Cancer Induction by an Organic Arsenic Compound, Dimethylarsinic Acid (Cacodylic Acid), in F344/DuCrj Rats after Pretreatment with Five Carcinogens

Shinji Yamamoto, Yoshitsugu Konishi, Tsutomu Matsuda, Takashi Murai, Masa-Aki Shibata, Isao Matsui-Yuasa, Shuizo Otani, Koichi Kuroda, Ginji Endo, and Shoji Fukushima

First Department of Pathology [S. Y., Ts. M., Ts. M., M.-A. S., S. F.], Second Department of Biochemistry [H. Y., S. O.], and Department of Preventive Medicine and Environmental Health [Y. K., G. E.], Osaka City University Medical School, 1-4-54 Asahi-machi, Abeno-ku, Osaka 545, and Osaka City Institute of Public Health and Environmental Sciences, Osaka 543 [K. K.], Japan

ABSTRACT

Arsenic (As) is environmentally ubiquitous and an epidemiologically significant chemical related to certain human cancers. Dimethylarsinic acid (cacodylic acid; DMA) is one of the major methylated metabolites of ingested arsenicals in most mammals. To evaluate the effects of DMA on chemical carcinogenesis, we conducted a multiorgan bioassay in rats given various doses of DMA. One-hundred twenty-four male F344/DuCrj rats were divided randomly into 7 groups (20 rats each for groups 1–5; 12 rats each for groups 6 and 7). To initiate multiple organs and tissues, animals in groups 1–5 were treated sequentially with diethylstilbestrol (100 mg/kg body weight, i.p., single dose at the commencement) and N-methyl-N-0-nitrosoureya (20 mg/kg body weight, i.p., 4 times, on days 5, 8, 11, and 14). Thereafter, rats received 1,2-dimethylhydrazine (40 mg/kg body weight, s.c., 4 times, on days 18, 22, 26, and 30). During the same period, the animals were sequentially administered N-butyl-N-(4-hydroxybutyl)nitrosamine (0.05% in the drinking water, during weeks 1 and 2) and N-bis(2-hydroxypropyl)nitrosamine (0.1% in the drinking water, during weeks 3 and 4; DMBDD treatment). After a 2-week interval, groups 2–5 were given 50, 100, 200, or 400 ppm DMA, respectively, in the drinking water; Groups 6 and 7, which were not given DMBDD treatment, received 100 and 400 ppm DMA during weeks 6–30. All rats were killed at the end of week 30. In the initiated groups (groups 1–5), DMA significantly enhanced the tumor induction in the urinary bladder, kidney, liver, and thyroid gland, with respective incidences in group 5 (400 ppm DMA) being 80, 65, 65, and 45%. Induction of preneoplastic lesions (glutathione S-transferase placental form-positive foci in the liver and atypical tubules in the kidney) was also significantly increased in DMA-treated groups. Ornithine decarboxylase activity in the kidneys of rats treated with 100 ppm DMA was significantly increased compared with control values (P < 0.001). In conclusion, DMA is acting as a promoter of urinary bladder, kidney, liver, and thyroid gland carcinogenesis in rats, and we speculate that this may be related to cancer induction by As in humans.

INTRODUCTION

As is ubiquitously distributed in nature (1, 2), and there have been various kinds of exposure for humans. Industrial (3), agricultural (4), environmental (1, 5, 6), and iatrogenic arsenical exposures (7) have been reported and discussed repeatedly. Residents in certain areas in Taiwan and Mexico are exposed to high amounts of As via the drinking water (5, 8). Moreover, the wide population in the United States may be supplied with water containing more than 50 µg/l As (6). Industrial arsenicals are used for smelting, glass making, and the manufacture of semiconductors (3, 9). For more than half a century, various carcinogenic effects of As for humans have been documented, mainly involving the skin and lung (7). In addition, recent epidemiological studies have indicated that there are significant dose-response relationships between inorganic As ingestion and cancer incidences of the kidney, urinary bladder, liver, and other internal organs (5, 6).

DMA is a major form of As in the environment (1) as well as being used as a general herbicide or pesticide for many years (4). Furthermore, DMA is one of the major methylated metabolites of ingested organic (10) or inorganic (11) As in most mammals, including humans (3, 4, 12, 13), and is eliminated by the kidney and finally excreted in urine. Despite most As compounds being negative in some mutagenicity assays (14), recent in vitro findings have revealed that DMA appears to be a potent clastogenic agent and induces chromosome aberrations, such as tetraploid formation (15, 16). Yamanaka et al. (17) suggested that single-strand breaks of DNA induced by DMA can be mainly attributed to DNA adduct formation. Therefore, it is a compound of some environmental significance, and we speculated on the possible participation of DMA in the tumorigenesis of some organs.

Although some investigators have undertaken to determine carcinogenic effects of DMA in vivo, no unequivocal data have been published (2, 18). To elucidate any carcinogenic effects of DMA in various organs, we conducted a multiorgan carcinogenesis bioassay in rats which has been developed for assessment of carcinogenic potential of test agents at a variety of target sites (19–21). We carefully investigated various organs by histopathological procedures. Moreover, we evaluated some biological markers of cell proliferation, including the two enzymatic activities ODC and SAT, in the kidneys and lungs, which are rate-limiting enzymes of polyamine biosynthesis and reported to be increased in the promoting stage of skin and bladder carcinogenesis (22, 23). Concentrations of three kinds of polyanines, putrescine, spermine, and spermidine in the kidney, were also measured.

MATERIALS AND METHODS

Animals. Male F344/DuCrj rats were obtained at 5 weeks of age from Charles River Japan, Inc. (Hino, Japan). Rats were divided randomly into 7 groups (20 rats each for groups 1–5; 12 rats each for groups 6 and 7), were housed 5/steel cage, and fed common basal diet MF (Oriental Yeast Co., Ltd., Tokyo, Japan) and water ad libitum. The animals were kept at a temperature of 23 ±1°C with a 12-h light/dark cycle. After a 1-week acclimation period, they were used in this study.

Chemicals. DEN and DMH were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), BBIN from Tokyo Kasai Kogyo Co., Ltd. (Tokyo, Japan), and MNU and DHF from Iwai Chemical Co. (Tokyo, Japan). DMA was purchased from Wako Pure Chemical Industries (purity 99%).
DIMETHYLARSINIC ACID ENHANCEMENT OF CARCINOGENESIS

Experimental Design. The experimental protocol of this bioassay system is shown in Fig. 1. To establish wide-spectrum initiation of organs and tissues, animals in groups 1–5 were treated sequentially with DEN (100 mg/kg body weight, i.p., single dose), MNU (80, 20 mg/kg body weight, i.p.; 4 times, on days 5, 8, 11, and 14). Thereafter, rats received DMH (40 mg/kg body weight, s.c., 4 times, on days 18, 22, 26, and 30). During the same period, the animals were sequentially administered BBN (0.05% in the drinking water, during weeks 1 and 2) and DHPN (0.1% in the drinking water, during weeks 3 and 4; DMBDD treatment). After a 2-week interval, groups 2–5 were given 50, 100, 200, or 400 ppm DMA during weeks 6–30. Throughout the experiment, all rats had free access to food and water, and body weights were measured each week. At week 10, fresh urine was collected by forced urination, immediately frozen, and stored until use for measurement of As concentrations. All survivors were killed by exsanguination under ether anesthesia at week 30.

Tissue Processing and Histopathological Diagnosis. All major organs were excised and fixed in 10% buffered formalin except for the forestomach and glandular stomach. After adequate fixation, the nose of each rat was removed from the head, trimmed of extraneous tissue, and decalcified for approximately 3 days. They were sectioned into three levels for further processing as described by Young (25). All organs and tissues excised were embedded in paraffin and stained with hematoxylin and eosin for histological examination. Liver slices fixed in ice-cold acetone and stomach slices in sublimated formaldehyde were embedded in paraffin and used for quantitative assessment of putative preneoplastic lesions, GST-P-positive foci in the liver (26) and PAPG in the glandular stomach (27). Frozen tissues of both kidneys and both lungs were used for measurements of enzymatic activity of ODC and SAT and concentrations of three kinds of polyamines.

Examination of GST-P-positive Foci in the Liver and PAPG in the Glandular Stomach. We evaluated preneoplastic lesion markers for assessment of carcinogenic activity, i.e., immunohistochemically demonstrated GST-P-positive foci in the liver (26) and PAPG in the glandular stomach (27). The liver tissues in groups 4 and 5 were not evaluable for preneoplastic lesions because tumor induction was so pronounced that the tumors occupied most of the liver. In groups 2 and 3, materials containing liver tumors (hyperplastic nodule or hepatocellular carcinoma) were excluded from the evaluation. For the assessment of PAPG, stomach tissues in groups 1, 3, and 5 were used. Sublimated formaldehyde for fixation of the stomach was made from 6% HgCl₂ in a mixture of 5% acetic acid and 4% formaldehyde. The avidin-biotin-peroxidase complex method (28) was used to determine the location of GST-P binding in the liver and PG1 binding in the pyloric mucosa. After deparaffinization, the liver and glandular stomach sections were treated with rabbit anti-rat GST-P (1:5,000) and rabbit anti-rat PG1 (1:10,000), respectively. The sites of peroxidase binding were demonstrated by the diaminobenzidine method. Sections were then counterstained with hematoxylin for microscopic examination. The numbers and areas of GST-P-positive foci in the liver sections and the length of pyloric mucosa in each histological slide for quantitative analysis of PAPG were measured with the aid of a color video image processor (VIP-21 C; Olympus-Ikegami Tsushin Co., Tokyo, Japan.). Areas of bilateral renal cortex sections stained with hematoxylin and eosin were also measured for quantitative analysis of the putative preneoplastic lesion, atypical tubules (29), by image analyzer.

Measurement of ODC and SAT Activities and Polyamine Biosynthesis. Two enzymatic activities, ODC and SAT, as biomarkers of cell proliferation in kidneys and lungs were measured by the methods of Matsui et al. (30) and Otani et al. (31), respectively. The animals without DMA administration (group 1) served as controls. DMBDD plus 100 ppm of DMA (group 3) was chosen for evaluation. At the time of terminal sacrifice, frozen pieces of rat kidney and lungs were suspended in 0.5 ml of 50 mM Tris (pH 7.5) containing 0.25 M sucrose and disrupted by 10 strokes with a Potter-Elvehjem homogenizer. The homogenized suspensions were centrifuged at 100,000 × g for 30 min, and the supernatant was assayed for ODC and SAT activity by measurement of the amount of radioactive putrescine produced from [5-14C]ornithine and the amount of acetyl moiety transferred from [1-14C]acetyl-CoA to spermidine, respectively. Some frozen kidney tissues were used for the measurement of three polyamines. They were homogenized in 4 volumes of ice-cold 5% trichloroacetic acid and centrifuged for removal of protein. The concentrations of polyamines in the acid extract were analyzed by HPLC (Shimadzu LC-6A; Shimadzu, Kyoto, Japan) equipped with a fluorescence detector.

Concentration and Speciation of As in Urine. Using the urine samples collected at week 10, five As species, MMA, DMA, TMA, arszenobetaine, and As³⁺ were analyzed with the aid of IC-ICP-MS (32, 33). We applied ion chromatography (Model IC 7000; Yokogawa Analytical Systems, Inc., Tokyo, Japan) with two anion exchange columns (Excelpak ICS-A35; Yokogawa Analytical Systems) as the separation technique and ICP-MS (Model PMS 2000; Yokogawa Analytical Systems) for detection. Briefly, urine samples were diluted 10-fold, and 50 μl were used for analysis. For separation of the five As species, IC was operated under the following conditions: mobile phase, 10 mM tartaric acid; flow-rate, 1.0 ml/min; and column temperature, 50°C. The eluent of the ion chromatography column was directly introduced into the nebulizer of the ICP-MS and analyzed at monitoring mass 75.

Statistical Analysis. The significance of differences between mean values was analyzed using Student's t test. The significance of differences in lesion incidence between groups was assessed by Fisher's exact probability test. Two-tailed Cochran-Armitage analysis was used to evaluate the dose-response relationships for lesion incidences in groups 1–5. For the assessment of mean values of preneoplastic lesions (GST-P-positive foci, PAPG, and atypical tubule), the one-tailed Dunnett multiple comparison procedure was used.

RESULTS

Survival and Main Cause of Death. In DMBDD-treated groups (groups 1–5), only seven animals were found dead or killed during the experiment. One animal of group 2 given 50 ppm DMA and three animals of group 3 given 100 ppm DMA died of thymic lymphoma during weeks 24–27. One animal of group 1 (control group) died at week 29 because of occlusion due to an esophageal carcinoma. In groups 4 and 7, which were not initiated but treated with DMA, all rats survived for 30 weeks and maintained a relatively healthy appearance throughout the experiment. All rats were considered in the effective number with one exception in Group 3. Some organs of one animal in group 3 were not evaluable because of postmortem changes.

Body Weight and Relative Organ Weight. During the DMBDD treatment, body weight gain in the treated groups was less compared to that of nontreated rats. We observed moderate body weight suppression by DMA at week 10; conversely, mean body weights became higher in the high-dose groups at the end of the experiment (groups 1 versus 3 and 4; P < 0.01; Student's t test). In the tumor-bearing organs, relative organ weights of liver and kidneys were higher in DMA high-dose groups; however, in the lungs and thymus, relative

![Fig. 1. Experimental protocol of multiorgan carcinogenesis bioassay (DMBDD mod.)](image-url)
organ weights were decreased as higher amounts of DMA were given (data not shown).

**Water Intakes and the Concentrations of As in Urine.** Water intake values (average of measurements made at weeks 12, 18, 24, and 30) in group 5 were about two times higher than in the control group 1 \( (P < 0.001) \). Higher amounts of water intake and DMA intake were related to DMA doses. In groups 1–5, concentrations of DMA in urine samples were 0.11, 5.4, 20.0, 35.4, and 54.7 \( \mu g/ml \) as As, respectively. Proportions of DMA in the total As content in urine of groups 1–7 were 49, 22, 46, 59, 59, 40, and 56%, respectively. Although the main As metabolite in urine samples was DMA as described in most previous reports \((10)\), the proportion of TMA was relatively high, and less As was excreted as inorganic As \((As^{III})\) in this experiment (data not shown). The amount of MMA was smaller, and arsenobetaine was not detected in any groups.

**Histopathological Examination and Tumor Incidences.** Neoplasms were found in several organs, suggesting a specific organotropism of DMA. Histopathology and tumor incidences in various organs are indicated in Table 1. In DMBDD-treated groups (groups 1–5), DMA significantly enhanced the tumor induction in the urinary bladder, kidneys, liver, and thyroid gland.

Macroscopically, most bladder tumors induced by DMA were whitish and showed papillary growth. Bladder calculi were not observed in any groups. Neoplastic urinary bladder-epithelial lesions were divided into papillomas and TCCs \((34)\). In DMBDD-treated animals, urinary bladder carcinogenesis was markedly enhanced by DMA, even in the lowest dose group (number of tumor-bearing animals, group 1 versus 2; \( P < 0.001 \); Table 1). A neoplastic lesion, papillary or nodular hyperplasia \((34)\), was significantly observed in all DMBDD plus DMA groups. We observed four invasive TCCs in the higher DMA groups (each two animals of groups 4 and 5), one squamous cell carcinoma arose within TCC, and one carcinoma \( \text{in situ} \) in the same animal of group 5. In groups 6 and 7 (without DMBDD treatment and DMA-treated groups), no tumor was observed, as well as no neoplastic lesions.

The numbers of kidney tumors (adenomas and/or adenocarcinomas) were significantly increased in a dose-dependent manner (Table 1; \( P < 0.001 \); two-tailed Cochran-Armitage analysis). All renal adenomas and adenocarcinomas were basophilic and solid. But all carcinomas were well differentiated, noninvasive, and revealed no evidence of metastasis to other distant organs. No tumor was observed in groups 6 and 7. Typical toxic lesions in the renal cortex, such as massive tubular necrosis, were not commonly observed, even in the

### Table 1 Incidences of preneoplastic and neoplastic lesions in various organs from rats that received DMBDD treatment

<table>
<thead>
<tr>
<th>Organ and Dx*</th>
<th>Groupb</th>
<th>Two-tailed Cochran-Armitage analysisc</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Urinary bladder</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PN hyperplasia</td>
<td>4 (20)</td>
<td>13 (65)f</td>
</tr>
<tr>
<td>Papilloma *</td>
<td>1 (5)</td>
<td>12 (60)f</td>
</tr>
<tr>
<td>TCC *</td>
<td>1 (5)</td>
<td>10 (50)f</td>
</tr>
<tr>
<td>No. of tumor-bearing animals</td>
<td>2 (10)</td>
<td>17 (85)f</td>
</tr>
<tr>
<td><strong>Kidney</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenoma *</td>
<td>1 (5)</td>
<td>3 (15)</td>
</tr>
<tr>
<td>Adenocarcinoma *</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Renal cell tumor</td>
<td>1 (5)</td>
<td>3 (15)</td>
</tr>
<tr>
<td>Nephroblastoma</td>
<td>4 (20)</td>
<td>0</td>
</tr>
<tr>
<td>No. of tumor-bearing animals</td>
<td>5 (25)</td>
<td>3 (15)</td>
</tr>
<tr>
<td><strong>Liver</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Altered cell foci</td>
<td>10 (50)</td>
<td>12 (60)</td>
</tr>
<tr>
<td>Basophilic foci</td>
<td>1 (5)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Eosinophilic foci</td>
<td>1 (5)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Hyperplastic nodule</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HCC</td>
<td>0</td>
<td>2 (10)</td>
</tr>
<tr>
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<td>0</td>
</tr>
<tr>
<td>Hemangioma</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No. of tumor-bearing animals</td>
<td>0</td>
<td>2 (10)</td>
</tr>
<tr>
<td><strong>Thyroid gland</strong></td>
<td></td>
<td></td>
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<tr>
<td>Hyperplasia</td>
<td>3 (15)</td>
<td>4 (20)</td>
</tr>
<tr>
<td>Adenoma</td>
<td>2 (10)</td>
<td>1 (5)</td>
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<tr>
<td>Adenocarcinoma</td>
<td>1 (5)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>No. of tumor-bearing animals</td>
<td>3 (15)</td>
<td>2 (10)</td>
</tr>
<tr>
<td><strong>Nasal cavity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperplasia</td>
<td>8/19/4 (42)</td>
<td>3/18 (16.6)</td>
</tr>
<tr>
<td>Papilloma/adenoma *</td>
<td>3/19 (15.8)</td>
<td>1/18 (5.6)</td>
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<tr>
<td>Carcinoma *</td>
<td>2/19 (10.5)</td>
<td>1/18 (5.6)</td>
</tr>
<tr>
<td>No. of tumor-bearing animals</td>
<td>5/19 (26.3)</td>
<td>2/18 (11.1)</td>
</tr>
<tr>
<td><strong>Lung</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperplasia</td>
<td>20 (100)</td>
<td>20 (100)</td>
</tr>
<tr>
<td>Adenoma</td>
<td>10 (50)</td>
<td>5 (25)</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>0</td>
<td>1 (5)</td>
</tr>
<tr>
<td>SCC</td>
<td>1 (5)</td>
<td>0</td>
</tr>
</tbody>
</table>

*a* Dx: diagnosis; PN: papillary or nodular; HCC: hepatocellular carcinoma; SCC: squamous cell carcinoma. Hematoxylin and eosin-stained sections are marked with (*) and are totaled in "No. of tumor-bearing animals." **: totaled in "Renal cell tumor."  
b Groups 1, 2, 3, 4, and 5: 0, 50, 100, 200, and 400 ppm DMA, respectively. Percentages are in parentheses.  
c Some organs of one animal in group 3, which died of thymic lymphoma, were not evaluable because of postmortem changes.  

d The significance of differences in lesion incidence between groups was assessed using the Fischer's exact probability test. To evaluate the dose-response relationships of lesion incidences in the kidney, liver, thyroid gland, and nasal cavity, two-tailed Cochran-Armitage analysis was used. NE, not examined.

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* Significantly different from group 1 at \( P < 0.05 \); \( P < 0.01 \), and \( P < 0.001 \).

** Significantly different from group 2 at \( P < 0.05 \), \( P < 0.01 \), and \( P < 0.001 \).

† Significantly different from group 3 at \( P < 0.05 \).

‡ Significantly different from group 4 at \( P < 0.05 \).

§ Significantly different from group 5 at \( P < 0.05 \).

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highest DMA groups (groups 5 and 7). Atypical tubules, which have a basophilic cytoplasm and occasionally contain granules, have been regarded as a preneoplastic lesion in the renal cortex (29). Numbers of atypical tubules/cm² renal cortex significantly increased with DMA administration in a dose-dependent manner (group 1 versus 5; P < 0.001; one-tailed Dunnett multiple comparison procedure; Table 2). The data for atypical tubules in groups 6 and 7 [groups 6 (0.11 ± 0.27; mean ± SD) and 7 (0.14 ± 0.18)] were lower than for group 1.

Immunohistochemically demonstrated GST-P-positive liver cell foci, a putative preneoplastic lesion, have been assessed as a marker for rat liver carcinogenesis (26). The moderate dose of DMA administration following DMBDD treatment (100 ppm; group 3) significantly enhanced the mean numbers and areas per unit area (cm²) of GST-P-positive foci compared to the DMBDD-alone group (group 1 versus 3; P < 0.001; Table 2). Neoplastic and preneoplastic lesions diagnosed on the hematoxylin and eosin sections were classified into categories as listed in Table 1. There were dose-response relationships between the given dose of DMA and occurrence of altered cell foci (P < 0.001). High incidences of hyperplastic nodules and hepatocellular carcinoma were observed in rats administered more than 200 ppm DMA (Table 1), usually bearing more than two tumors per liver. Most carcinomas were solid or trabecular; other types (papillary or tubular) were rare. Liver tissues in DMA-treated rats without DMBDD retained normal features without evidence of toxicological changes, except for one basophilic focus observed in group 7.

Follicular hyperplasia, which contains rich colloids and has ballooning follicles with atypical epithelial cells, was commonly observed in high DMA-treated groups (Table 1). The incidences of both follicular and C-cell adenoma/adenocarcinoma in DMA-treated groups were significantly higher than in the control group (group 1 versus 5; P < 0.05; Table 1). Solid and poorly differentiated adenocarcinoma was the most common type in the high DMA dose groups. Colloidal follicles of the thyroid gland appeared essentially normal. There was no remarkable pathological changes in the animals of groups 6 and 7.

Contrary to the positive effects of DMA described above, tumor induction of the nasal cavity tended to be suppressed by DMA (group 1 versus 5; P < 0.05; Table 1). Most of the tumors arose from the transitional epithelium and had both papillary and inverted growth patterns.

**Lung, Skin, and Other Organs.** All rats which received the DMBDD treatment developed alveolar hyperplasia of the lung (Table 1). In addition, there were no other remarkable pathological changes in DMA-treated lungs. We could find no evidence of promoting effects of DMA on respiratory carcinogenesis. Pathologically, no remarkable changes were observed in the skin. Only one sebaceous squamous cell carcinoma of the cheek and one s.c. fibroma on the back were present in the DMBDD plus DMA groups (group 2).

The alteration of Pgl₁, disappearing or preferentially decreasing in areas of rat pyloric mucosa, can be immunohistochemically detected as PAG during the early stages of stomach carcinogenesis (27). Numbers of PAPG/cm of mucosal length significantly increased in groups 3 and 5 [group 1 (4.76 ± 2.2: mean ± SD) versus group 3 (11.0 ± 3.1); P < 0.001; group 1 versus 5 (10.1 ± 3.8); P < 0.001; one-tailed Dunnett multiple comparison procedure]; however, no intergroup difference was observed between the DMBDD plus DMA groups (groups 3 and 5).

No remarkable histopathological changes were observed in other organs, even in high-dose DMA-treated rats. Overall, no metastases were observed for any tumors in any of the groups, and no neoplastic change was observed in rats without DMBDD treatment (groups 6 and 7), despite the relatively large amounts of DMA.

**ODC and SAT Activities and Polyamine Biosynthesis.** As shown in Fig. 2A, ODC activities of the kidney tissue in DMBDD plus 100 ppm DMA-treated rats were about three times higher than in DMBDD-only controls (P < 0.001). Kidney SAT activity did not differ between groups (Fig. 2B). Corresponding to these results, the concentrations of two polyamines, spermine and spermidine, in the kidney tissues were significantly higher in group 3 compared with control values (Fig. 2C). ODC and SAT activities in the initiated lung tissues displayed no increase with oral administration of DMA (Fig. 2, A and B).

**DISCUSSION**

Despite the numerous attempts to induce tumors by administration of DMA using experimental animals, no adequate data have been published (2, 18). Only a few reports using inorganic As compounds are available (35-37). Johansen et al. (18) suggested that DMA might be a promoter in rat kidney and liver carcinogenesis. The present study showed that DMA has significant enhancing effects on
As (3, 4, 9, 12, 13) and now corroborated by our data, DMA is the urothelium. In numerous reports about human exposure to inorganic mate metabolites of ingested chemicals being kept in the urinary of chemical carcinogenesis, probably related to the excreted proxi-
metabolism of ingested As show accumulation of DMA in the kidney (10-12), and most of the metabolites are significantly observed in the 400 ppm DMA group of this study. An remarkable induction of tumors was observed in the gastrointestinal tract, with the exception of preneoplastic lesions in the glandular stomach. Further examination will be required concerning As carcinogenicity of the skin as well as the gastrointestinal tract since some epidemiological studies have revealed an association of As with tumors in these organs (5-7).

Cell proliferation has long been regarded as playing an important role in each stage of chemical carcinogenesis (40) and is now thought to be reflected partially by two enzymatic activities, ODC and SAT (22, 23). We observed significantly high activity of ODC in the DMA-treated kidney. Our biochemical data indicate significant cell proliferation in 100 ppm DMA-treated kidneys, although tumor development was low. Cohen and Ellwein (40) suggested that an increase in cell proliferation can account for the carcinogenicity of nongenotoxic compounds; however, the actual mechanism of induction of cell proliferation by DMA is still unknown.

It is generally accepted that carcinogens have both initiating and promoting activities, and promoters have promoting activity without initiating activity (40, 41). In the present medium-term bioassay, we demonstrated promoting activity of DMA in carcinogenesis in four internal organs. However, considering the clastogenic (15, 16) or genotoxic (16, 17) activity of DMA, it may thus act as a carcinogen by inducing DNA strand breaks or as a promoter acting through other mechanisms for these organs.

For the purpose of evaluating any carcinogenic effects of chemical substances, various bioassay systems have been established (19, 20, 26, 27, 29). Recently, rat multiorgan carcinogenesis models have been developed for the detection of carcinogens (19, 20) and have already proven useful for this purpose. For example, Hirose et al. (21) reported that NaNO2 can enhance carcinogenesis when applied with certain phenolic antioxidants, particularly in the forestomach of rats. The lack of any possible interaction with carcinogen initiation allows effective assessment in a wide range of target organs (19). The results of such studies can be confirmed using various kinds of biomarkers, i.e., preneoplastic lesions or indications of cell proliferation as used in the present study.

There have been numerous reports concerning the possible carcinogenicity of As in limited areas on the southwest coast of Taiwan (5) and elsewhere (6, 8). Artesian well water with a high concentration of inorganic As has been used for more than 60 years in Taiwan, and these areas are known as having a high prevalence of some kinds of internal cancers. Our data are consistent with these epidemiological findings. Both inorganic and organic ingested As are methylated in the
body and mainly excreted in urine as DMA, although this methylation process had been believed to be a detoxification mechanism for inorganic As (10–13).

In summary, ingested As may act as a carcinogen or promoter in these four organs: urinary bladder, kidney, liver, and thyroid gland. DMA is used as a herbicide or pesticide in various areas of the world. Moreover, many people are consistently exposed to As by environmental or occupational routes (4–6, 8, 9). Now with the availability of an animal model of As carcinogenesis, progress might be more rapidly made in furthering our understanding of human risk to As exposures.

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