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Original Article

Dimethylarsinic acid may promote prostate carcinogenesis in rats

Shugo Suzuki[1]*, Takeshi Toyoda[2], Hiroyuki Kato[1], Aya Naiki-Ito[1], Yoriko Yamashita[1], Jun-ichi Akagi[2], Young-Man Cho[2], Kumiko Ogawa[2], and Satoru Takahashi[1]

[1] Department of Experimental Pathology and Tumor Biology, Nagoya City University Graduate School of Medical Sciences, 1 Kawasumi, Mizuho-Cho, Mizuho-Ku, Nagoya, Aichi 467-8601, Japan
[2] Division of Pathology, National Institute of Health Sciences, 3-25-26 Tonomachi, Kawasaki, Kanagawa 210-9501, Japan

Abstract: Arsenic is a known human carcinogen, inducing tumors of the lung, urinary bladder, skin, liver and prostate. However, there are no reports of prostate tumors induced by arsenicals in *in vivo* animal models. In a previous study, we found that HMGB2 expression was a predictive marker for prostate carcinogens in the rat 4-week repeated dose test. In this study, six-week-old male F344 rats were orally treated with a total of six chemicals (2-acetylaminofluorene (2-AAF), p-cresidine, dimethylarsinic acid (DMA), glycidol, N-nitrosodiethylamine and acrylamide) for four weeks. Animals were sacrificed at the end of the study, and HMGB2 and Ki-67 immunohistochemistry was performed. The numbers of HMGB2- and Ki-67- positive cells in all prostate lobes were significantly increased by DMA, one of the arsenicals, compared with the controls. Meanwhile, the number of Ki-67-positive cells in lateral and dorsal prostate lobes was significantly decreased by 2-AAF with the reduction of body weight, but HMGB2 expression was not. The other chemicals did not change HMGB2 and Ki-67 expression. These data indicate that DMA may have an ability to enhance prostate carcinogenesis. (DOI: 10.1293/tox.2018-0050; J Toxicol Pathol 2019; 32: 73–77)

Key words: dimethylarsinic acid (DMA), HMGB2, carcinogens, rat, prostate, carcinogenesis

Introduction

Arsenic is a known human carcinogen, inducing tumors of the lung, urinary bladder, skin, liver and prostate in individuals exposed to high concentrations, primarily through drinking water[1]. The significant dose-response relationships between the level of arsenic in drinking water and the risk for prostate cancer mortality were reported in Taiwan[2]. However, there were no reports of prostate tumors induced by arsenic in *in vivo* animal studies[1, 3].

Recently, we investigated alternative molecular markers for the detection of prostatic carcinogens in a short period in rats, and found that high-mobility group protein B2 (HMGB2) expression is an useful screening tool for the identification of prostate carcinogens[4]. In this report, we evaluated immunohistochemistry of HMGB2 in the prostate of rats which underwent the standard repeated dose 28-day oral toxicity study, and detected up-regulation of HMGB2 with prostate carcinogens. Meanwhile, there were no changes of HMGB2 positivity with chemicals that were carcinogens but not carcinogenic in the prostate. To increase the validity of the test method (using HMGB2 expression in prostate lobes with the 28-day toxicity study), we increased the number of test-chemicals and shared prostate samples from an experiment which investigated genotoxic urinary bladder carcinogens with immunohistochemistry for γ-H2AX[5]. The test-chemicals were carcinogens and mostly classified into the Group 2 category of carcinogenic risk evaluation by the International Agency for Research on Cancer (IARC; https://monographs.iarc.fr/list-of-classifications-volumes/), but were not reported as prostate carcinogens. It was found that HMGB2 and/or Ki-67 expression was changed in the prostate of rats treated with dimethylarsinic acid (DMA) and 2-acetylaminofluorene (2-AAF).

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*Corresponding author: S Suzuki
(e-mail: shugo@med.nagoya-cu.ac.jp)

Materials and Methods

Chemicals

The chemical used in the present study were as follows: 2-AAF (Tokyo Chemical Industry, Tokyo, Japan; Lot No. 243BD; purity, 99.8%), p-cresidine (Sigma-Aldrich, St. Louis, MO, USA; Lot No. BCBF1417V; purity, 99.5%), DMA (Sigma-Aldrich; Lot No. BCBJ3595V; purity, 100%), glycidol (Wako Pure Chemical Industries, Osaka, Japan; Lot No. PDM3910; purity, 97.0%), N-nitrosodiethylamine (DEN; Tokyo Chemical Industry; Lot No. FBMVM; purity,

99.9%), and acrylamide (AA; Wako; Lot No. PDJ0711; purity, 100%).

Animals

Five-week-old male F344/DuCrlCrlj rats were obtained from Charles River Laboratories Japan (Atsugi, Japan). They were housed in plastic cages with hardwood chip bedding in an air-conditioned room at 23 ± 2°C and 55 ± 5% humidity with a 12-h light/dark cycle and maintained on a basal certified diet (CRF-1; Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water *ad libitum*. The experimental design was approved by the Animal Care and Utilization Committee of the National Institute of Health Sciences, Japan, and the animals were cared for in accordance with institutional guidelines as well as the Guidelines for Proper Conduct of Animal Experiments (Science Council of Japan, June 1, 2006).

Animal experiment

At the beginning of the experiments, the animals were randomly allocated to seven groups of five rats each based on their body weights (measured just before starting chemical treatment). Animals were administered 0.025% 2-AAF (carcinogenic to the bladder, renal pelvis, liver, pancreas and lung)[6], 1.0% p-cresidine (bladder and olfactory neuroblastoma)[7], 0.02% DMA (bladder)[8], 0.04% glycidol (tunica vaginalis, mammary gland, brain and forestomach)[9], 0.001% DEN (liver, esophagus, kidney, lung and nasal cavity)[10], and 0.005% AA (thyroid gland, testes, heart, pancreas, clitoral gland, mammary gland, oral cavity and skin)[11] in their drinking water with light-shielded bottles (DMA, glycidol, DEN, and AA) or in basal diet (2-AAF and p-cresidine) for four weeks. All chemicals were administered at carcinogenic doses reported in previous studies. The diet and water were changed once and twice per week respectively. At experimental week 4, the animals were sacrificed by exsanguination under inhalation anesthesia with isoflurane, and subjected to laparotomy with excision of the prostate. The urogenital complex of each rat was removed as a whole together with the seminal vesicles, and then the ventral prostate was weighed. All prostate tissues were fixed in 10% formalin, routinely processed to paraffin-embedded sections, and stained with hematoxylin and eosin (H&E). Necropsy was performed on one rat which died on day 20; the liver, kidneys and urinary bladder were removed and fixed in 10% formalin, routinely processed to paraffin-embedded sections, and stained with H&E.

Immunohistochemistry

Prostate sections were treated with rabbit monoclonal HMGB2 antibody (Abcam plc, Cambridge, UK) or rabbit monoclonal Ki-67 for rats (Agilent Technologies, Santa Clara, CA, USA), followed by staining with BOND-MAX (Leica Biosystems, Wetzlar, Germany) according to the manufacturer's instructions. The numbers of HMGB2- or Ki-67-positive cells in at least 1,000 luminal cells of each prostate lobe were counted to determine their labeling indices and compared with the respective lobe of the control rats.

Statistical analyses

Statistical analyses were performed with Prism ver. 6 (GraphPad Software, Inc., La Jolla, CA, USA). Values are presented as mean ± SD values obtained using 1-way ANOVA and Dunnett's test. $P<0.05$ was considered statistically significant.

Results

One rat treated with 0.02% DMA died on day 20, and severe tubular necrosis of the kidney was histopathologically observed. As this was considered to be a toxic effect of DMA, the administration dose for the final week was changed to 0.01%, which is still in the range of the carcinogenic dose[12]. Body weight gain was significantly reduced in rats receiving 2-AAF, p-cresidine or DMA as compared with that of the controls (data not shown), and final body weights were significantly lower than that of the controls (Table 1). Ventral prostate weight was significantly reduced in 2-AAF-treated rats compared with that of the control (Table 1).

There were some atrophic changes in all prostate tissues of 2-AAF-treated rats (Fig. 1). In immunohistochemistry, DMA significantly increased the number of HMGB2-positive cells in all prostate lobes (ventral, lateral and dorsal) compared with the control in rats. In contrast, there was no statistical significant difference in HMGB2 positivity in the prostate lobes after exposure to the other chemicals compared with the control (Fig. 1 and Table 2). DMA also significantly increased the Ki-67 labeling index in all prostate lobes compared with the control. 2-AAF significantly reduced Ki-67 labeling index in lateral and dorsal prostate lobes compared to the control (Fig. 1 and Table 2). There was no statistically significant difference in Ki-67 expression after exposure to other chemicals in any prostate lobes compared with the respective controls (Table 2).

Discussion

Studies conducted in Taiwan (China) reported significant dose-response relationships between the level of arsenic in drinking water and the risk for prostate cancer mortality[2], however, the data from South America are not consistent with this observation[13]. Inorganic arsenic (arsenite and arsenate) is the most abundant form of arsenic in nature and is commonly present in soil, water, and food[1, 14]. Most arsenicals are metabolized and excreted into DMA in urine of humans and rodents[14–16]. DMA is also reported to be mutagenic and genotoxic at high concentrations in *in vitro* studies[17]. Therefore, the carcinogenicity of DMA is important for human risk of arsenic. Meanwhile, there are no reports of carcinogenic effects of inorganic arsenic on all organs including prostate with animal experiments[1, 3]. In the present study, we detected increased expression of

Table 1. The Final Body and Ventral Prostate Weights

Treatment	No. of rats	Body weight (g)	Ventral lobe weight (g)
Control	5	244.2 ± 9.5	0.17 ± 0.04
2-AAF	5	153.5 ± 7.3***	0.05 ± 0.02***
p-Cresidine	5	223.2 ± 7.2**	0.15 ± 0.00
DMA[a]	4	221.2 ± 9.5**	0.15 ± 0.02
Glycidol	5	230.9 ± 5.4	0.17 ± 0.01
DEN	5	239.9 ± 11.6	0.19 ± 0.01
AA	5	239.8 ± 6.2	0.18 ± 0.02

Values are means ± SDs. 2-AAF, 2-acetylaminofluorene; DMA, dimethylarsinic acid; DEN, N-nitrosodiethylamine; AA, acrylamide. [a]The administration dose was changed to 0.01% for the last week due to 1 death, which occurred because of the toxic effects of the chemical. **, ***: Significantly different from the Control at P<0.01 and 0.001, respectively.

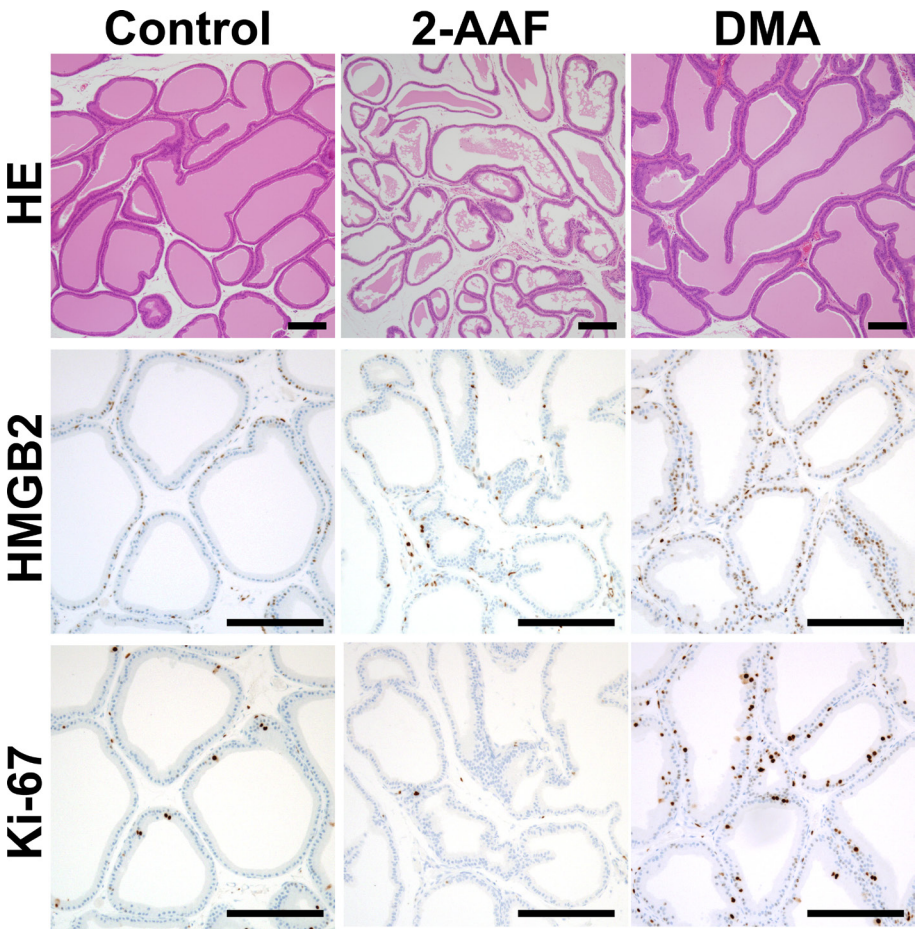

Fig. 1. Histopathology and immunohistochemistry of the ventral prostate. Photos of H&E and immunostaining of HMGB2 and Ki-67 in the ventral prostate of control, 2-AAF-treated and DMA-treated rats are presented. Bars=200 μm.

HMGB2 and Ki-67 in all prostate lobes of DMA-treated rats. In a previous study, we reported that increased expression of HMGB2 may be an useful tool for screening to identify potential prostate carcinogens with an animal model[4]. Increased expression of Ki-67, a cell proliferation marker, may be also related to some parts of carcinogenesis because chemicals are first evaluated for proliferative activity in various tissues[18]. These data suggest that DMA has a potential to induce prostate carcinogenesis. This report is the first to detect the possibility of arsenic carcinogenicity having an effect on prostate carcinogenesis in an animal model.

In the previous reports, treatment with DMA alone induced bladder tumors in rats[8, 12] and lung tumors in mice[19] but not other organs including the prostate. As we utilized a dose of DMA which was enough to induce rat urinary bladder tumorigenesis, the carcinogenic effect of DMA alone

Table 2. HMGB2 and Ki-67 Labeling Indices in Prostate

Treatment	No. of rat	HMGB2			Ki-67		
		Ventral	Lateral	Dorsal	Ventral	Lateral	Dorsal
Control	5	3.3 ± 1.8	7.5 ± 1.3	9.7 ± 1.3	2.8 ± 1.7	4.3 ± 1.4	7.2 ± 1.6
2-AAF	5	1.1 ± 0.6	5.0 ± 2.7	7.3 ± 1.5	0.6 ± 0.1	0.8 ± 0.5***	0.6 ± 0.2***
p-Cresidine	5	4.6 ± 1.5	9.4 ± 2.1	10.2 ± 1.4	2.9 ± 0.9	4.9 ± 0.5	7.6 ± 1.2
DMA	4	12.8 ± 5.6***	16.7 ± 3.0***	21.2 ± 1.5***	9.9 ± 5.7***	8.8 ± 1.8***	9.9 ± 1.8*
Glycidol	5	4.2 ± 1.9	8.6 ± 0.8	10.4 ± 1.8	2.5 ± 0.7	4.8 ± 0.7	7.9 ± 1.0
DEN	5	3.5 ± 1.2	8.6 ± 1.5	10.5 ± 1.9	2.2 ± 1.2	5.4 ± 0.7	8.1 ± 1.2
AA	5	3.3 ± 1.4	8.4 ± 0.9	9.9 ± 1.3	2.8 ± 1.3	5.1 ± 0.9	7.1 ± 0.7

Values are means ± SDs. 2-AAF, 2-acetylaminofluorene; DMA, dimethylarsinic acid; DEN, N-nitrosodiethylamine; AA, acrylamide. *, ***: Significantly different from Control group, $P<0.05$ and 0.001, respectively

may not be sufficient to induce prostate tumors in rodent animal models. Meanwhile, with regard to DMA as a promoter of carcinogenesis, DMA enhanced rat liver carcinogenesis induced by diethylnitrosamine and mouse skin carcinogenesis induced by dimethylbenz(a)anthracene[20, 21]. To investigate the promotion effect of DMA on prostate carcinogenesis, further work on experimental prostate carcinogenesis models with other genotoxic prostate carcinogens such as 3,2'-dimethyl-4-aminobiphenyl (DMAB) or 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) may be needed[22].

Regarding the carcinogenic mechanism of DMA, it has been reported that oxidative stress is one of the most important factors for arsenic metabolism and carcinogenesis[23, 24]. Some reports indicated that DMA administration caused an elevation of 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels, a biomarker for oxidative stress[25], in the lung, liver and urinary bladder[12, 26, 27]. Therefore, oxidative stress may play an important role in the tumorigenic process of DMA-induced carcinogenesis. Oxidative stress has also been reported to contribute to the development and progression of human prostate cancer[28, 29]. Additionally, we detected that apocynin, an NADPH oxidase inhibitor, inhibited oxidative stress in prostate tissue and suppressed rat prostate carcinogenesis[30]. These data suggest that DMA has the possibility to induce prostate carcinogenesis via oxidative stress.

The other chemicals are known to be carcinogens, and most are listed in the Group 2 category by IARC (Group 2A, glycidol, DEN and AA; Group 2B, p-cresidine). There was no information from animal experiments indicating that these chemicals were prostate carcinogens[6–10]. In addition, no epidemiological study data were available (glycidol, DEN, p-cresidine and 2-AAF), and there was no evidence of association with prostate cancer risk (AA)[31]. In a previous report, the four chemicals that are not carcinogenic to prostate did not induce to up-regulation of HMGB2[4]. These data support our results showing that up-regulation of HMGB2 expression is the specific response for prostate carcinogens.

In conclusion, we found that DMA has the possibility to enhance prostate carcinogenesis. Further work is needed to investigate the carcinogenicity of other arsenicals in prostate and the mechanisms of prostate carcinogenesis caused by DMA treatment.

Disclosure of Potential Conflicts of Interest: The authors declare no conflict of interest.

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