Distribution of N-Nitrosomethylbenzylamine Evaluated by Whole-Body Radioautography and Densitometry

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ABSTRACT

The distribution of N-nitrosomethylbenzylamine (MBN) was studied using whole-body radioautography and densitometry. Male Sprague-Dawley rats were given N-nitroso-[methyl-14C]-benzylamine ([methyl-14C]MBN) (3.3 mg, 1 mCi/kg body weight i.p.) or N-nitrosomethyl[benzyl-7-14C]amine (2.1 mg, 1 mCi/kg body weight i.p.), and sagittal sections were taken at 15 min, 60 min, 3.5 hr, 7.25 hr, 24 hr, and 3 days after injection.

Very high levels of [methyl-14C]MBN-derived radioactivity were present at all time periods (15 min to 3 days) in the three target tissues for MBN-induced tumorigenesis (nasal cavity, lung, and esophagus). The liver contained high and the kidney contained moderately high levels of radioactivity at all time periods. A considerable amount of radiolabel was present in a number of tissues at 24 hr. After 3 days, very high levels were observed only in the tissues in which MBN-induced tumors eventually developed.

Following administration of N-nitrosomethyl[benzyl-7-14C]-amine, levels of radioactivity in most tissues were lower than following injection of [methyl-14C]MBN; however, very high levels were present in the nasal cavity, liver, and kidney. Considerably less radiolabel remained at 24 hr and 3 days following injection of N-nitrosomethyl[benzyl-7-14C]amine than following [methyl-14C]MBN administration. At 24 hr, benzyl moiety-derived radiolabel was present in either the enterohepatic circulation or the tissues in which MBN-induced tumors arose. At 3 days, the highest level of radioactivity was contained by the liver, although detectable levels remained in the nasal cavity and lung, two tissues in which MBN induced carcinomas.

The findings of this study indicate that the carcinogenicity of MBN may be the result of the inability of the target organs (esophagus, nasal cavity, and lungs) to readily clear methylated macromolecules, benzylated macromolecules, or the oxidized metabolites which arise during nitrosamine metabolism.

INTRODUCTION

MBN is an unsymmetrically substituted nitrosamine which is mutagenic (13, 19, 21–24), toxic (5, 9), and carcinogenic (1, 5, 6, 14, 17). The compound has been shown to selectively induce esophageal tumors in rats (5), and the 2 α-acetoxy derivatives of MBN display striking differences in mutagenicity (19). Of interest because of its structure, toxicity, organ-specific carcinogenicity, and mutagenicity of oxidized derivatives, we undertook an extensive investigation of MBN which encompassed toxicity and carcinogenicity testing, pharmacokinetic and metabolic studies, and tissue distribution and persistence evaluations. The latter topic is the focus of the present report.

Whole-body radioautography was performed at 6 time points after injection of [14C]MBN. The 4 earlier time points were chosen to reflect tissue distribution and uptake. Tissue persistence of radiolabel was determined at the 2 later time periods. Densitometric analysis of the radioautograms permitted the calculation of half-lives of dpm equivalents in selected tissues. Whole-body radioautography has recently been used to investigate the distribution of DMN and MBN (9, 10). In the present study, a dose of the 50% lethal dose of MBN was administered to male Sprague-Dawley rats, and the use of [14C]MBN circumvented the problem of loss of tritium due to hydrogen exchange and metabolism of the nitrosamine.

MATERIALS AND METHODS

Animals and Diet. Male Sprague-Dawley rats (Charles River Breeding Laboratories, North Wilmington, Mass.) weighing 120 to 160 g were permitted food (Charles River Rat/Mouse Hamster Formula, Country Foods, Syracuse, N.Y.) and tap water ad libitum. The animals were housed in fume hoods in plastic tubs 13 x 11 x 5 inches (Superior Chemical Company, Somerville, Mass.) containing Ab-Sorb-Dri bedding.

Chemicals. [methyl-14C]MBN (specific activity, 6 mCi/mmol) and [benzyl-7-14C]MBN (specific activity, 5 mCi/mmol) were synthesized by Paul L. Skipper (15). The radiochemical purity of both compounds, determined by thin-layer chromatography on silica gel using acetone, was greater than 99%.

Whole-Body Radioautography. Twelve rats were given i.p. injections of [methyl-14C]MBN (3.3 mg, 1 mCi/kg body weight) or [benzyl-7-14C]MBN (2.1 mg, 1 mCi/kg body weight) and then frozen by immersion in a bath of dry ice and hexane at 6 intervals postinjection: 15 min, 60 min, 3.5 hr, 7.25 hr, 24 hr, and 3 days (1 rat/time period for each of the 2 compounds). The 4 earlier time points were selected based on whole-blood clearance of MBN. In approximately 10 min, 10% of an i.p. dose of MBN was cleared from whole blood. Fifty % was cleared in 65 min, 90% in 217 min, and 99% in 434 min.

Following immersion in dry ice-hexane, the animals were embedded in a mold containing methylcellulose which was

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4 The abbreviations used are: MBN, N-nitrosomethylbenzylamine; DMN, dimethyl nitrosamine; [methyl-14C]MBN, N-nitrosomethyl[14C]benzylamine; [benzyl-7-14C]MBN, N-nitrosomethyl[benzyl-7-14C]amine.

placed in a −25°C freezer until frozen. The frozen methylcellulose block was mounted on the stage of a Jung microtome (Kenneth A. Dawson, Inc., Belmont, Mass.) housed in a Harris cryostat (Harris Manufacturing Company, Cambridge, Mass.). Sagittal selections (60 μm thick), 60 to 80 at the first 4 time points and 30 to 40 at the latter 2 intervals, were picked up on Scotch 810 transparent tape (L. E. Muran, Boston, Mass.), transferred over dry ice, and stored in a low-humidity cold cryostat (Harris Manufacturing Company, Cambridge, Mass.). Alumina sheets were clamped between and exposed at room temperature. Film exposure resulting from the standardized discs provided a scale for evaluating densities in the whole-body section radioautogram. After suitable exposure, sections were removed, and the X-ray films were processed in Kodak X-ray developer and fixer (E. M. Parker). Exposure ranged from 2 to 24 hr, depending upon the densitometric quality of the radioautograms.

Densitometry. Radioautographic densities of the standard spots and selected tissues (the nasal cavity, the blood contained within the heart, the lung, and the musculature) were determined using a Macbeth densitometer, Model TD-502 LB (Macbeth Instrument Company, Newburgh, N. Y.). A linear-standard curve relating the dpm equivalents of the standardized discs and the corresponding densitometer readings enabled the calculation of dpm equivalents of tissues, including those lighter or darker than the standards, based on densitometer readings. Densitometric analysis required an area of uniformly darkened film 1 mm in diameter which precluded examination of a number of tissues including the esophagus, lung, and regions of the kidney at certain time points.

RESULTS

Whole-Body Radioautography. Semiquantitative data for the distribution of radioactivity following injection of [methyl-14C]MBN and [benzyl-7-14C]MBN is presented in Tables 1 and 2, respectively. Tissues were assigned values from 1 to 10 on the darkening of the X-ray film. Numbers were designated on the basis of dpm equivalents, determined by densitometry and by visual comparison to the 7 standard spots. Several tissues were either lighter or darker than were the standard discs which required expansion of the scale from 1 to 7 to 1 to 10. Radioautographs are shown in Figs. 1 to 3.

Following [methyl-14C]MBN administration, very high levels of radioactivity were present at all time points in the 3 tissues in which tumors developed following i.p. administration of MBM (nasal cavity, lung, and esophagus). Radioactivity distribution was not uniform throughout the lung; areas of radiolabel concentration were observed at all time periods. Label localization also occurred in the salivary glands at 3 days. The liver contained high and the kidney moderately high levels of radioactivity at all time periods. The radioactivity distribution pattern of the liver at 24 hr and 3 days indicated that intestinal radiolabel entered the enterohepatic circulation. A considerable amount of radioactivity was present in a number of tissues at 24 hr: the esophagus, nose, and lung, as well as the thymus, liver, kidney, salivary glands, and epithelium of the gastrointestinal tract. By 3 days, very high levels were observed only in tissues in which MBM-induced tumors ultimately arose.

In general, tissue levels of radioactivity following injection of [benzyl-7-14C]MBN were lower than following administration of [methyl-14C]MBN. Notable exceptions were the nasal cavity, liver, and kidney. The nasal cavity contained very high levels of radiolabel at 15 and 60 min. Radioactivity uptake by the tongue, pharynx, trachea, and esophageal epithelium was much less pronounced than that observed with [methyl-14C]MBN and had only begun to exceed tissue (tongue)
background at 3.5 hr. Areas of radiolabel localization also occurred in the lung following \([\text{benzyl-}^7\text{-}^14\text{C}]\text{MBN}\) injection, but only from 3.5 hr onward. The distribution of radioactivity in the liver at 3.5 hr and beyond indicated that \([\text{benzyl-}^7\text{-}^14\text{C}]\text{MBN}\)-derived radioactivity also entered the enterohepatic circulation. Considerably less radioactivity persisted (present at 24 hr and 3 days) following administration of \([\text{benzyl-}^7\text{-}^14\text{C}]\text{MBN}\) than following injection of \([\text{methyl-}^14\text{C}]\text{MBN}\). At 3 days, the highest level of radioactivity was contained by the liver. Barely detectable levels remained in the nasal cavity and lung.

**Densitometry.** Tissue dpm equivalents rose and fell more sharply following injection of \([\text{benzyl-}^7\text{-}^14\text{C}]\text{MBN}\) (Chart 1) than following administration of \([\text{methyl-}^14\text{C}]\text{MBN}\) (Chart 2). Following administration of \([\text{methyl-}^14\text{C}]\text{MBN}\), peak dpm equivalents were observed at 60 min in the blood (in the heart) and at 3.5 hr in the nasal cavity and liver. Musculature dpm equivalents probably reached a peak between 60 min and 3.5 hr (Chart 2). In contrast, following \([\text{benzyl-}^7\text{-}^14\text{C}]\text{MBN}\) injection, peak dpm equivalents were observed at 60 min in all 4 tissues analyzed (Chart 1).

Equations for clearance of dpm equivalents could only be calculated using data at time points at and after the observed peak, namely, 3.5, 7.25, and 24 hr for \([\text{methyl-}^14\text{C}]\text{MBN}\), and 60 min, 3.5 hr, and 7.25 hr for \([\text{benzyl-}^7\text{-}^14\text{C}]\text{MBN}\). Clearance equations calculated for \([\text{benzyl-}^7\text{-}^14\text{C}]\text{MBN}\) using data at 3.5, 7.25, and 24 hr had poorer correlation coefficients. Estimated tissue half-lives of \(^{14}\text{C}\) equivalents were calculated from clearance equation rate constants. The \(^{14}\text{C}\) equivalent half-lives were considerably shorter following administration of \([\text{benzyl-}^7\text{-}^14\text{C}]\text{MBN}\) than following injection of \([\text{methyl-}^14\text{C}]\text{MBN}\): 2 to 4 hr versus 18 to 34 hr, respectively (Tables 3 and 4). For a given tissue, there was favorable agreement between the clearance equation intercepts calculated for methyl and benzyl dpm equivalents. For example, intercepts for nasal cavity clearance equations were 9.9 and 9.8.

**DISCUSSION**

Whole-body radioautography was utilized to demonstrate the
Nitrosamines require metabolic activation to alkylating agents in order to exert their biological effects (8, 11). MBN can be oxidized to either a methylating or a benzylating species (7, 18) which could react with cellular nucleophiles yielding methylated or benzylated macromolecules or with water to produce methanol or benzyl alcohol. The alcohols can be oxidized further to the corresponding aldehydes and acids by alcohol and aldehyde dehydrogenases (4, 12). The oxidized metabolites would be generated within the cell and all are either irritants or capable of inflicting tissue damage (16). During ensuing regenerative processes, cell division may provide an opportunity for incorporation of the carcinogenic lesion into the genetic code. The oxidized metabolites of MBN may be acting as cocarcinogens; noncarcinogens which enhance the carcinogenicity of MBN, in this case by necessitating cell repair and division which may increase the probability of lesion fixation and eventual tumor development. Stimulation of liver cell proliferation via partial hepatectomy has been used to increase the yield of tumors following administration of DMN (2, 3) and aromatic hydrocarbons, nitrosamines and nitrosamides, aromatic amines, and miscellaneous carcinogens (20). Liver cell necrosis and the ensuing compensatory cell proliferation following administration of N-nitrosodiethylamine have been shown to play an important role in the induction of preneoplastic lesions (25). The carcinogenic effect of MBN may be enhanced by the inability of the esophagus, nasal cavity, and lung to readily clear the methylated or benzylated macromolecules or oxidized metabolites which arise during nitrosamine metabolism.
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REFERENCES

Fig. 1. Radioautographs of male Sprague-Dawley rats 15 min (a), 7.25 hr (b), and 24 hr (c) after i.p. injections of [methyl-14C]MBN (3.3 mg, 1 mCi/kg body weight).

Fig. 2. Radioautographs of male Sprague-Dawley rats 15 min (a), 7.25 hr (b), and 24 hr (c) after i.p. injections of [benzyl-7-14C]MBN (2.1 mg, 1 mCi/kg body weight).
Fig. 3. Radioautographs of male Sprague-Dawley rats 3 days after i.p. injection of [methyl-14C]MBN (3.3 mg, 1 mCi/kg body weight) (a, b) and [benzyl-7-14C]MBN (2.1 mg, 1 mCi/kg body weight) (c).
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