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# Decrease of Prostaglandin $E_2$ and 5-Bromo-2′-deoxyuridine Labeling but Not Prostate Tumor Development by Indomethacin Treatment of Rats Given 3,2′-Dimethyl-4-aminobiphenyl and Testosterone Propionate

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**The modifying effects of indomethacin (IM) on rat prostate carcinogenesis induced by 3,2′-dimethyl-4-aminobiphenyl (DMAB) were investigated. F344 rats were given 50 mg/kg body weight of DMAB at 2-week intervals for 20 weeks and then received IM at a dose of 20 ppm in the drinking water for 37 weeks. Separate groups additionally received testosterone propionate (TP) in Silastic tubes throughout the experiment. DMAB alone induced carcinomas *in situ* in the ventral lobe and in combination with TP caused invasive carcinomas of the dorso-lateral and anterior lobes and seminal vesicles. No clear suppression by IM of development of *in situ* carcinomas or invasive carcinomas was observed. In a short-term satellite experiment, it was revealed that prostaglandin $E_2$ ($PGE_2$) levels in the dorso-lateral prostate and seminal vesicles, but not the ventral prostate, were significantly reduced by IM and that TP itself also suppressed $PGE_2$ levels. The 5-bromo-2′-deoxyuridine labeling index in the ventral prostate was significantly decreased by IM administration. These results indicate that while IM can efficiently suppress tissue $PGE_2$ levels, it does not inhibit tumor development in the prostate or seminal vesicles of rats in the present model.**

Key words: Rat — Prostate — Indomethacin — Chemoprevention

Prostate cancer is the leading cause of cancer morbidity in males in the United States, with mortality being second only to that from lung cancer.[1, 2] In Japan, although the mortality due to prostate cancer is low, a tendency for increase with increased life expectancy has been noted.[3] Most prostate carcinomas are androgen-dependent and respond to androgen deprivation therapy. However, later they become hormone-insensitive. No effective chemotherapeutic drugs are available for hormone-independent prostatic cancers or those that become hormone therapy-insensitive. Therefore, the discovery of chemopreventive agents effective against prostate cancer is a high priority.

Prostaglandins (PGs) can enhance experimental chemical carcinogenesis, and themselves exert carcinogenic activity under certain conditions.[4] PGs are synthesized from membrane phospholipids through arachidonic acid (cyclooxygenase pathway) and indomethacin (IM), aspirin and ibuprofen are non-steroid anti-inflammatory drugs which block this synthesis.[5] They have been experimentally shown to reduce tumor development in the esophagus, colon, mammary gland, urinary bladder and pancreas.[6–12] High levels of PGs have been detected in cultured neoplastic cells.[13] Furthermore, it has been reported that the concentrations of the PG precursor arachidonic acid are significantly lower in malignant tissue than in benign prostate lesions.[13, 14]

We earlier reported that 3,2′-dimethyl-4-aminobiphenyl (DMAB) induces *in situ* carcinomas in the ventral prostate, and in combination with testosterone propionate (TP) results in the development of invasive adenocarcinoma in the dorso-lateral and anterior prostate, with occasional metastasis.[15–18] In the present study, we evaluated the chemopreventive potential of the anti-inflammatory drug IM on the development of invasive and *in situ* carcinomas in the accessory sex organs of rats in the DMAB/TP model.

## MATERIALS AND METHODS

**Animals** One hundred and sixty-two five-week-old male F344 rats were obtained from Charles River Japan, Inc., Atsugi. They were housed in plastic cages on hardwood chip bedding in an air-conditioned room at a temperature of 22±2°C with a 12-h light, 12-h dark cycle. They were maintained on Oriental MF basal diet (Oriental Yeast Co., Tokyo) and tap water *ad libitum*.

**Chemicals** DMAB was purchased from the NARD Institute Ltd., Osaka, TP from Tokyo Kasei Kogyo, Tokyo and IM from Wako Industries, Osaka.

**The prostate carcinogenesis study** The experimental protocol is shown in Fig. 1. The animals were randomly allocated to 4 groups of 25 rats each and another 2 groups of 15 rats. Rats in groups 1, 2, 4 and 5 were subcutaneously (s.c.) injected with DMAB dissolved in corn oil at a dose of 50 mg/kg body weight 10 times at 2-

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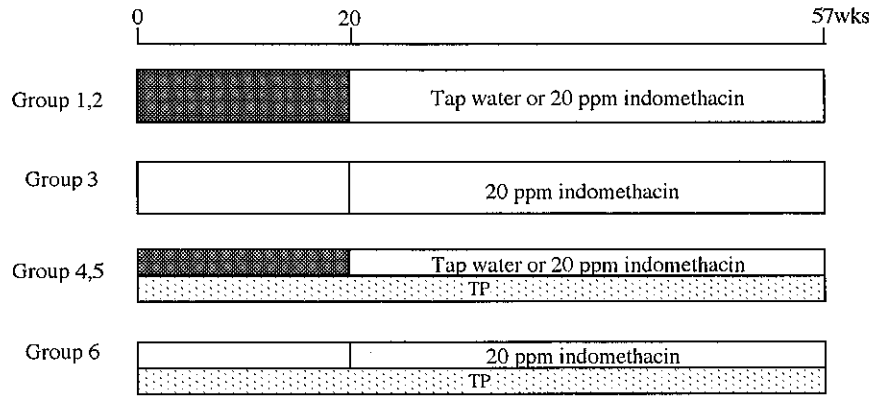


Fig. 1. Experimental protocol used for the investigation of the effects of IM on rat prostate carcinogenesis. ▒: 50 mg/kg b.w. DMAB s.c. injection 10 times 2 weeks interval. ⁞⁞: s.c. implantation of 2-cm-long Silastic tubes containing 40 mg of TP.

week intervals. The day following the first DMAB injection, in groups 4 and 5, s.c. implantation of 2-cm-long Silastic tubes containing 40 mg of TP was performed, these then being replaced at 6-week intervals until the termination of the experiment as previously described.[16-18] After the last DMAB injection, groups 2 and 5 were given 20 ppm IM in the drinking water for the 37 weeks until the end of the experiment. Groups 3 and 6 were controls given IM, and TP and IM, respectively, without the administration of DMAB. All surviving animals were killed under ether anesthesia at weeks 57. Animals that died earlier or were killed upon becoming moribund were also autopsied. All organs were examined for gross abnormalities, and the prostate, seminal vesicles, testes and gross lesions were taken and fixed in 10% buffered formalin. For tissue preparation of the accessory sex organs, two sagittal slices of the ventral prostate, sagittal samples of the dorso-lateral prostate including the urethra, and transverse samples from each side of the seminal vesicle including the coagulating glands were embedded in paraffin, and sections were stained with hematoxylin and eosin for histopathological examination.

**Investigation of DNA synthesis in the prostate and seminal vesicles** Thirty-two seven-week-old F344 rats were randomly allocated to 4 groups of 8 rats each. Rats in groups 1 and 2 were given TP as in the tumor induction experiment and, starting one week later, were respectively given 20 ppm IM in the drinking water or tap water until the end of the experiment. Groups 3 and 4 received 20 ppm IM or tap water without TP implantation. Starting 4 weeks later, 3 rats in each group were injected i.p. with 100 mg/kg b.w. 5-bromo-2′-deoxyuridine (BrdU) solution (Sigma Chemical Co., St. Louis, MO). One hour later, the animals were killed under ether anesthesia, and the prostate, seminal vesicles and liver were fixed in 10% buffered formalin. Immunohistochemical staining of incorporated BrdU using the avidin-biotin peroxidase complex method with a monoclonal antibody against BrdU was performed on paraffin-embedded sections. The numbers of cells with positively stained nuclei per 2000 cells were counted and labeling indices were expressed as percentage values.

**Measurement of $PGE_2$** The five remaining rats in each group in the satellite experiment were used for measurement of tissue $PGE_2$ levels. Samples of ventral and dorsolateral prostate, seminal vesicles and liver, about 50–100 mg, were rinsed in saline containing 0.1 m*M* IM and 10 m*M* EDTA. After the addition of 4 ml of 95% ethanol to each sample, they were homogenized and centrifuged at 3000 rpm for 15 min. The $PGE_2$ in the supernatants was measured by radioimmunoassay. The samples were stored at −80°C until measurement of $PGE_2$. Remaining tissues after centrifugation were dried at 37°C overnight. The dried tissues were completely dissolved in 5 ml of NaOH, and used to measure the total protein content of the tissues. $PGE_2$ levels were expressed as pg/mg wet tissue and pg/mg total protein.

**Statistical analysis** The significance of differences between groups in body and organ weights, values for DNA synthesis and $PGE_2$ levels was analyzed using Student's *t* test according to Welch. The significance of differences in lesion incidences between different groups was examined using Fisher's exact probability test.

## RESULTS

**The prostate carcinogenesis study** Table I summarizes final body weights and average food and water consumption data. The body weights were significantly decreased by TP implantation. Food consumption was similar in all groups, while increased water consumption was noted in the groups which received TP. Combined treatment with

Table I. Final Body Weights and Food and Water Consumption Data

| Group | Treatments | No. of[a] rats | Final b.w. (g) | Food consumption (g/rat/day) | Water consumption (g/rat/day) |
|---|---|---|---|---|---|
| 1 | DMAB | 13 | 493.3±43.3[b] | 15.5 | 22.4 |
| 2 | DMAB+IM | 18 | 424.6±34.6 | 15.1 | 20.7 |
| 3 | IM | 15 | 465.9±25.4 | 14.9 | 20.8 |
| 4 | DMAB+TP | 13 | 301.5±24.6[c] | 15.2 | 26.2 |
| 5 | DMAB+TP+IM | 9 | 321.6±19.5[c] | 14.4 | 24.5 |
| 6 | TP+IM | 13 | 330.8±16.9[c] | 15.4 | 26.8 |

*a*) At termination.
*b*) Data are mean ± SD values.
*c*) Significantly different from the respective control groups without TP implantation at $P<0.01$.

Table II. Relative Organ Weight Data

| Group | Treatments | No. of[a] rats | Relative organ weights (%) | |
|---|---|---|---|---|
| | | | testes | ventral prostate |
| 1 | DMAB | 13 | 0.76 | 0.10 |
| 2 | DMAB+IM | 18 | 0.73 | 0.14 |
| 3 | IM | 15 | 0.71 | 0.11 |
| 4 | DMAB+TP | 13 | 0.59[b] | 0.37[b] |
| 5 | DMAB+TP+IM | 9 | 0.63[b] | 0.35[b] |
| 6 | TP+IM | 13 | 0.58[b] | 0.37[b] |

*a*) At termination.
*b*) Significantly different from the respective control groups without TP at $P<0.01$.

DMAB and TP in groups 4 and 5 decreased the relative weight of the testes and increased the relative ventral prostate weight. IM treatment, however, did not clearly affect these organ weights (Table II).

Neoplastic and preneoplastic lesions in the accessory sex organs of animals given DMAB injections without TP (groups 1 and 2) were confined to the ventral and anterior prostate and seminal vesicles. The incidences of atypical hyperplasia of the ventral prostate in the DMAB and DMAB plus IM groups were 84% and 63%, and those of carcinomas were 32% and 13%, respectively. All carcinomas were of the *in situ* type. There was no statistically significant difference between the groups. The treatment with DMAB plus TP (groups 4 and 5) induced atypical hyperplasias and carcinomas of the dorsolateral and anterior prostate and seminal vesicle. The incidences of these lesions in groups 4 and 5 were similar (Table III). The carcinomas were invasive and metastatic. The main metastatic sites were the lung and liver.

Neoplastic lesions in the lung, salivary gland, small and large intestine, liver, skin/subcutis, Zymbal's gland, mammary gland and preputial gland were also observed in groups given DMAB (Table IV). However, no significant alteration in the tumor incidences was caused by the IM treatment.

**DNA synthesis in the prostate and seminal vesicles** The labeling indices of the ventral prostate assessed in terms of incorporation of BrdU into nuclei were lower than in the other lobes of the prostate and the seminal vesicles (Table V). Ventral prostate values in the groups given

Table III. Incidences of Neoplastic Lesions in the Prostate and Seminal Vesicles

| Group | Treatments | Effective no. of rats | Ventral prostate | | Dorso-lateral prostate | | Anterior prostate | | Seminal vesicles | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | AH[a] | Carcinoma | AH | Carcinoma | AH | Carcinoma | AH | Carcinoma |
| 1 | DMAB | 25 | 21 (84) | 8 (32) | 0 | 0 | 1 (4) | 0 | 23 (92) | 0 |
| 2 | DMAB+IM | 24 | 15 (63) | 3 (13) | 0 | 0 | 5 (21) | 0 | 24 (100) | 0 |
| 3 | IM | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | DMAB+TP | 24 | 4 (17) | 0 | 1 (4) | 3 (13) | 14 (58) | 11 (46) | 20 (83) | 6 (25) |
| 5 | DMAB+TP+IM | 25 | 11 (44) | 0 | 1 (4) | 3 (12) | 12 (48) | 7 (28) | 21 (84) | 8 (32) |
| 6 | TP+IM | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

*a*) AH, atypical hyperplasia.

Table IV. Tumor Incidences in Organs Other than the Accessory Sex Organs

| Group | Treatments | Effective no. of rats | Lung | Salivary gland | Small intestine | Large intestine | Liver | Skin/ subcutis | Zymbal's gland | Mammary gland | Preputial gland |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | DMAB | 25 | 1 (4) | 5 (20) | 3 (12) | 1 (4) | 0 | 6 (24) | 3 (12) | 0 | 2 (8) |
| 2 | DMAB+IM | 25 | 2 (8) | 6 (24) | 1 (4) | 1 (4) | 2 (8) | 11 (44) | 2 (8) | 1 (4) | 4 (16) |
| 3 | IM | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | DMAB+TP | 24 | 0 | 2 (8) | 1 (4) | 0 | 1 (4) | 7 (29) | 1 (4) | 0 | 0 |
| 5 | DMAB+TP+IM | 25 | 0 | 8 (32) | 2 (8) | 0 | 0 | 2 (8) | 0 | 0 | 0 |
| 6 | TP+IM | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Table V. Average BrdU Labeling Indices in the Epithelial Cells of the Prostate and Seminal Vesicles

| Group | Treatments | No. of rats | Labeling index (%) | | | |
|---|---|---|---|---|---|---|
| | | | Ventral prostate | Dorsolateral prostate | Anterior prostate | Seminal vesicle |
| 1 | TP→IM | 3 | 0.10±0.09[a, b] | 1.70±0.05 | 1.58±0.45 | 1.23±0.28 |
| 2 | TP | 3 | 0.58±0.19[c] | 1.88±0.13 | 2.12±0.48 | 0.83±0.40 |
| 3 | IM | 3 | 0.55±0.09[c] | 1.82±0.31 | 1.77±0.58 | 1.58±0.40 |
| 4 | — | 3 | 1.40±0.35 | 1.60±0.05 | 2.28±0.85 | 1.28±0.29 |

*a*) Data are mean±SD values.
*b*) Significantly different from the value for group 2 at $P<0.05$.
*c*) Significantly different from the value for group 4 at $P<0.05$.

IM (groups 1 and 3) were significantly reduced as compared with the respective control groups (groups 2 and 4). Labeling indices in the anterior prostate showed a tendency to decrease after IM treatment, but no differences were noted in the dorso-lateral prostate and seminal vesicles.

**$PGE_2$ concentrations in tissues** $PGE_2$ levels in the ventral prostate and liver were lower than in the dorso-lateral prostate and seminal vesicles (Fig. 2). TP treatment lowered the $PGE_2$ levels in all measured tissues, but without statistical significance. A significant decrease in the $PGE_2$ value due to administration of IM alone was observed in the dorso-lateral prostate and the seminal vesicle. Similar results were obtained when IM was given together with TP.

## DISCUSSION

IM has been demonstrated to be an effective agent for reducing or inhibiting chemically induced tumor development in the colon, esophagus, mammary gland, urinary bladder and pancreas.[6–12] This tumor suppression was associated with inhibition of the synthesis of PGs. Recently, the potential of non-steroidal anti-inflammatory drugs to cure colon polyposis in man has been stressed.[19] The available data thus suggest that inhibitors of the synthesis of PGs may have considerable potential as chemopreventive agents, acting on a variety of organs. However, the present results would suggest that IM does not significantly suppress development of DMAB-initiated carcinomas of the prostate and seminal vesicles in the rat.

In accordance with our previous findings,[16–18] the development of prostatic tumors after treatment with DMAB alone was limited to the ventral prostate and non-invasive *in situ* carcinomas, while combined treatment with TP induced invasive and metastasizing carcinomas of the dorso-lateral prostate, anterior prostate and seminal vesicle. No suppressive effect of IM on the development of either of these types of carcinoma was observed.

The satellite experiment revealed that $PGE_2$ levels in the prostate and seminal vesicles are reduced by IM treatment, particularly in the dorso-lateral prostate. Thus, IM certainly interferes with $PGE_2$ synthesis in the accessory sex organs, despite the lack of tumor inhibition. $PGE_2$ level in the ventral lobe was much lower than those in the dorso-lateral prostate and seminal vesicles, and there was also a partial lobe-dependent difference in the suppression of $PGE_2$ levels by IM. These findings indicate that the $PGE_2$ synthetic ability in the prostate is lobe-dependent.

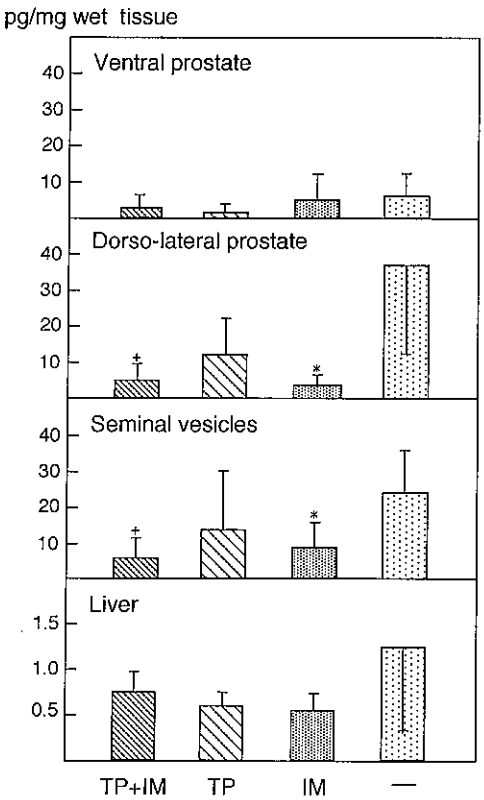


Fig. 2. $PGE_2$ concentrations per mg weight in the ventral and dorso-lateral prostate, seminal vesicles and liver. Data presented are mean±SD values. Significant difference between the IM group and control group, $^*P<0.05$; between the TP+IM group and control group, $^+P<0.05$.

Suppression of $PGE_2$ levels was also associated with administration of a high dose of TP, even though TP treatment was shown to play a key role in induction of invasive carcinoma. It is known that testosterone exerts regulatory effects on the level of PGs in the mouse male reproductive system, including the prostate.[20] In addition, cholesterol, which is a direct precursor of testosterone, is increased by the administration of exogenous testosterone and itself suppresses synthesis of PGs.[21–23] The reduction of $PGE_2$ levels observed in rats given TP may thus be the result of an increase of cholesterol. It is possible that IM did not exert any ability to block the development of prostatic tumors because cyclooxygenase activity was already reduced by TP treatment.

PG synthesis inhibitors have been reported to inhibit tumor development in colon carcinogenesis models using methylnitrosourea, 1,2-dimethylhydrazine or azoxymethane as initiators.[7, 8, 24, 25] Although DMAB also induced tumors of the large intestine in line with previous studies,[26, 27] no inhibition by IM of the colon tumor development was found in the present study. This might, however, have been related to the very low incidence of lesions.

In conclusion, our results suggest that IM treatment does not exert inhibitory effects on rat prostate carcinogenesis induced by DMAB with/without TP, despite an apparent suppression of $PGE_2$ synthesis and reduced cell proliferation. Further investigations of the mechanisms responsible for the organ specificity of IM chemopreventive potential appear warranted.

## ACKNOWLEDGMENTS

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