheter in a jugular vein beginning at 24 h after the first dose of isocarboxazid or placebo. Individual, sterile drug-reservoir bags (Travenol Laboratories, Deerfield, Ill.) containing 500 ml HMBA solution were attached to battery-operated Cormed M6–8 ambulatory infusion pumps (Cormed, Inc., Medina, N.Y.) which were set to deliver the drug solution at a rate of 3.3 ml kg^{-1} h^{-1} (40 mg kg^{-1} h^{-1}). The drug-reservoir bags and batteries were changed every 12 h.

Reagents. $(Na)_2HPO_4$ was obtained from MCB Manufacturing Chemists, Inc. (Cincinnati, Ohio); NaH_2PO_4 was supplied by Mallinkrodt, Inc. (St. Louis, Mo.); and trifluoroacetic anhydride, 2,2,2-trifluoroethanol, 6AcHA, 1,6-diaminohexane (DAH), 6-aminohexanoic acid (6AmHA), benzylamine, benzaldehyde, and cadaverine were obtained from Sigma Chemical Co. (St. Louis, Mo.). NADAH was synthesized from DAH and acetic anhydride as previously described [1].

Animals. Purebred beagle hounds (9.6–12.2 kg, two males and two females) aged 17–18 months were obtained from Springborn Institute for Bioresearch (Spencerville, Ohio) and were housed in individual stainless steel cages in an environmentally controlled room at Battelle Columbus Division (Columbus, Ohio) before and during the study. Food (approximately 400 g/dog daily) and water were available ad libitum. The animal protocol was approved by the Institutional Animal Care and Use Committee of the University of Maryland at Baltimore and at Battelle Columbus Division. Cage size and animal care conformed to the guidelines of the United States Department of Agriculture as specified in the Animal Welfare Act (Public Law 91-579) and Department of Health and Human Services (NIH) Publication 85-23 (revised 1985).

Experimental design. Animals were observed 24 h/day from the time of jugular catheter placement (day 1) through day 8. All clinical signs of toxicity were recorded. Body weights were measured on days –1 and 1–4. Blood samples were taken for determinations of MAO activity and plasma concentrations of HMBA and its metabolites both prior to the administration of isocarboxazid or placebo and at 0, 2, 4, 6, 8, 12, 24, 32, 48, 48.5, 49, 50, 52, 54, and 56 h after the start of the HMBA infusion. Blood samples were centrifuged at 2,000 *g* for 10 min, and plasma was removed and stored at –20° C until analysis. Urine was collected from all dogs every 6 h and stored at 4° C. Samples collected over each 24-h collection period were pooled, the combined samples were mixed, the volumes were recorded, and aliquots of each sample were frozen (–20° C) until assay for HMBA and its metabolites.

Gas chromatography. Concentrations of HMBA and its metabolites were determined in plasma and urine using the gas chromatographic method described elsewhere [1, 8, 9]. The internal standard for HMBA was pentamethylene bisacetamide (PMBA), and that for HMBA metabolites was cadaverine. Concentrations of HMBA and its metabolites in plasma were calculated from concomitantly performed standard curves. Determination of HMBA in urine required a 10-fold aqueous dilution of the urine prior to analysis.

Gas chromatography was performed on a Shimadzu mini-2 gas chromatograph (Shimadzu Corp., Columbia, Md.) fitted with a 1.8-m glass column (inside diameter, 3 mm) containing 3% SP2250 DB (for HMBA) or 3% SP2250 (for metabolites) on 100/120-mesh Supelcoport (Supelco, Bellefonte, Pa.). A nitrogen phosphorous-selective detector was used at a hydrogen flow rate of 3 ml/min, an air flow rate of 150 ml/min, a bead

voltage of 7.5 V, and a nitrogen carrier-gas flow rate of 40 ml/min. For analysis of HMBA, the oven was maintained at 240° C and the injector and detector, at 275° C. For analysis of HMBA metabolites, the oven was maintained at 230° C and the injector and detector, at 250° C. Peak areas were integrated using a SpectraPhysics 4290 Integrator (SpectraPhysics, Piscataway, N. J.). Under these operating conditions, the approximate retention times of HMBA and metabolites were: HMBA, 4 min; DAH, 6.9 min; 6AcHA, 8 min; 6AmHA, 2.8 min; and NADAH, 19.2 min. There were no endogenous interfering peaks.

Gas chromatography mass spectrometry. Trifluoroacetylation of amino and alcohol functional groups and trifluoroethyl esterification of carboxylic acid groups of HMBA metabolites in plasma and urine samples were accomplished as described elsewhere [1]. Gas chromatography was carried out on a Hewlett-Packard 5890 gas chromatograph (Hewlett Packard, Palo Alto, Calif.) equipped with a 15-m (inside diameter, 0.25 mm) Durabond 1701 capillary column (J & W Scientific Inc., Rancho Cordova, Calif.) using helium as the carrier gas. After a 1-min solvent delay, the column was heated from a starting temperature of 120° C to a temperature of 260° C at a rate of 45° C/min. The column was directly interfaced with a Hewlett-Packard 5970 mass-selective detector. Electron ionization spectra were obtained at an ionization voltage of 70 eV. Data acquisition was under the control of a Hewlett-Packard 9133 data system operating at a scan rate of 500 u/s over a mass range of 60–500. Confirmation of metabolite structure was accomplished by comparison of spectral and relative retention times for authentic standards [1, 9]. Parallel control experiments excluded the possibility of artifactual results attributable to endogenous materials or to sample preparation and derivatization procedures.

Plasma MAO activity: Plasma amine oxidase activity was determined using modification of the method of McEwen and Cohen [12], which quantifies such activity by spectrophotometrically measuring the oxidation of benzylamine to benzaldehyde. Briefly, 0.1 ml plasma was mixed with 1.25 ml 0.2 M phosphate buffer (pH 7.2) and 0.15 ml 0.008 M benzylamine in 0.2 M phosphate buffer (pH 7.2). The solution was incubated for 2 h in a shaker bath at 37° C, and the reaction was stopped by the addition of 0.15 ml 60% (v/v) perchloric acid. Following the addition of 1.5 ml cyclohexane, the mixture was allowed to stand at room temperature for 15 min. Denatured protein was sedimented by centrifugation at 2,000 *g* for 10 min, and the absorbance of the cyclohexane extract was measured at 242 nm. The absorbance of a control, to which benzylamine had been added immediately before perchloric acid and cyclohexane, was subtracted from that of the test sample to correct for background absorbance. MAO activity was calculated by reference to a concomitantly performed standard curve for benzaldehyde.

Results

Toxicity

Emesis occurred at approximately 1–1.5 h after isocarboxazid doses 2, 3, 4, 7, and 9 in the male dog that was given HMBA and isocarboxazid and at approximately 1 h after isocarboxazid dose 3 in the female dog that received HMBA and isocarboxazid. The male was excitable and had soft stool after the second dose of isocarboxazid (prior to the HMBA infusion). Both of the dogs receiving HMBA and isocarboxazid were restless after 32.5 and 43 h of HMBA infusion, respectively. The male developed convulsions at 47 h after the start of the infusion and exhibited hyperactivity, confusion, disorientation, difficulty in standing, salivation, and repeated convulsions. For humane reasons, this animal was killed at 24 h after the completion of the HMBA infusion. The female dog was restless and barked constantly during the final 5–6 h of HMBA infusion and for at least 48 h thereafter. This animal appeared normal at 72 h after the completion of the HMBA infusion. No severe toxicity was noted in either dog that received HMBA alone.

Table 1. Hematology and blood pH values at the end of a 48-h continuous i. v. infusion of HMBA (40 mg/kg/h)

Parameter	HMBA alone		HMBA + isocarboxazid	
	Male	Female	Male	Female
WBC ($\times 10^3$/mm^3)	7.5	14.7	17.9	21.4
Hb (g/dl)	14.3	12.5	14.3	15
PLT ($\times 10^3$/mm^3)	287	244	273	333
Band ($\times 10^3$/mm^3)	0	0	1.61	0.43
PMN ($\times 10^3$/mm^3)	4.3	10.3	14.3	16.9
Lymphocytes ($\times 10^3$/mm^3)	2.8	4.3	1.8	3.6
pH	7.39	7.39	7.36	7.25

Body weights

Body weights decreased by 0.4 and 0.6 kg, respectively, in the dogs receiving HMBA and isocarboxazid, whereas a decrease of 0.6 kg and an increase of 0.4 kg were observed in the dogs receiving HMBA alone. Food consumption of all dogs decreased during the study period.

Pathology

Blood pH and hematology values were determined for all dogs (Table 1). WBC values were higher in the two HMBA/isocarboxazid-treated animals, whereas blood pH values were slightly lower in these animals. At necropsy, gross and microscopic examination of tissues taken from the male dog that had experienced convulsions revealed no abnormal findings other than a small depressed area in one lobe of this animal's lungs.

Pharmacokinetics of HMBA and its metabolites

The pharmacokinetic parameters for HMBA given to dogs as a 48-h continuous i.v. infusion with or without isocarboxazid were determined (Table 2). Steady-state concentrations (c_{ss}) of HMBA in plasma had been achieved in all dogs by 8–12 h after the start of the infusion. The mean c_{ss} for all animals was 0.84 ± 0.08 mM (range, 0.72–1 mM) and was not statistically different for dogs that were treated with HMBA alone as compared with animals that received HMBA and isocarboxazid. Plasma HMBA concentrations were fit to a one-compartment model using the ADAPT [6] computer program and nonlinear least-squares regression. The elimination half-life ($t_{1/2}$) ranged from 1.57 to 2.51 h and total body clearance ranged from 0.19 to 0.28 l kg^{-1} h^{-1}. Neither parameter was significantly different between the groups (repeated-measures ANOVA). The AUC for HMBA ranged from 35.7 to 53 mM × h. In all, 14%–22%

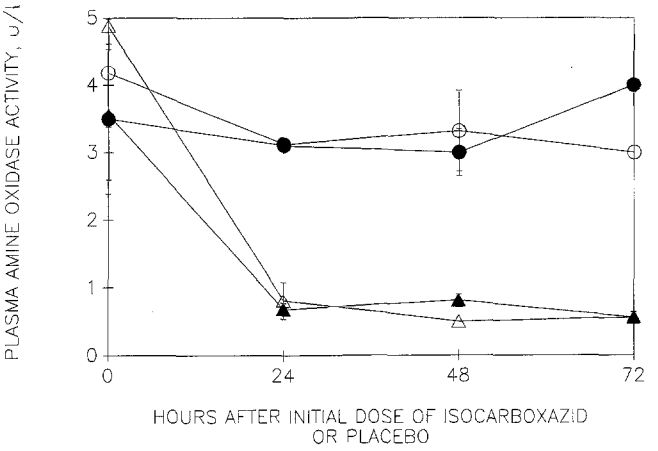

Fig. 2. Comparison of plasma amine oxidase activity in male (○) and female (●) dogs that received HMBA alone with that in male (△) and female (▲) dogs that received HMBA + isocarboxazid. HMBA (40 mg kg^{-1} h^{-1}) was given continuously between 24 and 72 h following the initial dose of isocarboxazid or placebo. Error bars represent the standard deviation of duplicate determinations

Table 2. Pharmacokinetic parameters of HMBA given as a continuous 48-h infusion to dogs with and without concurrent isocarboxazid

Parameter	HMBA alone		HMBA + isocarboxazid	
	Male	Female	Male	Female
c_{ss} (mM)	0.83 ± 0.09	0.72 ± 0.03	0.81 ± 0.06	1 ± 0.13
c_{TB} (l kg^{-1} h^{-1})	0.22	0.28	0.25	0.19
$t_{1/2}\beta$ (h)	2.16	1.74	1.57	2.51
AUC (mM × h)	43.84	35.69	39.34	53.04
Percentage of delivered HMBA excreted in urine in 48 h	20.6	21.9	16.1	14.5

Table 3. Plasma concentrations of HMBA and its metabolites in dogs receiving 48-h infusions of HMBA with or without isocarboxazid

		HMBA alone		HMBA + isocarboxazid	
		Male	Female	Male	Female
32 h	NADAH	40	10	60	20
	DAH	ND	ND	90	10
	6AcHA	20	10	ND	ND
	6AmHA	ND	ND	ND	ND
	HMBA	820	740	930	980
48.5 h	NADAH	20	10	50	40
	DAH	ND	10	10	10
	6AcHA	30	10	ND	ND
	6AmHA	ND	ND	ND	ND
	HMBA	910	710	620	980

All data are expressed as μM values; ND, none detected

Table 4. Urinary excretion of HMBA and its metabolites

		HMBA alone (mmol)		HMBA + isocarboxazid (mmol)	
		Male	Female	Male	Female
24 h	NADAH	0.14	0.15	0.67	0.16
	DAH	ND	0.01	0.07	0.03
	6AcHA	0.26	0.38	0.03	0.02
	6AmHA	0.2	0.34	0.34	0.17
	HMBA	7.69	10.995	8.96	6.99
48 h	NADAH	0.26	0.18	0.92	0.45
	DAH	0.02	0.01	0.08	0.06
	6AcHA	0.78	0.49	0.12	0.11
	6AmHA	0.67	0.36	0.4	0.21
	HMBA	13.94	9.91	9.34	4.95

ND, None detected

of the delivered dose of HMBA was excreted as the parent compound in urine within 48 h. These observations are similar to previous results obtained after the administration of HMBA to beagle dogs [13].

Effect of isocarboxazid on HMBA metabolism

Plasma MAO inhibition. Plasma amine oxidase activity in dogs was determined at the start of the study, prior to the administration of HMBA, and during the infusion. This activity was reduced by 86% ± 3% in the isocarboxazid-treated animals (Fig. 2).

Urine and plasma metabolites. Analysis of plasma samples by both gas chromatography and gas chromatography/mass spectrometry indicated that the metabolism of HMBA was altered in dogs that received isocarboxazid and HMBA concurrently (Table 3). Analysis of urine samples obtained at 24 and 48 h after the start of the HMBA infusion revealed a marked difference between the two groups of dogs in the relative amounts of metabolites excreted (Table 4).

The concentrations of 6AcHA in the plasma of dogs that were treated with HMBA alone were measurable at 32 and 48.5 h, whereas those in the plasma of animals that received HMBA and isocarboxazid were below the limit of detection (Table 3). Gas chromatography/mass spectrometry of plasma samples taken at 24 h after the start of the HMBA infusion documented the presence of 6AcHA in the plasma of dogs receiving HMBA alone, whereas this compound was not detected in the plasma of animals receiving both HMBA and isocarboxazid (data not shown). Plasma concentrations of NADAH were uniformly low and were not different in the two groups of dogs.

An alteration in HMBA metabolism was clearly demonstrated by the amounts of different metabolites excreted in the urine of dogs that were given HMBA with isocarboxazid and those that received HMBA alone (Table 4). Dogs receiving HMBA and isocarboxazid excreted less 6AcHA than did animals receiving HMBA alone; this finding was consistent for both 24-h and 48-h samples. The percentage of total drug infused over 48 h that was excreted as 6AcHA amounted to 0.79% and 0.92% in dogs receiving HMBA alone vs 0.13% and 0.16% in animals receiving HMBA and isocarboxazid. In contrast to the

findings in plasma, there was a marked increase in the urinary excretion of NADAH and DAH in dogs that were treated with the MAO inhibitor. Total amounts of NADAH detected in the urine at 48 h were 3 times greater for animals receiving isocarboxazid than for dogs receiving HMBA alone. The percentage of total drug infused over 48 h that was excreted as NADAH was 0.37% and 0.35% in dogs receiving HMBA alone vs 1.38% and 0.74% in those receiving HMBA and isocarboxazid. The amounts of urinary 6AmHA in the two groups of dogs were not significantly different.

Discussion

The results of the present study are consistent with the hypothesis that MAO is involved in the metabolism of HMBA in vivo as well as in vitro. Isocarboxazid treatment was effective in producing sufficient inhibition ($\geq 80\%$) of plasma MAO to test the effect of MAO inhibition on the metabolism and toxicity of HMBA. Inhibition of MAO resulted in lower urinary concentrations of 6AcHA and higher urinary concentrations of NADAH. Dogs receiving both isocarboxazid and HMBA produced more DAH than did animals that received HMBA alone, which implies increased HMBA metabolism via the deacetylase pathway involved in the conversion of NADAH to DAH (Fig. 1).

The pharmacokinetic parameters obtained in our study are consistent with those obtained in previous studies using similar doses of HMBA in beagle dogs [13]. In preclinical studies, these animals tolerated 120-h infusions of HMBA at 40 mg kg^{-1} h^{-1} with little evidence of toxicity, whereas convulsions were seen in dogs that received 120-h infusions at a rate of ≥ 60 mg kg^{-1} h^{-1}. The plasma half-life of HMBA in dogs was 1.8 h and 40% of the delivered dose was recovered as unchanged drug in the urine [13]. The plasma c_{ss} of HMBA in dogs receiving HMBA at 40 mg kg^{-1} h^{-1} over 120 h was between 0.8 and 1 mM.

Although the desired modulation of the metabolism of HMBA was achieved, it resulted in more, rather than less, neurotoxicity in both of the dogs that were given HMBA and isocarboxazid as compared with those that received HMBA alone. Although some neurotoxicity was seen in one dog (restlessness) prior to the HMBA infusion, published data indicate that similar neurotoxicity is seen only in dogs that receive higher (10–20 mg/kg) isocarboxazid doses for longer periods (6–13 weeks) than those used in the present study [11]. Therefore, isocarboxazid is unlikely to have been the sole cause of the observed neurotoxicity. Although the number of dogs investigated was small, the current study implies that 6AcHA was not responsible for the neurotoxicity that was associated with HMBA administration. Although plasma concentrations of isocarboxazid were not measured, it is possible that HMBA affected the metabolism or excretion of this drug in such a way that toxicity was augmented. Alternatively, NADAH may have been responsible for the neurotoxicity seen, either by competitive inhibition of MAO itself or by a completely different and as yet undefined mechanism. The male dog that exhibited seizures while receiving HMBA and isocarboxazid showed a higher plasma concentration of NADAH at 32 h after the start of the infusion and excreted more urinary NADAH than did the other animals.

Most clinically useful MAO inhibitors, such as isocarboxazid, are not selective for either MAO-A or MAO-B and most therapeutic doses achieve >80% inhibition of MAO-B [15], which is irreversible. Thus, measurement of MAO-B inhibition [24] as carried out in the present study gives an indication of overall MAO inhibition.

It is interesting to note that venous blood pH was lower at the end of the HMBA infusion in one dog that received isocarboxazid as compared with the corresponding value for animals that received HMBA and placebo (Table 1). This observation implies that 6AcHA may not be the sole cause of the acidosis seen during HMBA administration and that acidosis cannot be ruled out as a contributor to the neurotoxicity of HMBA in dogs. The finding that WBC and polymorphonuclear cell (PMN) counts were higher in isocarboxazid-treated dogs may be related to demargination caused by an excess of unmetabolized catecholamines due to MAO inhibition, although plasma catecholamines were not measured in this study.

The results obtained in the present study indicate that although MAO inhibition augments the amount of differentiating agents (HMBA + NADAH) in plasma, concomitant administration of an MAO inhibitor may also exacerbate the neurotoxicity associated with HMBA treatment. Alternatively, because increased amounts of the active differentiating agent NADAH were observed when MAO inhibitors were given, perhaps the HMBA dose could be significantly decreased. This would presumably result in less neurotoxicity than was seen during the present study. Because HMBA is effective in vitro at concentrations that are achievable in the plasma of patients who receive this drug and because some tumor responses have been documented, continued efforts aimed at blocking HMBA toxicity and maintaining optimal exposure to this agent are worthwhile.

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