Methylated Purines in Human Liver DNA after Probable Dimethylnitrosamine Poisoning

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ABSTRACT

DNA, isolated from two samples of human liver obtained from a suspected dimethylnitrosamine poisoning, contained 1363 to 1373 μmol of 7-methylguanine per mol of guanine and 273 to 317 μmol of O6-methylguanine per mol of guanine. Liver and kidney DNA obtained from unrelated cases contained no detectable methylated purines. From the DNA methylation levels, it is estimated that the dimethylnitrosamine-poisoning victim had been exposed to a dose of 20 mg or more of dimethylnitrosamine per kg of body weight. The results indicate for the first time that humans, like rodents, appear to activate dimethylnitrosamine metabolically to a strong methylating agent, resulting in methylation of liver DNA at both the 7- and O6- positions of guanine.

INTRODUCTION

The hepatocarcinogenicity of DMN has been demonstrated experimentally in 10 animal species (6), yet, in spite of known industrial and laboratory uses, no human cancers have been associated with exposures to this compound. It is now recognized that DMN is widely present in the environment in the food and air supplies and, under appropriate conditions, can form in the human stomach (8). Biochemical evidence suggests that this compound is a strong carcinogen in animals because of its metabolic activation to a highly reactive agent, which methylates target organ DNA at base-pairing sites such as the O6 position of guanine (6). A recent case in forensic medicine in which 2 victims died of suspected DMN poisoning has now provided in vivo evidence that human beings seem to activate this nitrosamine metabolically in the same way, at least qualitatively, as do laboratory animals. Analysis of liver DNA prepared from one of the victims of suspected DMN poisoning indicates the presence of 7-methylguanine and O6-methylguanine.

MATERIALS AND METHODS

Twelve frozen samples of human liver, kidney, and heart were received from the Center for Disease Control, Atlanta, Ga. and Poison Lab., Denver, Colo. Tissue analyses were performed without knowledge of the illness or cause of death. The phenolic extraction method of Kirby (4) as modified by Swann and Magee (10) was used to isolate and purify tissue DNA. Nucleic acid could not be isolated from 5 of the samples, which were severely autolyzed. DNA from the remaining 7 tissues was hydrolyzed in 0.1 M HCl (5 mg/ml) for 30 min at 70°, thus releasing all purines as free bases. Chromatographic separation of hydrolysates was carried out using a Partisil-10 strong cation-exchange column (inner diameter, 25 cm × 4.5 mm; Whatman, Inc., Clifton, N.J.) and 0.06 M ammonium phosphate (pH 2.0) at 2.0 ml/min (3). Elution of fluorescing bases was monitored using a 286-nm emission wavelength with a 366-nm emission interference filter. Quantitation was achieved using electronic integration calibrated with standard solutions of authentic guanine, 7-methylguanine, and O6-methylguanine.

RESULTS AND DISCUSSION

Only 2 DNA samples contained detectable amounts of methylated purines (Table 1). These samples were prepared from 2 liver specimens taken from a 23-year-old male victim of probable DMN poisoning.4 The DNA samples from the other tissues were prepared from 2 cases of Reye's syndrome and one case of methyl bromide poisoning; none of these samples contained either 7-methylguanine or O6-methylguanine. The amounts of these methylated bases found in the liver from the DMN victim were readily detectable and quantifiable (Chart 1).

This is the first report of the occurrence of O6-methylguanine in human liver resulting from DMN exposure. Until recently, methylation of DNA following in vivo exposure to DMN was demonstrable only by using radioactively labeled carcinogen or large amounts of tissue, neither of which was available in the human poisoning considered here. The analytical method used in this study achieved high sensitivity by taking advantage of high-pressure liquid chromatography coupled with fluorescence detection (3).

An in vitro determination of metabolic activation of [14C]DMN by rat and human liver slices was reported by Montesano and Magee (7). They found that human liver slices metabolized DMN at a rate such that 0.13% of the DNA guanine was methylated at position 7 in 1 hr compared to 0.17% of the DNA guanine in rat liver slices.

We have attempted to determine whether our data are consistent with an exposure to an acutely toxic dose of DMN. Craddock (1) has determined that a dose of 5 mg of DMN per kg of body weight results in the formation of approximately 1300 μmol of 7-methylguanine per mol of guanine in rat liver DNA 5 hr after administration of carcinogen p.o. If the human metabolizes DMN at only 68% of the rate at which the rat forms the active methylating agent as suggested by the study of Montesano and Magee (7) mentioned above, then the human dose could approximate 5 mg + 0.68 or 7 mg/kg of body weight. Since the victim died 5 days after presumed exposure,
and since 7-methylguanine spontaneously depurinates from DNA (half-life, 24 to 48 hr), the human dose probably was considerably greater than 7 mg/kg of body weight. Pegg (9) has shown a dose-dependent relationship between DMN exposure and the \( O^6 \)-methylguanine:7-methylguanine ratio in rat liver DNA. This ratio approaches 0.1 at about 20 mg of DMN per kg of body weight. The ratio in the human liver DNA reported here was 0.2; if extrapolation can be made from rat data, this provides additional evidence that the human exposure was probably greater than 20 mg/kg of body weight. The 50% lethal dose for DMN administered p.o. in the rat is 27 to 41 mg/kg of body weight (2); hence, the finding of the amounts of methylated purines in the human liver DNA reported here is consistent with an exposure to a level of DMN likely to be fatal.

In view of current theories of chemical carcinogenesis, the detection of methylated bases, particularly \( O^6 \)-methylguanine, in human tissue after a probable exposure to DMN has important implications. Considerable attention has been focused on the presence and quantitative analysis of alkylated bases in DNA of animals treated with chemical carcinogens. Several attempts have been made to associate levels and sites of DNA alkylation with carcinogenicity. Although quantitatively a minor product, \( O^6 \)-methylguanine may be more closely associated with carcinogenicity than the more frequently occurring 7-methylguanine (5). Both rats and humans, then, appear capable of metabolically activating DMN to a strong methylating agent, which interacts with the same sites in liver DNA in both species.

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