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SHORT COMMUNICATION

Urinary bladder carcinogenicity of dimethylarsinic acid in male F344 rats

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The present study was conducted to determine the carcinogenicity of dimethylarsinic acid (DMA) administered to male F344 rats in a 2 year bioassay. A total of 144 rats (10 weeks old at the start) were divided into four groups of 36 rats each. Groups 1–4 received DMA (purity 100%) at concentrations of 200, 50, 12.5 and 0 p.p.m. in the drinking water, respectively, for 104 weeks. From weeks 97 to 104, urinary bladder tumors were observed in 12 of 31, eight of 31 and none of 33 in groups 1–3, respectively. No bladder tumors were observed in group 4. The present study demonstrated that long-term p.o. administration of DMA induced urinary bladder carcinomas in male F344 rats. Therefore, the results indicate that DMA is carcinogenic for the rat urinary bladder, which may be related to the human carcinogenicity of arsenicals.

Inorganic arsenicals are well known as carcinogens to humans, especially for the skin and lung (1,2). Studies in Taiwan have also found an association between high levels of arsenic in the drinking water and development of cancers in the bladder, liver, kidney, lung, nasal cavity, prostate and other internal sites (3–7). However, unlike most substances classified as carcinogens, classification of arsenic is based on human data, animal data being inadequate. In fact, attempts to induce tumors in experimental animals with inorganic arsenic compounds have mostly failed (1), except for a few studies in which animals were given arsenic trioxide by intratracheal instillation (8,9). Arsenic carcinogenicity in experimental animals has remained uncertain.

Dimethylarsinic acid (DMA) is a major form of organic arsenic in the environment (10). Humans may be exposed to DMA by many means. One source is exposure to arsenic in the drinking water. A number of studies have indicated that most mammals, including humans, methylate inorganic arsenic compounds to methylated arsenicals in the liver and DMA is the major metabolite (11–16). Other sources include production or use of arsenic-containing herbicides (17–20) and ingestion of food which has been contaminated with these herbicides or foods in which DMA occurs naturally, such as certain types of seaweed (21,22). These multiple sources suggest that humans could be continuously exposed to DMA in their general environment. Increasing evidence suggests that DMA exposure may be extremely important with regard to arsenic exposure-associated development of tumors and that the study of DMA carcinogenicity may provide a clue for our understanding of the mechanism of arsenic carcinogenesis in humans.

Recent *in vitro* findings have revealed that DMA is probably a potent clastogenic agent (23,24) capable of inducing DNA damage such as strand breaks (25,26) and crosslink formation between DNA and proteins (27). However, numerous studies indicate that DMA and other chemicals do not directly react with DNA and are negtive in mutagenesis assays such as the Ames assay (28,29). Yamamoto *et al.* (30) recently found that in an *in vivo* multi-organ carcinogenesis bioassay based on the two-stage model of carcinogenesis, DMA promoted carcinogenesis of the urinary bladder, kidney, liver and thyroid gland in rats initiated by five sequential treatments with initiators. DMA promotional activity was further supported by the finding of significantly increased renal ornithine decarboxylase activity in rats treated with DMA. Wanibuchi *et al.* (31,32) indicated that DMA showed promoting activities in a dose-responsive manner in urinary bladder and liver carcinogenesis in rats, possibly via a mechanism involving stimulation of cell proliferation and DNA damage caused by oxygen radicals. These findings indicated that DMA itself may be a promoter or a carcinogen in rats and may provide clues to the carcinogenic mechanism of arsenic in humans. Although some investigators have undertaken the determination of the carcinogenic effect of DMA *in vivo*, no unequivocal data have been published (1). It had been reported that DMA treatment given in water for 6 months did not result in formation of any tumors in male Wistar rats (33), but an extended treatment time or a higher dose may be necessary for tumorigenesis to occur. Therefore, a long-term carcinogenicity bioassay is needed to confirm whether DMA is a carcinogen or a promoter.

The urinary bladder has long been described as one of the major target organs of arsenic carcinogenicity in humans (34,35). This is probably related to the proximate metabolites of the ingested chemicals being excreted in the urine and retained in the urinary bladder for relatively long duration with continuous exposure to the urothelium. In our previous studies, DMA stimulated cell proliferation in the urinary bladder epithelium (31), but no bladder epithelial lesions were observed in the urinary bladder of F344 rats treated with DMA at concentrations of 100 or 400 p.p.m. in the drinking water for 24 or 32 weeks (30,31); the chronic effects of DMA administration on rats are not known.

The present study was therefore undertaken to determine whether DMA induces neoplastic lesions in various organs in F344 rats, particularly in the urinary bladder, when orally administrated in the drinking water for a 2 year period.

A total of 144 male, 6-week-old, F344/DuCrj rats were obtained from Charles River Japan (Hino, Japan). They were housed four per steel cage with woodchip bedding in an animal room with a 12 h light/dark cycle at 22 ± 2°C and 44 ± 5% relative humidity. The animals were fed common basal pelleted diet (CE2; Clea Japan,Tokyo, Japan) and water *ad libtium*. Our previous data showed that chronic DMA administration

Abbreviations: DMA, dimethylarsenic acid; TCC, transitional cell carcinoma.

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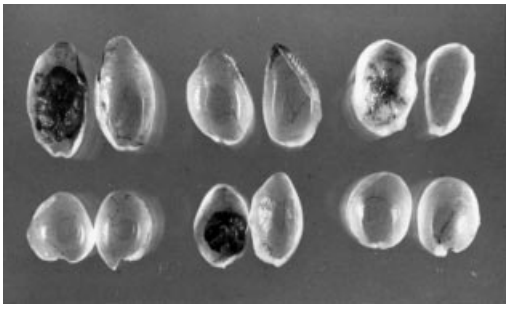

Fig. 1. Macroscopic view of rat urinary bladder tumors induced by 200 p.p.m. DMA treatment.

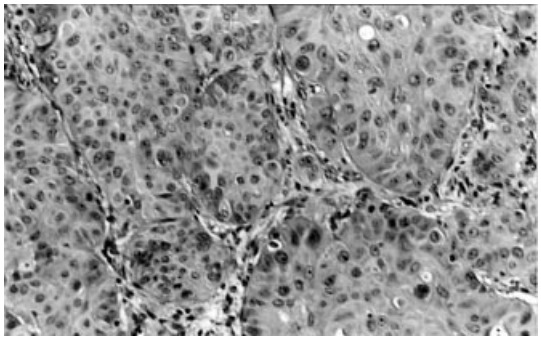

Fig. 2. Photomicrograph of transitional cell carcinoma of urinary bladder in a DMA-treated male F344 rat (hematoxylin and eosin, ×160).

Table I. Data for average body weight, food and water intake and total DMA intake of rats treated with DMA

Group	DMA (p.p.m.)	Body wt (g)		Food intake (g/rat/day)[a]	Water intake (g/rat/day)[a]	Total intake of DMA (mg/rat)
		Initial	Final			
1	200	226	387	13.3 ± 0.9	20.7 ± 1.5[b]	274.8
2	50	223	384	12.9 ± 0.9	20.7 ± 2.0[b]	70.6
3	12.5	222	403	13.4 ± 0.7	18.9 ± 1.1	16.3
4	0	219	398	13.7 ± 0.6	18.4 ± 1.4	0

[a]Values are mean values ± SD.
[b]Significantly different from groups 3 and 4 at $P < 0.001$.

is toxic to young rats (32), so rats received DMA after a 4 week acclimation period, during which time they were not administrated DMA. Administration began at 10 weeks of age in the present study. DMA was purchased from Wako Pure Chemical Industries (purity 100%). The highest dose of DMA given in the present study was determined based on guidelines for long-term carcinogen bioassays (36) and results of our previous studies (30–32). Rats were divided randomly into four groups, each consisting of 36 rats. Groups 1–4 received DMA at concentrations of 200, 50, 12.5 and 0 p.p.m. in the drinking water for 104 weeks, respectively. The body weight of the rats and their water and food consumption were recorded every week for the first 12 weeks of the study and subsequently once every 4 weeks. Urine was collected by forced urination at the end of 30, 60 and 100 weeks; pH was immediately measured with a pH meter (model F-15; Horiba, Tokyo, Japan) and then samples were stored at –80°C until analysis. Rats that had died or were killed when becoming moribund during the study or killed at the end of the study at week 104 were autopsied for macroscopic and histopathological examinations.

At autopsy, the rat urinary bladders were inflated and fixed with 10% phosphate-buffered formalin (pH 7.4). After adequate fixation, they were weighed, cut and processed for paraffin embedding and sectioned. Sections were stained with hematoxylin and eosin for light microscopic examination. Histopathological lesions of the urinary bladder epithelium were classified into three categories: papillary or nodular (PN) hyperplasia, papilloma and carcinoma, as described previously (37).

The significance of differences between mean values was analyzed using Fisher's PLSD method. Log rank (Mantel–Cox) analysis was used to analyse the survival rates. The significance of differences in lesion incidences between groups was assessed by χ^2 probability analysis or Fisher's exact probability test.

The numbers of surviving rats at week 104 were 25, 28, 28 and 24 rats in groups 1–4, respectively. There were no statistically significant differences for the overall survival rates between groups during the experiment.

We observed transient body weight suppression in the highest dose DMA-treated groups (groups 1 and 2) at weeks 3–4. After that the growth rates were comparable among all groups until week 60. From week 60 to the end of study the increase in body weight was slightly lower in groups 1 and 2 than in group 3 treated with the lower concentration of DMA or in the controls, group 4. Nevertheless, the final body weights were not significantly different between the groups. Water intake was significantly ($P < 0.001$) increased in groups 1 and 2 and total DMA intake was related to the DMA dose. Administration of DMA did not affect food intake during the experiment (Table I).

Urinary pH did not differ significantly between groups during the experiment. Bladder calculi were not observed in any of the rats.

Macroscopically, most urinary bladder tumors observed in groups 1 and 2 showed papillary growth. Most of the larger tumors were observed in group 1 (Figure 1). At weeks 97 and 100, two animals in group 1 were found to have urinary bladder tumors, while one urinary bladder tumor was observed in an animal in group 2 at week 103. The other tumors were found in rats at the terminal killing at week 104. The incidences and multiplicities of urinary bladder tumors in rats treated with DMA are summarized in Table II. The effective number of rats in each group was considered to be the number alive at week 97, when the first bladder tumor was found. Urinary bladder tumors, including papillomas and carcinomas, were observed in 12 of 31, eight of 31 and none of 33 in groups 1–3, respectively. The two rats in group 1 with bladder papillomas also had carcinomas. The two rats in group 2 with papillomas were different from the rats with carcinomas. In group 4, no urinary bladder tumors were observed. Incidences and numbers of urinary bladder carcinomas were significantly higher in groups 1 and 2 compared with group 4. The incidences and numbers of carcinomas observed in DMA treatment groups increased in a dose-responsive manner. Histologically, the carcinomas were transitional cell carcinoma (TCC) (Figure 2). One rat in group 1 had an invasive TCC and histiocytic sarcoma.

The results of the present study demonstrated that long-term p.o. administration of DMA induced urinary bladder carcinomas in male F344 rats. The results were consistent with a recent report of a 2 year carcinogenicity study that DMA induced an increased incidence of bladder tumors in rats (38). We therefore conclude that DMA is carcinogenic to the urinary bladder in the male F344 rat.

Table II. Incidence of urinary bladder tumors in rats treated with DMA

Group	DMA (p.p.m.)	Effective no. of rats[a]	Total (%)	No. of rats bearing		Papilloma incidence (%)	Carcinoma incidence (%)
				One tumor	Two tumors		
1	200	31	12 (39)[b]	10	2[e]	2 (6)	12 (39)[b]
2	50	31	8 (26)[c]	8	0	2 (6)	6 (19)[d]
3	12.5	33	0	0	0	0	0
4	0	28	0	0	0	0	0

[a]Rats that survived more than week 97.
[b~d]Significantly different from groups 3 and 4 at [b]$P < 0.001$, [c]$P < 0.01$, [d]$P < 0.05$.
[e]Two rats had both papilloma and carcinoma.

The exact mechanism of DMA carcinogenesis remains unclear. A number of studies have indicated that oxygen radicals may participate in the carcinogenic process, including the stages of initiation, promotion and progression (39–42). Yamanaka *et al.* (25–27) revealed that DMA induced DNA damage in the mouse and rat lung due to both oxygen radicals and subsequent radicals produced in the metabolism of DMA. Wanibuchi *et al.* (32) reported that DMA treatment significantly increased the formation of 8-hydroxydeoxyguanosine, which is formed by agents producing oxygen radicals. These results also indicated that DMA or its metabolites maybe cause DNA damage via oxygen radicals in rat hepatocarcinogenesis (32,43). Therefore, it is reasonable to suggest that DMA might induce urinary bladder tumors via a mechanism involving the generation of oxygen radicals.

Although DMA has shown DNA damage effects in many *in vitro* studies (23–27), DMA is negative in most mutagenicity studies and does not appear to react with DNA directly (28,29). However, DMA indirectly damages DNA by generating active oxygen radicals, as mentioned above (25–27). Our previous results indicated that DMA stimulated cell proliferation in the urinary bladder epithelium (31). Cohen and Ellwein (44) suggested that an increase in cell proliferation can account for the carcinogenecity of non-genotoxic compounds. To understand the actual mechanism of induction of cell proliferation by DMA, further study is necessary. As is well known, some non-genotoxic compounds exert carcinogenic activity only at high doses (45–47). This is particularly true for non-genotoxic chemicals. Non-genotoxic compounds such as 4-ethylsulfonyl-naphthalene-1-sulfonamide (47), uracil (48) and melamine (49) induce urinary bladder tumors by producing urinary calculi in rats when given p.o. at high doses for long periods. Previous studies involving 32 weeks treatment with DMA at high doses induced neither formation of bladder tumors nor preneoplastic lesions (31). The present results suggest that exposure to DMA for long periods is required for formation of urinary bladder tumors. Indeed, the first urinary bladder tumor was observed in a rat that died at week 97. In addition, even the rats bearing urinary bladder tumors in the high dose DMA treatment groups survived until the end of the present experiment. In this respect, DMA is most likely to be a weak carcinogen. However, in the present study there was no calculus formation in the urinary bladder. In this sense it differs from non-genotoxic chemicals such as uracil and melamine.

In conclusion, our present study revealed that DMA is carcinogenic for the urinary bladder of male rats. It provides experimental support for the epidemiological data associating arsenic exposure to the development of bladder cancer. The possibility, therefore, exists that DMA plays a role in arsenic carcinogenicity in humans.

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