Brief Communication: Detection and Estimation of Azomethane in Expired Air of 1,2-Dimethylhydrazine-Treated Rats

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SUMMARY—Azomethane (AM) gas was identified as a major metabolite of 1,2-dimethylhydrazine (1,2-DMH) in the expired air of F344 rats. The compound was characterized by high-pressure liquid chromatography, gas chromatography, and mass spectrometry, in comparison to a synthetic standard. At a dose of 21 mg 1,2-DMH/kg sc, approximately 14 and 11% of the dose were exhaled as AM and CO₂, respectively, in 24 hours. At 200 mg 1,2-DMH/kg, 23 and 4% of the dose appeared as AM and CO₂, respectively, in the expired air within the same period. Most AM was seen in the first 6 hours, but the CO₂ evolution was more progressive, especially after the higher dose of 1,2-DMH.—J Natl Cancer Inst 56: 1271–1273, 1976.

1,2-Dimethylhydrazine (1,2-DMH) is a powerful colon carcinogen in several rodent species (1–3). This agent is extremely useful for the study of the mechanism of colon carcinogenesis in animal models. It has a high degree of organospecificity, as well as the ability to induce neoplasms in the colon after several small doses (1) or a single moderate-to-large dose (1, 4). The lesions produced are histologically similar to those found in man (5). The organospecificity of 1,2-DMH, a typical procarcinogen, is presumably due to tissue-specific activation and detoxification reactions. Druckrey (1), on the basis of the biologic effects of 1,2-DMH and its chemical derivatives, postulated that the activation pathway of this carcinogen proceeds by a series of oxidations through azomethane (AM), azoxy methane, and methylazoxy methanol to form the proximate carcinogen methyl diazonium hydroxide. Although such a scheme appears plausible, no direct experimental evidence exists for this sequence. We report here the identification and estimation of AM, a major 1,2-DMH metabolite, in the expired air of rats given 1,2-DMH.

MATERIALS AND METHODS

1,2-DMH·2HCl was obtained from Aldrich Chemical Co. (Metuchen, N.J.). 14C-labeled 1,2-DMH was obtained from New England Nuclear Corp. (Boston, Mass.), and its radiochemical purity was verified by thin-layer chromatography (6). AM gas (bp, 1.5° C) (7) was prepared, for standard reference purposes, by the oxidation of 1,2-dimethylhydrazine (1,2-DMH)·14C (3.5 mCi/mmole). The rats were placed in metabolism cages (Delmar Scientific Glass Products, Maywood, Ill.). Air, dried and freed of CO₂, was drawn through the cages at approximately 250–300 ml/minute. For the first 5 or 6 hours, air exiting the chamber was drawn through a train of three gas washing bottles. The first contained 150 ml ethanol cooled to −72° C by a surrounding Dry-Ice–ethanol bath, the second contained 150 ml of 1 N NaOH, and the third contained 150 ml of 1 N H₂SO₄. The contents of the first gas washer were changed every hour for the first 5 or 6 hours; thereafter, this gas washer was removed from the train. The contents of the other two gas washers were sampled every hour and changed at 5 or 6 and 24 hours. The 1 N H₂SO₄ gas washer was routinely used to ensure a more complete recovery of AM, which is highly soluble in dilute acid; however, in practice, less than 2–3% of the total 14C was found in this trapping agent. Most AM appeared in the preceding cold ethanol scrubber. AM, as such, cannot be recovered from the H₂SO₄ trap, since the gas converts to monomethylhydrazine and formaldehyde in the presence of dilute acid. For analysis of expired AM by gas chromatography (GC)—mass spectrometry (MS), spectrograde n-hexane at −72° C was used in the first gas washer of the train rather than ethanol, and the expired air was passed through the gas washer for 3 hours rather than 1 hour to accumulate sufficiently high concentrations (≥0.1 µg/µl) for adequate MS response without overloading the GC column. During the first 5 or 6 hours in the metabolism chambers, rats were given neither food nor water. After this period, both water and Purina Laboratory Chow were provided.

We performed high-pressure liquid chromatography (HPLC) of AM collected in ethanol by applying a 100-µl aliquot from the ethanol gas washer to two serially connected µBondapak C₁₈ columns (Waters Associates, Milford, Mass.), each 4 mm (inside diameter) × 30 cm, equilibrated with aqueous 1% ethanol, and eluted at 1 ml/minute at approximately 2,000 pounds/square inch pressure with the same solvent (9). To provide a qualitative indication of the elution of AM, we monitored the effluent at 205 nm (the absorption maximum of AM is 185 nm) (10) using the Model SF 770 dual-beam variable wavelength flow analyzer (Schoeffel Corp., Westwood, N.J.). For quantitative estimation of AM-14C, 1-ml fractions were collected directly in scintillation vials containing 10 ml Scintisol cocktail (Isolab Incorp., Akron, Ohio), and radioactivity was determined by liquid scintillation counting with the use of external standard–channels ratio method to establish efficiency.

For GC/MS analysis, aliquots up to 10 µl n-hexane containing AM were analyzed on a 4-mm (inside diameter) × 122-cm column of Chromosorb 105 (Johns-Manville, Denver, Colo.), 60/80 mesh, at 120° C with helium carrier at 40 ml/minute. A Hewlett Packard Model HP 5982 GC/MS system was used in conjunction with an HP-5983A computer to obtain the mass spectrum of the metabolite and the synthetic AM. Mass spectra of expired AM were obtained only at the higher dose of 21 mg 1,2-DMH/kg.

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1,2-DMH (200 mg/kg) because of limitations in the sensitivity of the mass spectrograph. When n-hexane samples containing AM from rats treated with 21 mg 1,2-DMH/kg were analyzed by GC, a flame ionization detector was substituted for the mass spectrograph. The retention time of the metabolically produced AM in this instance, as well as at the higher dose level, was identical to that of the synthetic AM standard.

In contrast to a single dose of 21 mg 1,2-DMH/kg which, to our knowledge, has not yet been observed to induce colon tumors, larger single doses of 40 and 200 mg/kg produced these lesions in BD rats (1, 4). Single doses of 150 and 200 mg/kg have produced colon tumors in F344 rats. Thus we compared the production of CO₂ and AM from 1,2-DMH at both dose levels of 21 and 200 mg/kg.

RESULTS

The appearance of ¹⁴CO₂ (trapped by the 1 N NaOH and AM-¹⁴C (trapped by the cold ethanol and the 1 N H₂SO₄) in the expired air of male F344 rats given a single dose of either 21 or 200 mg 1,2-DMH-¹⁴C/kg is shown in text-figure 1.

Although the excretion of AM in the expired air commenced almost immediately after administration of 1,2-DMH, CO₂ production showed a lag, apparently reflecting the time required for the complete metabolism of the carcinogen or perhaps the time necessary for induction of appropriate metabolic enzymes. At both dose levels, the evolution of AM was most rapid during the first 4 or 5 hours after administration of 1,2-DMH and decreased thereafter. With 21 mg 1,2-DMH/kg, approximately 11 and 14% of the carcinogen was metabolized to CO₂ and AM, respectively, during 24 hours. At the 200-mg/kg level, approximately 4% of the dose appeared as CO₂ and 23% appeared as AM during this time. Our values for the amounts of CO₂ derived in vivo from 1,2-DMH agreed with those obtained by Hawks and Magee (11) in their detailed work on the distribution of label from 1,2-DMH-¹⁴C in the urine, bile fluid, and tissues of rats. As the 1,2-DMH dose was increased from 21 to 200 mg/kg, the amount of CO₂ exhaled did not increase in proportion, whereas the amount of AM was disproportionately higher. This effect, which may be due to enzyme saturation, is under study.

Evidence for the identity of AM was obtained by HPLC for both the 21- and 200-mg/kg dose levels (text-fig. 2). In each case, 94–97% of the radioactivity applied...
to the column was recovered in a single peak corresponding in retention volume to authentic AM standard. Moreover, oxidation of metabolically produced AM-14C with m-chloroperbenzoic acid (12) and submission to HPLC on P.Bondapak C18 caused a peak corresponding to azoxy-methane-14C as would be expected. Final proof of the identity of AM came from analysis by GC and GC/MS system (text-fig. 3) for the sample collected in n-hexane. A peak giving current response at m/e 58 (molecular ion) was observed at a retention time of 3.3 minutes for the sample collected in n-hexane. The retention time was identical to that of the synthetic AM standard. The relative abundance of the main ions was as follows [values from (13) are given in parentheses]: at m/e 15, 100% (100%); at 45, 45% (35%); at 28, 26% (30%); at 58, 18% (16%).

DISCUSSION

Although 1,2-DMH is easily oxidized to AM by oxygen, especially in the presence of trace amounts of metal catalysts (14), the finding of AM in the expired air is not due to artifactual contamination of the dose given with this compound to the animals. Indeed, the purity of the 1,2-DMH used was checked by HPLC before being administered to the rats, and it had an AM content of less than 1%

The demonstration that AM is a major metabolite of 1,2-DMH supports Druckrey's proposal (1) for the metabolic activation of this carcinogen. Although the carcinogenic properties of AM have not been studied, there is every reason to believe that this compound, like 1,2-DMH and azoxymethane, is also a carcinogen. In this respect, we suggest that investigators treating many animals with 1,2-DMH should take the special precautions of performing this procedure in a well-ventilated area to avoid exposure to AM gas that is expired by the animals in high yields for at least 4 hours after treatment.

REFERENCES

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* m/e = mass of the ion divided by its charge.