Effects of the Prostaglandin Synthetase Inhibitor Indomethacin on Tumorigenesis, Tumor Proliferation, Cell Kinetics, and Receptor Contents of 7,12-Dimethylbenz(a)anthracene-induced Mammary Carcinoma in Sprague-Dawley Rats Fed a High- or Low-Fat Diet

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ABSTRACT

The effects of indomethacin on tumorigenesis, tumor proliferation, cell kinetics, and receptor content of 7,12-dimethylbenz(a)anthracene-induced mammary carcinoma have been examined in female Sprague-Dawley rats. The rats were fed either a high-fat (20% corn oil) or low-fat (0.5% corn oil) diet with or without 0.005% indomethacin starting 7 days after intragastric administration of a single dose of 5 mg 7,12-dimethylbenz(a)anthracene. The results demonstrated that indomethacin completely blocked the stimulatory effect of fat on tumorigenesis, as demonstrated by a decreased tumor incidence, a decreased number of tumors per group, and an increased latency. Contrary to what had been expected, however, indomethacin promoted tumor proliferation in both the high- and low-fat diet groups, as evidenced by an increased tumor size, an increased bromodeoxyuridine-labeling index, and a decreased potential tumor-doubling time. No significant difference in either the estrogen receptor or progesterone receptor content of the tumor was noted. It can be concluded, therefore, that indomethacin significantly reduced tumorigenesis in the high-fat diet group but significantly promoted tumor proliferation in both the high- and low-fat diet groups.

INTRODUCTION

Breast cancer differs greatly in incidence and prognosis between Western and Japanese women (1–3), and the difference between them possibly derives from the different dietary fat intake (4–6). In experimental studies, polyunsaturated fatty acids in a high-fat diet proved influential only on the promotional phase of mammary tumorigenesis, since no stimulation was observed when high-fat diets were administered prior to carcinogen treatment (7–9). It may be postulated that the stimulation of mammary tumorigenesis by feeding diets rich in polyunsaturated fat takes place via an increased synthesis of prostaglandins. Carter et al. (10) reported that the prostaglandin synthetase inhibitor, indomethacin, completely blocked the dietary fat enhancement of DMBA2-induced mammary tumorigenesis in female Sprague-Dawley rats. The prostaglandins have been shown to exert an inhibitory effect on natural killer cells, components of the host defense system presumably playing a role in immunosurveillance (11). In addition to an indirect mechanism through the immune system (12, 13), it has been shown that polyunsaturated fatty acids have a direct influence on the tumor cell growth process. Furthermore, Tanaka et al. (14) recently reported that indomethacin inhibited the growth of an early transplant of colon 26 adenocarcinoma but that it facilitated the growth of the tumor at an advanced stage. The effect of high dietary fat content on the estrogen and/or progesterone receptor content of mammary tumors has also been addressed. Diets high in fat have been shown not to influence the levels of estrogen and/or progesterone receptor in several studies (15, 16), although there seems to be a general consensus that the presence of estrogen and/or progesterone receptor is inversely related to the proliferative rate of tumors (17–24). Thus, the effects of high dietary fat content (25) and prostaglandin synthetase inhibitor (10, 14, 26, 27) on tumor cell proliferation, and the effect of high dietary fat content on hormone receptors (15, 16) need further study for mechanistic reasons. In this paper, indomethacin was investigated for its effects on the tumorigenesis, tumor proliferation, cell kinetics, and receptor contents of DMBA-induced mammary carcinoma in female Sprague-Dawley rats fed on either a high- or low-fat diet.

MATERIALS AND METHODS

Animals and Tumor Induction. This experiment was designed to determine whether the administration of indomethacin would inhibit the stimulatory effect of fat on tumorigenesis, tumor proliferation, and cell kinetics. Fifty-day-old virgin female Sprague-Dawley rats (Shizuoka Laboratory Animal Center, Hamamatsu, Japan) were given a single dose of 5 mg of DMBA (Sigma Chemical Co., St. Louis, MO) via intragastric intubation. All rats were maintained on laboratory chow and were housed in suspended metal cages at a temperature [23 ± 2°C (SE)] and humidity-controlled facility on a 12-h-light, 12-h-dark cycle. Seven days after the DMBA administration, the rats were switched from the laboratory chow to either a high-fat (20% corn oil) or low-fat (0.5% corn oil) diet (Oriental Yeast Co., Tokyo, Japan). The diet for one-half of the rats of each group was supplemented with 0.005% (w/w) indomethacin (Sigma). The rats were thus divided into four groups [a high-fat group (n = 32); an indomethacin-treated, high-fat group (n = 33); a low-fat group (n = 33); and an indomethacin-treated, low-fat group (n = 32)]. Indomethacin was started 7 days after the DMBA administration. The diets were used throughout the rest of the experimental period. The low-fat diet contained, in percentage by weight: corn oil, 0.5%; vitamin-free casein, 25.0%; α-potato starch, 10.0%; β-corn starch, 49.125%; cellulose powder, 2.375%; mineral mixture, 6.0%; vitamin mixture, 2.0%; and granulated sugar, 5.0%. The high-fat diet contained in percentage by weight: corn oil, 20.0%; vitamin-free casein, 25.0%; α-potato starch, 10.0%; β-corn starch, 25.5%; cellulose powder, 26.75%; mineral mixture, 6.0%; vitamin mixture, 2.0%; and granulated sugar, 5.0. Thus, the diets were formulated on an assumption that the rats would consume

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2 The abbreviations used are: DMBA, 7,12-dimethylbenz(a)anthracene; BrdUrd, bromodeoxyuridine; ER, estrogen receptor; PgR, progesterone receptor; PBS, phosphate-buffered saline; Prdf, propidium iodide; P-buffer, phosphate buffer, pH 7.5, 0.02 mM KH2PO4-Na2HPO4 containing 0.5 mM dithiothreitol and 0.25 mM sucrose; P'-buffer, P-buffer without sucrose.

2 The mineral mixture contains in each kg: potassium, 4.2 g; phosphorus, 9.9 g; calcium, 5.6 g; sodium, 2.5 g; magnesium, 749 mg; iron, 270 mg; zinc, 51 mg; manganese, 22 mg; copper, 5.7 mg; iodine, 4.6 mg.

3 The vitamin mixture contains in each kg: vitamin A acetate, 10,000 IU; vitamin D3, 2,000 IU; vitamin B1, 24 mg; vitamin B6, 80 mg; vitamin B12, 16 mg; vitamin B12, 0.01 mg; vitamin C, 600 mg; vitamin E, 100 mg; vitamin K1, 104 mg; biotin, 0.4 mg; folate acid, 4 mg; calcite pantothenate, 100 mg; p-aminobenzoic acid, 100 mg; niacin, 120 mg; inositol, 120 mg; choline chloride, 4.0 g.

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an equal number of calories, but the rats receiving the high fat diet also had a high fiber diet. Indomethacin was added to the diets by premixing with the vitamins and minerals, followed by the addition of this whole mixture to the bulk of the diet ingredients. The diets were stored in sealed, plastic containers in the dark and were maintained at 4°C. The rats had free access to the diets and water throughout the rest of the experimental period.

Tumor Measurement and BrdUrd Staining. Body weights, tumor incidence, and measurements were recorded weekly throughout the experimental period. Each tumor location was recorded and the size was measured with a vernier caliper in 2 perpendicular dimensions. Tumor diameter was calculated by averaging these 2 measurements. Weekly tumor measurements were added up for each rat, and the values were expressed by summing the average diameter of all tumors for tumor-bearing rats of each group and by summing the average diameter of only initial tumors (first palpable tumors) for tumor-bearing rats of each group, respectively. Mammary tumorogenesis was also assessed as average tumor number and percentage of tumor incidence. In addition, the average latent period to tumor appearance was calculated for the first palpable tumors in tumor-bearing rats of each group. This value represents the interval in weeks between the DMBA administration and the appearance of the first palpable tumor.

Twenty weeks after the DMBA administration, 20 mg/kg of BrdUrd (Sigma) were injected i.p. The rats were sacrificed 6 h later, and only the initial tumor in each rat was extirpated. Then, a part of each tumor was immediately stored at -80°C until assayed for estrogen receptor and progestrone receptor by the radioreceptor assay. Another part of each tumor was immediately cut into small pieces and fixed in 70% ethanol for 30 min. It was then incubated in physiological saline with 0.5% pepsin (pH 1.5) for 2 h and dissociated with pipetting. After it was filtered through a 40-μm nylon mesh, and centrifuged at 1500 x g for 5 min, the cells were suspended in 70% cold ethanol (4°C). Another part of each tumor was fixed in 70% ethanol, sectioned, and stained with hematoxylin-eosin for routine histological examination.

BrdUrd Staining. The cell suspensions were stained by a modification of Dolbeare's method (28). Approximately 2 x 10⁶ cells were removed from the ethanol and washed twice with phosphate-buffered saline containing 0.5% Tween 20 (Sigma). The cells were resuspended in 2 N HCl at 37°C for 20 min and then neutralized with 0.1 N Na₂B₄O₇. After a washing with PBS, the cells were replaced into 0.5 ml of the same solution containing 10 μl of monoclonal anti-BrdUrd (Becton Dickinson Immunocytometry System, Mountain View, CA) at room temperature for 60 min. The cells were washed twice with PBS and then resuspended in PBS containing 0.5% Tween 20 for 30 min. After a washing with PBS, the cells were resuspended in 0.5 ml of PBS containing 10 μl of fluorescein-labeled goat anti-mouse γ-globulin (Cappel; Cooper Biomedical Inc., Malvern, PA), 0.5% Tween 20, and 0.5% normal goat serum for 60 min. After 2 washes with PBS, the cells were resuspended in 4 ml of PBS containing 20 μg/ml of Pdrl (Sigma Chemical Co.) and 0.1% RNase (Sigma) for 30 min and analyzed by flow cytometry.

Flow Cytometry and DNA Data Analysis. Bivariate distribution of BrdUrd content (green) versus DNA content (Pdrl labeling; red), was measured by use of an EPICS flow cytometer (Coulter Co., Hialeah, FL). Approximately 20 x 10⁶ cells were analyzed with each specimen. The 488 nm argon ion laser line of 400 mW was used for fluorochrome excitation. The red fluorescence from Pdrl was collected through a 630 nm band-pass filter and recorded as a measure of total DNA content; the green fluorescence from fluorescein was collected through a 530 nm band-pass filter and recorded as a measure of the amount of incorporated BrdUrd. All data were analyzed by use of a computer. The resulting data were accumulated to form a bivariate 64 x 64 channel distribution showing the distribution of DNA (red fluorescence) and BrdUrd (green fluorescence) among the cells to the population. S phase was determined by selecting a region around the significantly green-fluorescing cells. The lower limit of the region was determined by processing through the staining procedure the cells from rodent tumors that had not been exposed to BrdUrd. In virtually no cases in which this nonspecific staining was done there was a significant green fluorescence above channel 10 on the vertical scale. The BrdUrd labeling index (LI) was then calculated from the cytogram as the percentage of cells in the regions

\[
\text{BrdUrd LI} = \frac{S}{G_{G_1} + S + G_{M}} \times 100
\]

DNA synthesis time \((T_s)\) and potential doubling time \((T_{pot})\) were measured by the method of Begg et al. (29, 30):

\[
T_s = \frac{0.5}{RM - 0.5} \times 6 \text{ (h)}
\]

Relative movement \((RM)\) =

\[
\frac{F_S - F_G}{F_G - F_S}
\]

where \(F\) is the mean red fluorescence of the corresponding phase of the cell cycle.

\[
T_{pot} = \frac{T_s}{\text{BrdUrd LI} \times 100/24 \text{ (day)}}
\]

ER and PgR Assay. [\(^3H\)]Estradiol ([2,4,6,7-\(^3H\)]estradiol; specific activity, 99 Ci/mmol) and [\(^3H\)]progesterone ([17α, \(^3H\) ]estradiol); specific activity, 87 Ci/mmol) were purchased from New England Nuclear. P-buffer was used throughout. [\(^3H\)]Estradiol and [\(^3H\)]progesterone were first dissolved in ethanol, respectively, and then diluted with P-buffer to the required concentrations. Ethylene glycol was added to the P-buffer to dissolve the steroids. The final concentrations of ethanol and ethylene glycol at incubation were below 4 and 10 volume %, respectively. To stabilize the steroid receptors, sodium molybdate (10 mM final concentration) was added to the \(^3H\)-ligand solution in the ER assay and to the homogenization buffer in the PgR assay (31). Cancer tissue (approximately 0.5 g, wet weight) was homogenized with efficient cooling in 4 volumes of P-buffer by use of a glass homogenizer (Yamashita Co., Osaka, Japan) for five 30-s periods, each followed by a 30-s cooling period. The homogenate was centrifuged at 0°C at 45,000 rpm (Model L-8-70, Sw 55Ti; Beckman, Fullerton, CA) for 30 min, and the supernatant fraction was retained as the cytosolic preparation. The cytosols as obtained were diluted with an equal volume of P-buffer. Protein concentrations of the cytosols were determined according to the method of Lowry et al. (32). In the competitive binding assay, 100 μl of each cytosol were incubated with 50 μl of P-buffer containing varying concentrations of [\(^3H\)]estradiol (0.05–2 pmol), [\(^3H\)]progesterone (0.05–2 pmol), and 50 μl of 40 nm \(^3H\)-ligand. For the Scatchard plot analysis, each cytosol was incubated with P-buffer and various concentrations (0.05–2 pmol) of \(^3H\)-ligands. Nonspecific bindings were determined in a parallel incubation with 1000-fold excess of unlabeled steroid and were subtracted from the total bindings. After incubation for 16 h at 0°C, bound and free steroids were separated by the dextran-coated charcoal method; the cytosol was treated with dextran-coated charcoal (1% Norit Sx-3 in 0.1% Dextran T-70) for 10 min and centrifuged at 0°C at 3000 rpm for 10 min. The supernatant was mixed with 10 ml of scintillation fluid (Scintisol EX-H; Dojin De Laboratories, Kumamoto, Japan) in 20-ml glass vials, and the supernatant radioactivities were quantitated with a scintillation counter (Tri-Carb 4640; Packard Instrument Co., Downers Grove, IL). The results were analyzed according to the procedure of Scatchard (33).

**Statistical Analysis.** Statistical differences in mammary tumor size, average tumor number, mean tumor latent period, BrdUrd labeling index, DNA synthesis time, potential doubling time, and ER and PgR levels were analyzed by Student's t test. Statistical difference in tumor incidence was analyzed by the x² test.

**RESULTS**

**Body Weight and Histology of Tumors.** Total body weight did not differ significantly between the high-fat and low-fat diet
groups of rats. Indomethacin exerted no effect on the body weight of the rats (Fig. 1). The induced mammary tumors were histologically identified as adenocarcinomas. No fibroadenoma was found. Mammary tumors from the high-fat diet group showed a dense proliferation of ductal cells. The ductal cells with arrangement of solid cell nests and glandular structures were intermingled within the solid tumor cell nests. Some mitotic figures of the nuclei were also observed (Fig. 2a). In the tumors from the low-fat diet group, well-developed glandular structures of ductal cells were found upon histological examination. Mucinous materials were contained in their lumina. The density of tumor cells was low and mitoses were infrequent (Fig. 2b). In the tumors from the indomethacin-treated high-fat diet group, a more extensive proliferation of ductal cells was noted than that found in the low-fat diet group. The tumor cells were frequently associated with an arrangement of large and small solid cell nests and central necrosis of the nests. Mitosis of tumor cells was also frequent. Only small lumina were occasionally associated with the nests (Fig. 2c). In the tumors from the indomethacin-treated low-fat diet group, a somewhat extensive proliferation of ductal cells was seen, compared with that from the low-fat diet group. However, the proliferation was of far lower degree than that observed in the high-fat diet group. Well-developed glandular structures and moderate proliferation of ductal cells were also observed (Fig. 2d).

Tumor Incidence, Latency, and Size. Fig. 3 shows the cumulative weekly incidence of palpable tumors in each group of rats after the administration of DMBA. The feeding of rats on the high-fat diet stimulated the tumor incidence significantly ($P < 0.01$), compared with the low-fat diet group. The addition of 0.005% indomethacin to the high-fat diet resulted in the complete blocking of this effect. However, indomethacin had no effect on the low-fat diet groups. Fig. 3 depicts also that mammary tumors first appeared approximately 8 weeks after the DMBA administration in the high-fat diet group but between 10 and 12 weeks after the DMBA administration in the indomethacin-treated high-fat diet, the low-fat diet, or the indomethacin-treated low-fat diet group (Fig. 3).

The experiment was terminated 20 weeks after DMBA administration. Table 1 further shows the data on tumorigenesis. Impalpable tumors were not included in this analysis. The tumor incidences in the high-fat diet, the indomethacin-treated high-fat diet, the low-fat diet, and the indomethacin-treated low-fat diet group were 81, 30, 27, and 34%, respectively. The total number of tumors per rat was over 4-fold larger in the high-fat diet group than in the low-fat diet group. The addition of indomethacin to the high-fat diet resulted in a reduction of the total number of tumors by one-half. The average tumor latent period was significantly shorter in the high-fat diet group, compared with the low-fat diet, the indomethacin-treated low-fat diet, or the indomethacin-treated high-fat diet group. Feeding a high-fat diet thus stimulated tumorigenesis significantly. Furthermore, the addition of 0.005% indomethacin completely blocked the stimulatory effect of the high-fat diet on tumorigenesis but exerted no effect on the low-fat diet groups. The number of tumors per tumor-bearing rat was, however, not statistically different among the groups. The effect of indomethacin on tumor proliferation is noteworthy in both the high- and low-fat diet groups.

The effects of indomethacin are further illustrated in Fig. 4, which shows the cumulative tumor diameters for each group at various stages after the administration of DMBA. The values were obtained by summing the average diameter of all tumors for tumor-bearing rats of each group. Indomethacin proved to exert an inhibitory effect on the high-fat diet groups (Fig. 4). The striking inhibitory effect of indomethacin on the high-fat diet groups was again noteworthy, and it was similar to the findings by Carter et al. (10). The cumulative tumor size was, however, determined by both tumor incidence and tumor size.

Fig. 5 shows the effects of indomethacin on the initial tumor for tumor-bearing rats of each group. The values were obtained by summing the average diameter of only the initial tumors for tumor-bearing rats of each group. The striking stimulatory effect of indomethacin on the tumor proliferation is noteworthy in both the high- and low-fat diet groups.

**BudUrd Labeling Index, DNA Synthesis Time, Potential Doubling Time, and ER and PgR Contents in Initial Tumors.** Furthermore, the BrdUrd labeling indices of tumors in the high-fat diet group were significantly higher than those in the low-fat diet group ($P < 0.001$); the labeling indices of tumors in the indomethacin-treated high-fat diet group were significantly higher than those in the high-fat diet group; and the indices in the indomethacin-treated low-fat diet group were significantly higher than those in the low-fat diet group. The potential doubling times of tumors in the high-fat diet group were significantly shorter than those in the low-fat diet groups ($P < 0.01$); the times in the indomethacin-treated high-fat diet group were significantly shorter than those in the high-fat diet group; and the times in the indomethacin-treated low-fat diet group were significantly shorter than those in the low-fat diet group (Table 2). The DNA synthesis time was not, however, different among these groups. The average level of ER and PgR binding activity in each group is also shown in Table 2. The ER and PgR contents of tumors were not statistically different between the
Fig. 2. Histology of 7,12-dimethylbenz(a)anthracene-induced mammary carcinoma in Sprague-Dawley rats. a, high-fat diet group; b, low-fat diet group; c, indomethacin-treated high-fat diet group; d, indomethacin-treated low-fat diet group.

Fig. 3. Effect of indomethacin on the cumulative incidence of palpable mammary tumor in rats fed high- or low-fat diets. *, P < 0.01; □, high-fat; ■, high-fat plus indomethacin; ○, low-fat; ● low-fat plus indomethacin.

DISCUSSION

In experimental studies, the polyunsaturated fatty acids contained in high-fat diets have been shown to promote mammary tumorigenesis and tumor proliferation (7–9). It may be postulated that the stimulation of mammary tumorigenesis by feeding diets rich in polyunsaturated fat takes place via an increased synthesis of prostaglandins. The effect of some prostaglandins is to inhibit the immune response of the host, allowing the tumor to escape immunological rejection. However, polyunsaturated fatty acids have also been shown to have a direct influ-
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Table 1 Effect of high dietary fat and the prostaglandin synthetase inhibitor, indomethacin, on tumorigenesis and tumor proliferation of DMBA-induced mammary carcinoma in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of rats</th>
<th>No. of tumors</th>
<th>Tumor incidence (%)</th>
<th>No. of tumors in a rat</th>
<th>No. of tumors/tumor-bearing rat</th>
<th>Latent period (wk)</th>
<th>Av. tumor diameter of all tumors (mm)</th>
<th>Initial tumor diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HF</td>
<td>32</td>
<td>26</td>
<td>81</td>
<td>66</td>
<td>2.3 ± 2.8</td>
<td>12.6 ± 2.1</td>
<td>13 ± 5</td>
<td>16 ± 4*</td>
</tr>
<tr>
<td>HF + Ind</td>
<td>33</td>
<td>30</td>
<td>91</td>
<td>30</td>
<td>0.9 ± 1.9</td>
<td>15.3 ± 2.6</td>
<td>15 ± 9</td>
<td>25 ± 8</td>
</tr>
<tr>
<td>LF</td>
<td>33</td>
<td>9</td>
<td>27</td>
<td>13</td>
<td>0.5 ± 0.9</td>
<td>15.3 ± 1.8</td>
<td>12 ± 3</td>
<td>12 ± 2*</td>
</tr>
<tr>
<td>LF + Ind</td>
<td>32</td>
<td>11</td>
<td>34</td>
<td>23</td>
<td>0.7 ± 1.4</td>
<td>15.0 ± 2.7</td>
<td>12 ± 5</td>
<td>17 ± 5</td>
</tr>
</tbody>
</table>

* Initial tumor diameter is defined as the average diameter of the first palpable tumor in each tumor-bearing rat of each group measured at 20 weeks after DMBA administration.

HF, high-fat group; HF + Ind, indomethacin-treated high-fat group; LF, low-fat group; LF + Ind, indomethacin-treated low-fat group.

P < 0.01 in comparing HF versus LF.

P < 0.05 in comparing HF versus LF, or HF + Ind.

P < 0.01 in comparing HF versus LF.

P < 0.01 in comparing LF versus LF + Ind.

P < 0.05 in comparing HF versus LF, or HF + Ind.

P < 0.01 in comparing HF versus LF.

P < 0.01 in comparing LF versus LF + Ind.

Fig. 4. Effect of indomethacin on the cumulative diameter of palpable mammary tumor in rats fed high- or low-fat diets. Since each group differed in number of rats, the cumulative tumor diameter in the high-fat diet group or the indomethacin-treated low-fat diet group was corrected by multiplying 33/32. D, high-fat; •, high-fat plus indomethacin; O, low-fat; •, low-fat plus indomethacin.

Fig. 5. Effect of indomethacin on the mean diameter of initial tumor in rats fed high- or low-fat diets. D, high-fat; •, high-fat plus indomethacin; O, low-fat; •, low-fat plus indomethacin.

ence on the tumor cell growth in vitro (12, 13), suggesting that in vivo, diets high in polyunsaturated fat may stimulate mammary tumorigenesis by both direct and indirect mechanisms. The technique of flow cytometry provides two important pieces of information on the malignant intensity (defined by proliferative rate and metastatic potential) of a tumor: S-phase fraction which correlates with tumor growth; and ploidy which correlates with malignant aggressive tumor behavior (17). In a previous study, we demonstrated that a high dietary fat content not only promoted tumorigenesis but also stimulated tumor proliferation (13). This suggests that the effect of dietary fat may be direct, perhaps through an alteration in intercellular communication (34, 35) or some as yet undefined mechanism.

When the feeding of diets rich in polyunsaturated fat stimulates mammary tumorigenesis via an increased synthesis of prostaglandins, the prostaglandin synthetase inhibitor, indomethacin, may produce tumor growth retardation, thereby permitting the restoration of host immunity. Carter et al. (10) investigated the effect of indomethacin on the dietary fat enhancement of DMBA-induced mammary tumorigenesis in female Sprague-Dawley rats and reported that indomethacin blocked completely the stimulatory effect of fat on tumorigenesis. The influence of indomethacin on chemical carcinogenesis in the mammary gland, however, appears to be somewhat more complex. Abou-El-Ela et al. (36) studied the effect of 0.004% indomethacin in a 20% corn oil diet on DMBA-induced mammary tumor development in Sprague-Dawley rats and reported that indomethacin exerted no effect on tumor incidence, although tumor prostaglandin production was reduced by 45%. The apparent discrepancy between the findings could be explained by several differences in protocol between the study by Carter et al. (10) and that by Abou-El-Ela et al. (36), most notably different doses of DMBA (5 versus 10 mg, respectively) and a different time of initiation of indomethacin administration (3 days versus 3 weeks post-DMBA, respectively). According to a model for tumor formation, only initiation and stage I promotion are essential for tumorigenesis, whereas stage II promotion enhances tumor proliferation (37). While a low dose of DMBA only initiates tumorigenesis, a completely carcinogenic dose both initiates tumorigenesis and promotes tumor proliferation. It is possible that indomethacin is a less effective inhibitor of mammary tumorigenesis when used at a high carcinogen dose as in the study by Abou-El-Ela et al. (36). Indomethacin has been shown to inhibit DMBA-induced mammary carcinogenesis in both early and late stages (38). In early stages, indomethacin appears to modulate carcinogen metabolism through the inhibition of prostaglandin H synthase (38).
The possible late stage effect of indomethacin has been explained by the fact that it inhibits cell proliferation in a variety of normal and neoplastic mammalian cells in vitro, but the effects of indomethacin in vitro still remain controversial (39-43). Fulton (39, 40) and Rose et al. (41) showed that indomethacin stimulates tumor cell proliferation in vitro. Bayer et al. (42) and Hial et al. (43) found, in contrast, that higher levels of indomethacin inhibited cell proliferation in vitro. The suppression of cell proliferation in vitro may contribute to the therapeutic and/or toxic effects of indomethacin (42). It was recently suggested that higher indomethacin concentrations inhibit mammary tumor cell growth by affecting enzymes other than the cyclooxygenase which regulates entry into the prostaglandin-synthesizing pathway (41).

In the present study, we demonstrated that indomethacin blocked completely the stimulatory effect of fat on tumorigenesis, but contrary to what had been expected, it promoted tumor proliferation in either the high- or low-fat diet group. When the cumulative tumor size was obtained by summing the average diameter of all tumors for tumor-bearing rats of each group, the inhibitory effect of indomethacin was manifest in the high-fat diet groups. The observation was similar to the findings by Carter et al. (10). The cumulative tumor size is, however, determined by both tumor number and tumor size and thus does not represent tumor proliferation per se. The average diameter of tumors was different among all tumors and the initial tumors for tumor-bearing rats of each group. When the tumor size was determined by measuring the average diameter of only the initial tumors for tumor-bearing rats of each group, the tumors in the indomethacin-treated high-fat diet group or the indomethacin-treated low-fat diet group were significantly larger than those in the high-fat diet group or the low-fat diet group, respectively. The stimulatory effect of indomethacin on tumor proliferation was striking in both the high- and low-fat diet groups, and it was supported by an increased BrdUrd-labeling index and a decreased potential doubling time of tumor in the present study. McCormick et al. (38) studied the effect of indomethacin on DMBA-induced mammary tumor in Sprague-Dawley rats and reported that the administration of indomethacin resulted in an inhibition of both mammary tumor incidence (tumorigenesis) as well as normal mammary differentiation. Tumor proliferation was not evaluated in these experiments. Although other prostaglandin synthetase inhibitors (44) as well as other eicosanoid-synthetase inhibitors (45) have been well shown to affect tumor incidence, moreover, their effects on tumor proliferation have not been evaluated. Tanaka et al. (14) recently reported that indomethacin inhibited the growth of an early transplant of colon 26 adenocarcinoma but that it facilitated the growth of the tumor at an advanced stage. The antitumor activity of indomethacin is not simply dependent on the ability of the drug to restore immune functions by inhibiting prostaglandin production but depends on the stage of growth of the tumor treated. In the model for tumor formation, as previously mentioned, only the initiation and stage I promotion are essential for tumorigenesis, and stage II promotion enhances tumor proliferation (37). Our study showed that indomethacin inhibited tumorigenesis, but it facilitated tumor proliferation. Although there is an apparent discrepancy between the inhibitory effect of indomethacin on tumorigenesis on the one hand and the stimulatory effect on tumor proliferation on the other, it is suggested that indomethacin inhibits the former through immune resistance mechanisms by inhibiting the production of immunosuppressive prostaglandins, but lower or nontoxic levels of indomethacin facilitate the latter by its direct effect. It has been well demonstrated in the animal system that all tumor immunity can be overcome when the tumor is sufficiently large (46). It is doubtful whether the immunological recognition of tumor antigen occurs in the stage of tumor proliferation following tumorigenesis. In some models in vivo, indomethacin did not inhibit tumor growth or it inversely facilitated tumor growth (47, 48) while significantly reducing prostaglandin levels.

There seems to be a general consensus that the presence of estrogen and/or progesterone receptor is inversely related to the proliferative rate of a tumor (17-24). However, it is unlikely that the effect of a high-fat diet is mediated through a change in estrogen and/or progesterone receptor levels since we have shown previously (15), as well as in this study, that dietary fat level does not affect receptor level or affinity. Ip and Ip (16) also reported that the levels of ER in tumors were not significantly different between the group given 5% and that given 20% fat. In the present study, we have also demonstrated that indomethacin has no influence on tumor hormone receptors levels. Further studies may be essential for understanding the interrelationship between high dietary fat content and hormone receptors.

It may be concluded that indomethacin reduced significantly DMBA-induced mammary tumorigenesis in the high-fat diet group but that it promoted significantly the proliferation of the thus produced tumors in both the high- and low-fat diet groups. The findings, therefore, suggest that indomethacin may not benefit patients with breast cancer.

REFERENCES


2. Mean ± SD.
3. Numbers in parentheses, number of tumors.
4. *P < 0.01 in comparing HF versus LF + Ind.
HIGH DIETARY FAT AND INDOMETHACIN

Effects of the Prostaglandin Synthetase Inhibitor Indomethacin on Tumorigenesis, Tumor Proliferation, Cell Kinetics, and Receptor Contents of 7,12-Dimethylbenz(a)anthracene-induced Mammary Carcinoma in Sprague-Dawley Rats Fed a High- or Low-Fat Diet

Masakuni Noguchi, Takao Taniya, Naohiro Koyasaki, et al.