In Vivo Metabolism and Whole-Blood Clearance of N-Nitrosomethylbenzylamine in the Rat

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ABSTRACT

The pharmacokinetics and metabolism of N-nitrosomethylbenzylamine, N-nitrosomethyl[benzyl-7-14C]benzylamine, and N-nitrosomethyl[benzyl-7-14C]amine were studied in male Sprague-Dawley rats, and a major urinary metabolite was identified. N-Nitrosomethylbenzylamine (4.7 mg/kg body weight i.p.) was distributed throughout extracellular water and cleared from the whole blood by metabolism with a half-life of 66 min. Less than 1% of the administered dose of N-nitrosomethylbenzylamine (4.7 mg/kg i.p. or 3.3 mg/kg intragastric intubation) was excreted and expired as the parent compound. In the 24-hr period following injection of N-nitrosomethyl[benzyl-14C]benzylamine (3.4 mg, 1 mCi/kg i.p.), 46% of the radioactivity administered was excreted with a half-life of 2.1 hr. In contrast, 81% of the radioactivity from a dose of N-nitrosomethyl[benzyl-7-14C]amine (2.4 mg, 1 mCi/kg i.p.) was excreted in the urine with a half-life of 4.2 hr. Hippuric acid accounted for 80% of the radioactivity recovered in the urine.

INTRODUCTION

MBN was among the 65 N-nitroso compounds synthesized and tested by Druckrey et al. (4). MBN, an unsymmetrically substituted nitrosamine, selectively induced esophageal tumors in rats given the compound daily in drinking water. In addition, of the compounds included in this study, MBN was one of the most toxic and carcinogenic (4).

Of interest because of its structure, toxicity, and esophagus-specific carcinogenicity, MBN was extensively investigated in our laboratory. As part of this undertaking, we conducted classical pharmacokinetic and metabolic studies. Equations for the clearance of MBN from whole blood were derived, and the apparent volume of distribution of MBN was calculated. Metabolic studies were conducted with MBN to determine the excretion and expiration of unmetabolized parent compound and with [methyl-14C]MBN and [benzyl-7-14C]MBN to determine the fate of those groups of the molecule. A major urinary metabolite was identified by thin-layer chromatography, permitting elucidation of biochemical pathways likely to be involved in MBN metabolism.

MATERIALS AND METHODS

Animals and Diet. Male Sprague-Dawley rats weighing 95 to 145 g (Charles River Breeding Laboratories, North Wilming-}

ton, Mass.) were permitted food (Charles River Rat/Mouse/ Hamster Formula, Country Foods, Syracuse, N. Y.), and tap water ad libitum.

Chemicals. MBN, synthesized using the procedure of Druckrey et al. (4), gave a single nitrosamine-positive peak when checked by the gas chromatography-thermal energy analyzer (detector) system, and purity was determined by nuclear magnetic resonance spectroscopy to be greater than 98%. [methyl-14C]MBN (specific activity, 4.6 mCi/mmol) and [benzyl-7-14]

C]MBN (specific activity, 6.73 mCi/mmol) were obtained from New England Nuclear, Boston, Mass. The radiochemical purity, determined by thin-layer chromatography on Silica Gel G using hexane:ether:methylene chloride (4:3:3, v/v/v), was greater than 98.5% for the methyl-labeled compound and greater than 98% for the benzyl-labeled compound. MBN was diluted with dimethyl sulfoxide (Burdick and Jackson Laboratories, Inc., Muskegon, Mich.) such that animals received dimethylsulfoxide 1 ml/kg body weight i.p. and 2 ml/kg body weight i.g.

Blood Sampling and Analysis. Rats were given MBN (4.7 or 0.9 mg/kg i.p. and 3.3 mg/kg i.g.). Blood samples (1.0 or 2.0 ml) were obtained by heart puncture 10, 30, 60, 90, 150, 210, and 240 min after dosing and were extracted with 1.0 or 4.0 ml hexane. Extracts were analyzed by gas chromatography performed on a column (0.125 inch x 10 ft) packed with 3% OV-17 on Chromosorb G and using the nitrosamine-specific thermal energy analyzer (Thermo-Electron Corp., Waltham, Mass.) as the detector. Peak areas obtained from the hexane extracts and a standard MBN solution were compared using an electronic computing integrator (Columbia Scientific Industries, Austin, Texas).

Metabolic Studies. Rats fitted with tail cuffs were given MBN (4.7 mg/kg i.p. or 3.3 mg/kg i.g.), [methyl-14C]MBN (3.4 mg; 1 mCi/kg i.p.), or [benzyl-7-14C]MBN (2.4 mg; 1 mCi/kg i.p.) and housed in glass metabolism cages. Following MBN administration, urine and feces were collected for 24 hr. One ml of urine was extracted with 1.0 ml of hexane. Feces (3.5 g) were combined with 7.0 ml of distilled water, homogenized with a Sorvall Omni-Mixer (Ivan Sorvall, Inc., Norwalk, Conn.), and extracted with 10 ml of hexane. Expired air was drawn through a liquid nitrogen trap for the time periods required to clear 90% of MBN from the blood. The defrosted trap was rinsed with distilled water, which was extracted with methylene chloride, and the methylene chloride phase was dried (MgSO4), filtered, and concentrated. All extracts were analyzed for unmetabolized MBN by the gas chromatography-thermal energy analyzer (detector) system.

Following administration of [14C]MBN, urine samples were collected periodically and counted (Beckman LS 8100) in
Biofluor (New England Nuclear, Boston, Mass.). Tail cups were removed 24 hr after dosing, and the feces were combusted in a Searle Analytic Model 6550 combustor. Room air was drawn through an Ascarite (Arthur H. Thomas Co., Philadelphia, Pa.)/Drierite (W. A. Hammond Drierite Compnay, Xenia, Ohio) column, into the metabolic cage, and through 2 CO2 traps connected in series (containing 75 and 40 ml, respectively, of approximately 8 M KOH). Aliquots of KOH were removed periodically and counted in modified Bray’s solution (2).

Urinary metabolites were identified by adding cold hippuric acid to urine collected 8.5 hr after administration of [benzy1-7-14C]MBN and recrystallizing 3 times to constant specific activity. Alternatively, urine excerted 3.5 hr after administration of [benzy1-7-14C]MBN was chromatographed on silica gel using hexane:ethanol:acetic acid (10:5:1, v/v/v). UV-absorbing markers (unlabeled hippuric and benzoic acid) were added to the plate before elution. Radioactivity was determined by cutting the plates into 0.5-cm sections which were counted in 10 ml Aquasol (New England Nuclear, Boston, Mass.).

RESULTS AND DISCUSSION

Pharmacokinetics. The pharmacokinetic parameters calculated for whole-blood clearance of MBN are summarized in Table 1. At all 3 doses, the disappearance of MBN followed first-order kinetics and all of the rate constants were of approximately the same order of magnitude. The rate constants obtained for clearance of the i.p. doses differed from the rate constant for clearance of the i.g. dose by roughly a factor of 2. In addition, the apparent volume of distribution of MBN was larger following i.g. than following i.p. administration. These 2 latter observations suggest that the gastrointestinal tract may have acted as a storage depot from which MBN absorption into and clearance from the blood were delayed.

The disappearance from the blood of MBN and N-nitrosodibutylamine injected i.p. is comparable and slower than the clearance of either DMN or DEN; the first-order rate constants for N-nitrosodibutylamine, DEN, and DMN have been reported as 0.010, 0.017, and 0.018 min⁻¹, respectively (21). Metabolism. At the dosages studied, less than 1% of the administered dose of MBN was recovered as the unmetabolized parent compound in the urine, feces, and expired air. Clearance of MBN from the whole blood could therefore be attributed to metabolism and, by inference, the first-order rate constant for metabolism of MBN by the rat can be estimated as 0.011 min⁻¹.

Radioactivity from [methyl-14C]MBN appeared primarily in the expired air (Table 2) with a half-life of 2.1 hr, whereas radiolabel from [benzy1-7-14C]MBN was excreted in the urine (Table 3) with a half-life of 4.2 hr. The initial rapid expiration of [methyl-14C]MBN-derived radioactivity was followed by a plateau, a pattern also observed in metabolic studies of 14C-labeled DMN, DEN, N-nitroso-n-butylmethyamine, and N-nitroso-t-butylmethyamine (8). Within 24 hr, 81% of the administered dose of [benzy1-7-14C]MBN had been excreted in the urine. When the urine was chromatographed, hippuric acid accounted for 80% of the total radiolabel, 20% remained at the origin, and none cochromatographed with benzoic acid. The metabolism of a number of nitrosamines, frequently

Table 1

<table>
<thead>
<tr>
<th>MBN (mg/kg)</th>
<th>Route</th>
<th>k0 (min⁻¹)</th>
<th>eV0 (% of body wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.7</td>
<td>i.p.</td>
<td>0.011 (n = 21, r = 0.98)</td>
<td>39</td>
</tr>
<tr>
<td>0.9</td>
<td>i.p.</td>
<td>0.009 (n = 28, r = 0.74)</td>
<td>58</td>
</tr>
<tr>
<td>3.3</td>
<td>i.g.</td>
<td>0.006 (n = 28, r = 0.75)</td>
<td>120</td>
</tr>
</tbody>
</table>

a K, first-order rate constant for clearance from blood; eV0, apparent volume of distribution defined as dose (mg)/C0 (mg/ml), where C0 is the concentration at zero time determined by extrapolation of first-order rate plots; n, number of animals used to calculate rate constant; r, correlation coefficient.

b Not different from 0.011 min⁻¹; t = 0.75 with 45 d.f.

Table 2

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Cumulative urinary excretion</th>
<th>Cumulative expiration</th>
<th>Fecal excretion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>31.1</td>
<td>42.4</td>
<td>43.7</td>
</tr>
<tr>
<td>7.25</td>
<td></td>
<td>42.4</td>
<td>43.7</td>
</tr>
<tr>
<td>24.0</td>
<td></td>
<td>42.4</td>
<td>43.7</td>
</tr>
<tr>
<td>Animal 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.5</td>
<td>2.7</td>
<td>37.9</td>
<td>45.5</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>37.9</td>
<td>45.5</td>
</tr>
<tr>
<td>7.25</td>
<td></td>
<td>45.5</td>
<td>0.5</td>
</tr>
<tr>
<td>24</td>
<td></td>
<td>45.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Table 3

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Cumulative urinary excretion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal 1</td>
<td>38.2</td>
</tr>
<tr>
<td>9</td>
<td>67.8</td>
</tr>
<tr>
<td>24</td>
<td>80.6</td>
</tr>
<tr>
<td>Animal 2</td>
<td>12.2</td>
</tr>
<tr>
<td>6.25</td>
<td>74.0</td>
</tr>
<tr>
<td>24</td>
<td>80.6</td>
</tr>
</tbody>
</table>
administered in toxic doses, has been investigated in vivo (7, 8, 11, 13, 18, 19). In the present study, the clearance from the blood and metabolism of MBN were studied at relatively low doses (one-third to one-half of the 50% lethal dose), minimizing the possibility of overburdening metabolic pathways and thereby increasing the relevance of the findings to the mechanism of carcinogenesis rather than toxicity.

According to the currently accepted mechanism for nitrosamine activation (8, 9, 14, 17), MBN would undergo α-hydroxylation in either the benzyl (5, 20) or methyl (20) moiety, thereby generating a methylating species and benzaldehyde (Chart 1) or a benzylating species and formaldehyde (Chart 2), respectively. The electrophiles would react with cellular nucleophiles to yield benzylated or methylated macromolecules, or react with water to produce benzyl alcohol or methanol. These alcohols could be oxidized to benzaldehyde or formaldehyde by alcohol dehydrogenase (15). Methanol and formaldehyde have been identified as metabolites of DMN (1, 12), and it has been suggested that these compounds enter the one-carbon pool and are incorporated into tissue constituents via normal biosynthetic pathways (8, 10). Alternatively, the aldehydes could be oxidized to the corresponding acids by aldehyde dehydrogenase (3). The resulting acids may be oxidized further or conjugated and excreted. The radioactivity from [methyl-14C]MBN was expired as 14CO2, suggesting that the methyl moiety had undergone prerequisite oxidation to formic acid (16). Urinary excretion of [benzyl-7-14C]MBN derived radiolabel as hippuric acid necessitated oxidation of the benzyl moiety to benzoic acid and its subsequent conjugation with glycine (16).

The expiration of 14CO2 and the urinary excretion of radiolabeled hippuric acid following administration of [14C]MBN is consistent with metabolism of MBN via oxidation at the position α to the nitroso function; however, the methods used in the present study cannot distinguish the relative proportion of methyl and benzyl oxidation.

ACKNOWLEDGMENTS

We wish to thank Dr. William M. Rand for assistance with the statistical analyses.

REFERENCES

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