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Enhancing Effects of an Organic Arsenic Compound, Dimethylarsinic Acid (Cacodylic Acid), in a Multi-organ Carcinogenesis Bioassay

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The modifying effects of dimethylarsinic acid (DMA) on tumor induction in various organs were examined using a multi-organ rat carcinogenesis bioassay. A total of 124 six-week-old male F344/DuCrj rats were divided randomly into seven groups. For establishment of wide-spectrum initiation, animals in Groups 1–5 were treated with five carcinogens, namely *N*-nitrosodiethylamine (DEN), *N*-methyl-*N*-nitrosourea (MNU), 1,2-dimethylhydrazine (DMH), *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN) and *N*-bis(2-hydroxypropyl)nitrosamine (DHPN) in the first four weeks. After a two-week interval, Groups 1–5 were then given 0, 50, 100, 200 and 400 ppm DMA, respectively, in drinking water. Groups 6 and 7 received 100 and 400 ppm DMA without any carcinogen pretreatment. All rats were sacrificed at the end of week 30. In the initiated groups (Groups 1–5), DMA enhanced tumor development in the urinary bladder, kidney, liver and thyroid gland. The main arsenic species in urine samples was DMA itself. In conclusion, the observed enhancement of carcinogenesis in the urinary tract as well as in the liver and thyroid gland may be directly due to this arsenic compound.

Keywords: Dimethylarsinic acid, two-stage carcinogenesis, carcinogenicity, urinary bladder, multi-organ carcinogenesis promotion

INTRODUCTION

Dimethylarsinic acid (cacodylic acid; DMA) has been used as a general herbicide or pesticide for many years.[1,2] As one of the major methylated metabolites of ingested organic or inorganic arsenic, it is eliminated by the kidney and finally excreted in the urine.[3–11] Therefore it is a compound of some environmental significance. *In vitro* examinations have revealed that DMA is not mutagenic as evaluated by the Ames test or Rec-assay but it has induced mitotic arrest, and chromosomal aberrations such as tetraploid formation and sister–chromatoid exchange.[12] Although some investigators have undertaken the determination of the carcinogenic effects of DMA *in vivo*, no unequivocal data have been presented.[13–16]

For the purpose of evaluating any modifying effects of DMA, we conducted the present multi-organ carcinogenesis bioassay in rats administered various concentrations of DMA. In addition to investigating all the organs by histopathological procedures, we also analysed five arsenic species in urine samples with the aid of inductively coupled plasma (ICP) mass spectrometry (MS) with ion chromatography (IC ICP MS).

MATERIALS AND METHODS

Experimental design

A total of 124 male F344/DuCrj rats (six weeks of age, from Charles River Japan Inc., Hino, Japan) were divided randomly into seven groups (20 rats each for Groups 1–5, 12 rats each for Groups 6

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and 7). For the establishment of wide-spectrum initiation of tissues, animals in Groups 1–5 were treated sequentially with saline DEN (100 mg kg^{-1} body weight, i.p., single dose at the commencement) and saline MNU (20 mg kg^{-1} body weight, i.p., four doses at days 5, 8, 11 and 14). Thereafter, rats received saline DMH (40 mg kg^{-1} body weight, s.c. four doses at days 18, 22, 26 and 30). During the same period, the animals were sequentially administered BBN (0.05% in drinking water, weeks 1 and 2) and DHPN (0.1% in drinking water, weeks 3 and 4). After a two-week interval, Groups 1–5 were then given 0, 50, 100, 200 and 400 ppm DMA, respectively, in drinking water. Group 1 received basal diet and tap-water without any chemical supplement after the carcinogen treatment (control group—no DMA). Groups 6 and 7, respectively, were given 100 and 400 ppm DMA in drinking water during weeks 6–30. Throughout the experiment, the animals had free access to food and water. At week 10, urine was collected, immediately frozen and stored until use for measurement of arsenic concentrations. All survivers were sacrificed by exsanguination under ether anesthesia at week 30.

All major organs were routinely excised, fixed in 10% buffered formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin for histopathological examination.

Concentration and speciation of arsenic in the urine

Using the urine samples collected at week 10, five arsenic species—trimethylarsine oxide, $(CH_3)_3AsO$ (TMA), DMA, monomethylarsinic acid (MMA), arsenobetaine (AsBe) and arsenite—were analysed by IC ICP MS; Yokogawa Analytical Systems Inc.). For separation of the five arsenic compounds, an anion exchange column and tartaric acid were used. The eluent of the IC column was directly introduced into the nebulizer of the ICP-MS and analysed at mass 75. Urine samples collected at 10 wks were used for study, with the aid of Yokogawa Analytical Systems Inc., Tokyo, Japan.

RESULTS AND DISCUSSION

Water intake values (averages of measurement made at weeks 12, 18, 24 and 30) in the group administered the highest DMA dose were about twice as high as the non-administered group level [Group 5 (102.8 $cm^3 kg^{-1} day^{-1}$) compared with Group 1 (57.3 $cm^3 kg^{-1} day^{-1}$); $P<0.001$; Student's *t*-test]. In DMA-administered groups, total arsenic content in the urine samples correlated with DMA concentrations in the drinking water.

In the initiated groups (Groups 1–5), DMA significantly enhanced tumor development in the urinary bladder, kidneys, liver and thyroid gland. This was particularly marked in the urinary bladder even in the lowest-dose group (50 ppm) in which the number of tumor-bearing animals (papilloma and/or carcinoma) was significantly increased ($P<0.001$; Fischer's exact probability test, Fig. 1). The tumor size was correlated with the DMA dose. Significantly high incidences of renal cell and liver tumors were observed in 200 ppm and/or 400 ppm animals (Group 4). Although carcinogenicity of DMA for the lung has been suggested, no intergroup differences were observed for tumor induction in this organ. Skin carcinogenesis was not evaluable in this bioassay system because of the lack of any initiation treatment for the skin. There were no notable changes in non-initiated groups (Groups 6 and 7).

There have been numerous reports concerning possible carcinogenesis by arsenic not only in the limited endemic area of poisoning on the southwest coast of Taiwan[17,18] but also worldwide.[19,20] Despite epidemiological evidence of carcinogenicity, no adequate confirmatory data using experimental animals have hitherto been gained.[13–16] However, the present study clearly shows that DMA has significant modifying effects on carcinogenesis in various organs.

In our experiment, the main metabolite in the urine was DMA in most of the DMA-administered groups. Taking account of the observed high incidences of urinary bladder cancer, we can conclude that this compound was responsible for the enhancement. Most reports of metabolism of arsenic species have indicated marked accumulation of DMA in the kidneys,[8] and Murai *et al.* earlier described renal lesions after high-dose administration of DMA to F344 rats.[21]

In conclusion, the present multi-organ carcinogenesis bioassay revealed the organic arsenic compound DMA enhances carcinogenesis in the urinary bladder, kidney, liver and thyroid gland of treated rats. We therefore speculate that this arsenic metabolite is a carcinogen or carcinogen

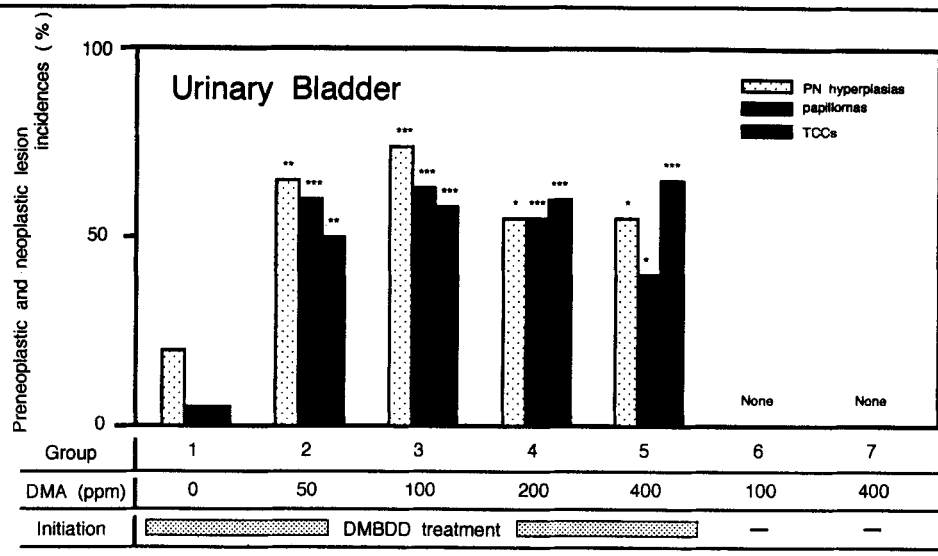

Figure 1 Incidence of preneoplastic and neoplastic lesions of the urinary bladder. Asterisks indicate groups significantly different from Group 1: $*p<0.05$; $**p<0.01$; $***p<0.001$. TCCs, transitional cell carcinomas.

promoter for these organs. Further investigations are required to ascertain the mechanisms of action.

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