Effects of D.L-2-Difluoromethylornithine and Indomethacin on Mammary Tumor Promotion in Rats Fed High n-3 and/or n-6 Fat Diets

Soad H. Abou-El-Ela, Keith W. Prasse, Robert L. Farrell, Richard W. Carroll, Adelbert E. Wade, and Opal R. Bunce

ABSTRACT

Virgin female Sprague-Dawley rats (50 days of age) were administered a single intragastric 10-mg dose of 7,12-dimethylbenz(a)anthracene (DMBA). Twenty-one days later they were placed on diets containing either 20% corn oil (CO), 15% menhaden oil plus 5% corn oil (MO + CO), 20% CO plus 0.5% w/w of the irreversible ornithine decarboxylase inhibitor, D,L-2-difluoromethylornithine (CO + DFMO), 20% CO plus 0.004% w/w of the cyclooxygenase inhibitor indomethacin (CO + INDO), 20% CO + 0.004% INDO + 0.5% DFMO (CO + INDO + DFMO), or 15% MO + 5% CO + 0.5% DFMO (MO + CO + DFMO). The incidence of DMBA-induced mammary tumors was significantly reduced in rats fed diets containing DFMO but not in rats fed the diet containing indomethacin. Incidences of mammary tumors at 16 weeks post-DMBA were 86% in rats fed the CO diet, 83% in rats ingesting the diet containing CO + INDO, 28% in rats fed CO + DFMO, 32% in rats fed diet containing CO + INDO + DFMO, 59% in rats fed the MO + CO diet, and 24% in rats fed the MO + CO + DFMO diet. The average number of tumors and tumor burden per tumor-bearing rat were reduced and tumor latency was increased in all rats fed diets containing DFMO. Body weight gain, but not food intake, of rats fed the 20% fat + 0.5% DFMO diets was significantly less than in rats fed the 20% fat diets. Prostaglandin E and leukotriene (LTB4) synthases, ODC activity and mammary tumorigenesis were significantly inhibited by feeding the diet containing menhaden oil or by adding 0.5% DFMO to any of the high fat diets. Feeding a 20% CO diet containing 0.004% INDO significantly reduced prostaglandin synthesis and ODC activity and increased LTB4 synthesis of mammary tumors but did not inhibit mammary tumorigenesis. This study suggests that the 5-lipoxygenase product LTB4 may be involved in mammary tumor production. Whereas a decrease in LTB4 appears to be associated with a decrease in tumorigenesis, an increase (as seen in the indomethacin group) was not associated with any change in the tumorigenic response.

INTRODUCTION

The promotion of carcinogen-induced, spontaneous, and transplantable mammary tumors is enhanced in rats fed increasing levels of the n-6 fatty acid linoleate (1). This fatty acid may, in part, promote tumor growth and development by increasing synthesis of eicosanoids, particularly arachidonic acid products that have been shown to enhance cell division, depress immune responses and promote tumor growth (2). Alternatively, diets containing high levels of n-3 fatty acids have been shown to inhibit development of several carcinogen-induced cancers, action which appears to be mediated through its ability to inhibit arachidonic acid metabolism by both cyclooxygenase and lipoxygenase (2, 3).

Indomethacin, an inhibitor of cyclooxygenase, has been shown to inhibit DMBA-induced mammary carcinogenesis in both the early and late stages. In the early stage, indomethacin appears to modulate carcinogen metabolism through the inhibition of prostaglandin H synthase (4–6). The possible late-stage effects of indomethacin may be explained by the fact that it inhibits cell proliferation in a variety of normal and neoplastic mammalian cells in vitro (7–9). Furthermore, it has been demonstrated that indomethacin can inhibit the growth of DMBA-induced mammary tumors in vivo (6) as well as the stimulatory effect of fat on DMBA-induced mammary tumor development (10–12). In contrast to these studies, others have reported that indomethacin did not inhibit tumor growth (13–16) although PGE levels were significantly reduced (13, 14, 16).

In the multistage carcinogenesis model for mouse skin, phorbol esters have been shown to inhibit intercellular communication (17, 18) through alterations in biochemical responses involving phospholipid metabolism, fatty acid and eicosanoid synthesis, and activation of ODC (19–23). Furthermore, the activity of ODC correlates with eicosanoid synthesis (23, 24) and is elevated in various proliferative cell systems including proliferative stages I and II of neoplastic growth (25–27). The biochemical mechanism of ODC induction is not fully understood. However, it appears that phospholipase A2 stimulation (19, 28) and resultant production of arachidonic metabolites (i.e., cyclooxygenase and lipoxygenase products) are involved (24, 28, 29). Moreover, indomethacin has been shown to block the induction of ODC (23, 30).

ODC catalyzes the formation of putrescine from ornithine, the immediate precursor of polyamines spermidine and spermine (31). Polyamines are essential for cell growth and proliferation of several tissues of the body, including the breast (32, 33). Accumulation of these polycationic amines appears essential for rapid neoplastic growth (31, 34). Therefore, interference with polyamine accumulation can inhibit tumor development (31, 34, 35). In particular, inhibition of ODC activity with a specific enzyme-activated irreversible inhibitor, e.g., D.L-2-difluoromethylornithine (DFMO), inhibits growth of chemically induced mammary tumor during promotion (36–39).

Since both ODC activity and eicosanoid levels are increased in cancerous tissues and blockade of their activation/synthesis is associated with reduced tumor incidence, these biochemical processes may be phenomena that are mechanistically related to mammary tumor promotion by high n-6 polynsaturated fat diets in rats. Therefore, the objectives of this study were to block one or more events in the mammary tumor promotion and progression mediated by diets containing a high level of linoleate in the form of 20% corn oil, in the following manner: (a) by adding to the diet high levels of n-3 fatty acids, as found in menhaden oil, to competitively block cyclooxygenase and lipoxygenase; (b) by adding to the diet 0.004% indomethacin.

The abbreviations used are: DMBA, 7,12-dimethylbenz(a)anthracene; DFMO, D,L-2-difluoromethylornithine; ODC, ornithine decarboxylase; CO, corn oil; MO, menhaden oil; INDO, indomethacin; PGE, prostaglandin E; LTB4, leukotriene B4; i.g., intragastric; EFA, essential fatty acids.

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to inhibit arachidonic acid metabolism by cyclooxygenase; (c) by adding to the diet containing n-3 and/or n-6 fatty acids, 0.5% DFMO to inhibit ODC activity; (d) by administering both indomethacin and DFMO in a 20% corn oil diet to establish whether additive or synergistic effect will result from inclusion of two inhibitors that act on tumor promotion by different but interrelated mechanisms.

MATERIALS AND METHODS

Diets, Feeding, and Tumor Induction. Two hundred forty female Sprague-Dawley rats, 40 days old, were purchased from Charles River Laboratories, Wilmington, MA. All animals were housed (four/cage) in suspended metal cages in a temperature-regulated (23 ± 0.5°C) and light controlled (12-h light/dark cycle) room and fed standard rat chow (Ralston Purina Co., St. Louis, MO). At 50 days of age, 180 rats were given a single dose of 10 mg of DMBA (Sigma Chemical Company, St. Louis, MO) via intragastric intubation in 0.5 ml corn oil. Sixty sham-treated rats each received 0.5 ml corn oil. After DMBA administration, the animals were randomly placed in 10- × 16-inch plastic cages on Absorb Dri Litter and housed three per cage for the duration of the experiment. Six rats were found dead 48 h after DMBA administration. As outlined in Fig. 1, at 21 days post-DMBA administration, the rats were randomly divided into six groups of 29 rats each and fed six diets: (a) 20% corn oil diet (CO); (b) 20% CO diet containing 0.004% (w/w) indomethacin (CO + INDO); (c) 20% CO diet containing 0.5% (w/w) DFMO (CO + DFMO); (d) 20% CO diet containing 0.004% INDO plus 0.5% DFMO (CO + INDO + DFMO); (e) diet containing 15% MO and 5% CO (MO + CO); and (f) diet containing 15% MO plus 0.5% CO (MO + 0.5% DFMO). Sham-treated animals were divided into six groups of 10 rats each and fed the same diets. The DFMO level chosen was based on method and results reported by Thompson et al. (38). In their study, 0.5% DFMO in the drinking water delivered approximately 130 mg of DFMO/day/rat, and significantly reduced tumor incidence, average number of cancers and tumor weight per rat, and increased tumor latency without producing systemic toxic effects. In the present study, feeding 0.5% DFMO in the diet delivered approximately 75 mg of DFMO/day/rat, which is 57% of the dose given by Thompson et al. (38). The rats remained on their respective treatments without interruption until the experiment was terminated 112 days after DMBA administration.

Twenty kg batches of each of the 20% fat diets were prepared by ICN Nutritional Biochemical, Cleveland, OH. The diets were cold pressed into jumbo pellets, sealed under nitrogen, and shipped frozen. One kg bags of pellets were placed in Seal-N-Save bags, flushed with nitrogen, sealed, and stored frozen at −20°C until used. Measured amounts of frozen diet (20 g/rat/day) were placed in the cage food dispenser each morning after uneaten pellets were discarded. Food consumption was determined over an 8-week period beginning 6 weeks post-DMBA. Food intake was measured for one 24-h period each week by weighing the uneaten food per cage, from which the approximated food consumption per rat per day was calculated. Basic diet formulation was based on the AIN 76 semipurified rat diet and has been reported previously (40). The fatty acid composition of the oils in the diets is given in Table 1.

α-Tocopherol was added with the AIN vitamin mix so that each diet contained 310 IU of vitamin E/kg of diet. All diets were isocaloric and contained the recommended level of nutrients with a constant amount per kilocalorie of casein, fat, carbohydrate, salts, vitamins, and fiber. Corn oil (5%) was added to the menhaden oil diet to provide adequate

### Table 1 Fatty acid composition of dietary oils

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Corn oil †</th>
<th>Menhaden oil ‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>11.2</td>
<td>8.35</td>
</tr>
<tr>
<td>16:0</td>
<td>15.0</td>
<td>16.18</td>
</tr>
<tr>
<td>16:1</td>
<td>11.62</td>
<td>10.83</td>
</tr>
<tr>
<td>16:2</td>
<td>2.37</td>
<td>2.47</td>
</tr>
<tr>
<td>16:3</td>
<td>1.96</td>
<td>2.04</td>
</tr>
<tr>
<td>18:0</td>
<td>2.1</td>
<td>2.67</td>
</tr>
<tr>
<td>18:1</td>
<td>25.0</td>
<td>9.5</td>
</tr>
<tr>
<td>18:2 n-6</td>
<td>59.9</td>
<td>1.81</td>
</tr>
<tr>
<td>18:3 n-3</td>
<td>1.82</td>
<td>2.61</td>
</tr>
<tr>
<td>20:4</td>
<td>3.12</td>
<td>3.47</td>
</tr>
<tr>
<td>20:4 n-6</td>
<td>2.30</td>
<td>2.92</td>
</tr>
<tr>
<td>20:5 n-3</td>
<td>16.03</td>
<td>10.83</td>
</tr>
<tr>
<td>22:5</td>
<td>3.92</td>
<td>1.81</td>
</tr>
<tr>
<td>22:5 n-3</td>
<td>10.83</td>
<td>2.04</td>
</tr>
<tr>
<td>Others</td>
<td>0.1</td>
<td>4.37</td>
</tr>
</tbody>
</table>

† Purchased from Seaway Foods, Cleveland, OH.
‡ Supplied by Zapata Haynie Corp., Reedville, VA.
* Carbon chain length: number of double bonds.
The reaction was stopped by addition of 0.4 ml 2 M citric acid and of substrate. Incubations were routinely carried out for 60 min at 37°C. 

\(^{14}C\)i of L-[l-14C]ornithine hydrochloride (58.6 mCi/mmol) and 0.1-0.6 contained 50 mM phosphate buffer, pH 7.2, 0.3 mM pyridoxal phos.

Antistatic Analyses. Malignant mammary tumors from DMBMA-treated rats and fat pads of sham-treated animals were finely minced and an appropriate tissue aliquot was incubated in 1 ml Krebs buffer (with millimole/liter concentrations of NaCl, 118.1; KCl, 4.7; MgSO\(_4\), 1.2; CaCl\(_2\), 2.9; KH\(_2\)PO\(_4\), 1.2; NaHCO\(_3\), 2.5; and dextrose, 5.6; pH 7.4) for 1 h at 37°C under 95% O\(_2\)/5% CO\(_2\). The reaction was stopped by acidification to pH 3 with 0.8 M phosphoric acid and the tissue-buffer mixture was extracted once with 4 volumes of ethyl acetate by vigorous shaking (44). The organic phase was removed, and 50 \(\mu\)l of 0.1 M Tris-HCl buffer (pH 7.4) was added. The organic phase was evaporated under nitrogen, and the residue was stored sealed under nitrogen at -80°C. The samples were appropriately diluted in assay buffer and the tissue supernatant was analyzed by radioimmunoassay using kits purchased from Amersham Corp., Arlington, Houston, Texas. The sensitivity of the antibodies is 1.6 pg for LTB\(_4\) and 43 pg for PGE. The PGE\(_2\) antibody was provided by Amersham and showed 100% cross-reactivity with PGE\(_i\), thus PGE\(_2\) values were within the acceptable range for commercial oils as used by Zapata Haynie Corporation, Reedville, VA.

RESULTS

Animals and Diets. The effects of high fat diets, DFMO, and indomethacin on weight gain and final body weights are shown in Fig. 2 and Table 2. The addition of indomethacin and/or DFMO to diets containing corn oil or menhaden oil had no significant effects on food consumption. DMBA-treated rats fed MO + CO ate less than those fed CO alone but their weight gains (Fig. 2) and final body weights (Table 2) were similar. However, body weight gain in DMBA-exposed animals were uniformly depressed by simultaneous incorporation of DFMO in the diet (Fig. 2). The weight gain of sham-treated rats was similar to those treated with DMBA in that the final body weights of both DMBA- and sham-treated rats were less in rats fed DFMO than in animals not fed DFMO (Table 2).

Diet and Tumorigenesis. Palpable mammary tumor incidences during the experiment are shown in Fig. 3. Rats fed either CO or CO + INDO had significantly higher palpable mammary tumor incidences than rats fed diets containing 0.5% DFMO or the MO + CO diet. The final tumor incidence at 16-weeks post-DMBA was highest in the CO and CO + INDO groups, 86 and 83%, respectively, while the lowest incidences were noted in rats receiving 0.5% DFMO in their diet (Table 3). Feeding the diet containing 15% MO + 5% CO reduced tumor incidence by 31% compared to feeding the 20% CO diet. The addition of 0.5% DFMO reduced tumor incidence 72% in animals fed MO and 67% in rats fed CO compared to feeding

\| WEEKS AFTER DMBA ADMINISTRATION |
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</tbody>
</table>

\( \Delta \) 20% CO + 0.5% DFMO (CO + DFMO); \( \Phi \) 20% CO + 0.004% INDO (CO + INDO); \( \triangle \) 20% CO + 0.5% DFMO (CO + DFMO); \( \square \) 20% CO + 0.004% INDO + 0.5% DFMO (CO + INDO + DFMO); \( \varpi \) 15% menhaden oil plus 5% corn oil (MO + CO); \( \mathbf{\varpi} \) 15% MO plus 5% CO + 0.5% DFMO (MO + CO + DFMO).
INHIBITION OF MAMMARY TUMOR PROMOTION BY DFMO AND n-3 FATTY ACIDS

Table 2 Effect of diets and treatments on food intake during the experiment and final body weights at 16 weeks post-DMBA of sham- and DMB4-treated animals

<table>
<thead>
<tr>
<th>Diet*</th>
<th>No. of rats</th>
<th>Body weight (g)</th>
<th>Food intake (g/rat/day)</th>
<th>No. of rats</th>
<th>Body weight (g)</th>
<th>Food intake (g/rat/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20% CO</td>
<td>29</td>
<td>365.5 ± 8.4d</td>
<td>14.8 ± 0.3b</td>
<td>29</td>
<td>372.0 ± 9.6d</td>
<td>14.4 ± 0.4b</td>
</tr>
<tr>
<td>20% CO + INDO</td>
<td>28</td>
<td>310.4 ± 7.9b</td>
<td>14.0 ± 0.2c</td>
<td>29</td>
<td>293.2 ± 6.4d</td>
<td>13.4 ± 0.6c</td>
</tr>
<tr>
<td>20% CO + DFMO</td>
<td>29</td>
<td>363.8 ± 7.4d</td>
<td>13.2 ± 0.7b</td>
<td>29</td>
<td>308.4 ± 7.5c</td>
<td>14.5 ± 0.4d</td>
</tr>
<tr>
<td>15% MO + 5% CO</td>
<td>29</td>
<td>379.7 ± 9.5c</td>
<td>14.5 ± 0.2b</td>
<td>29</td>
<td>303.9 ± 8.6d</td>
<td>14.3 ± 0.9c</td>
</tr>
<tr>
<td>15% MO + 5% CO + DFMO</td>
<td>29</td>
<td>300.9 ± 8.6d</td>
<td>13.6 ± 0.4c</td>
<td>29</td>
<td>308.4 ± 7.5c</td>
<td>14.5 ± 0.4d</td>
</tr>
</tbody>
</table>

* CO, corn oil; INDO, 0.004% indomethacin; DFMO, 0.5% D.L-2-difluoromethylornithine; MO, menhaden oil.

Fig. 3. Effect of diets and treatments on palpable mammary tumor incidences. Virgin female Sprague-Dawley rats were given 10 mg DMBA i.g. at 50 days of age. Mammary tumor-promoting high fat diets were begun 3 weeks post-DMBA and were continued until 162 days of age (experiment terminated). ○, 20% CO (CO); ●, 20% CO + 0.04% INDO (CO + INDO); △, 20% CO + 0.5% DFMO (CO + DFMO); ◊, 20% CO + 0.04% INDO + 0.5% DFMO (CO + INDO + DFMO); ●, 15% Menhaden oil + 5% CO (MO + CO); □, 15% MO + 5% CO + 0.5% DFMO (MO + CO + DFMO).

CO alone. However, feeding 0.004% indomethacin did not change tumor incidence compared to feeding 20% CO diet, nor did adding 0.004% INDO along with 0.5% DFMO to the 20% CO diet enhance the inhibition afforded by DFMO.

The tumor multiplicity (number of tumors/tumor bearing rat) and tumor burden (weight) were lowest in the rats which received 0.5% DFMO in their diets (Table 3). The average time (in weeks) for the appearance of the first tumor (tumor latency) in the CO + DFMO group was significantly longer than in the CO and CO + INDO groups (Table 3).

Eicosanoid and ODC Analyses. The effects of the high fat diets, with or without inhibitors, on eicosanoid synthesis and ODC activity of sham- and DMB4-treated rats are shown in Tables 4 and 5. PGE production rates in mammary tumors were highest in CO-fed rats. Indomethacin and DFMO each inhibited PGE synthesis, and when both were included in the CO diet, the inhibition was additive. Incorporation of MO in the diet inhibited PGE synthesis to a greater extent than either indomethacin or DFMO, and this inhibition was enhanced by incorporation of DFMO into the MO diet. The addition of indomethacin to the CO diet appeared to enhance LTB4 synthesis while depressing PGE synthesis and ODC activity. In contrast, DFMO and MO significantly depressed LTB4 synthesis of mammary tumors.

Ornithine decarboxylase activity was not detected in the mammary fat pads of sham-treated animals (Table 4). However, ODC activity in the tumors of CO-fed rats (Table 5) was significantly higher (P < 0.05) than in the tumors of rats fed any of the other five diets. ODC activity was depressed by 36 and 25% in tumors of rats fed indomethacin or menhaden oil, respectively. The incorporation of DFMO suppressed ODC activity by 75%, 81%, 91% for CO + DFMO, CO + INDO + DFMO and MO + DFMO, respectively, suggesting that suppression was additive with that induced by indomethacin or menhaden oil (Table 5). When ODC activities were compared among all dietary groups, no significant differences were observed among the DFMO dietary groups. However, when ODC activities of only tumors from rats fed diets containing DFMO were compared, the MO + CO + DFMO diet significantly (P < 0.05) reduced ODC activity compared to feeding CO + DFMO or CO + INDO + DFMO and MO + DFMO (Table 5). When the Pearson correlation coefficient was determined between mammary tumor incidences and ODC activities, the value was r = 0.92 (P < 0.01). A correlation also exists between PGE level and ODC activity (r = 0.652, P < 0.001).

Table 3 Effects of 20% fat diets and treatments on mammary tumor development at 16 weeks after DMBA administration

<table>
<thead>
<tr>
<th>Diet and treatment*</th>
<th>Tumor incidence</th>
<th>Total no. of tumors</th>
<th>Latency periodd (weeks)</th>
<th>No. of tumors/tumor-bearing rat</th>
<th>Tumor burden/tumor-bearing rat (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20% CO</td>
<td>25/29 (86%)d</td>
<td>83</td>
<td>11.9 ± 0.6d</td>
<td>1.79 ± 0.10d</td>
<td>2.36 ± 0.28d</td>
</tr>
<tr>
<td>20% CO + INDO</td>
<td>24/29 (83%)d</td>
<td>75</td>
<td>12.0 ± 0.6d</td>
<td>1.75 ± 0.12d</td>
<td>2.29 ± 0.35d</td>
</tr>
<tr>
<td>20% CO + DFMO</td>
<td>8/29 (28%)d</td>
<td>13</td>
<td>14.3 ± 0.6d</td>
<td>1.23 ± 0.13d</td>
<td>1.50 ± 0.46d</td>
</tr>
<tr>
<td>20% CO + INDO + DFMO</td>
<td>9/28 (32%)d</td>
<td>14</td>
<td>13.8 ± 1.0%</td>
<td>1.23 ± 0.07d</td>
<td>0.68 ± 0.14d</td>
</tr>
<tr>
<td>15% MO + 5% CO</td>
<td>17/29 (59%)d</td>
<td>54</td>
<td>12.6 ± 0.7%</td>
<td>1.72 ± 0.14d</td>
<td>1.92 ± 0.26d</td>
</tr>
<tr>
<td>15% MO + 5% CO + DFMO</td>
<td>7/29 (24%)d</td>
<td>13</td>
<td>12.7 ± 1.0%</td>
<td>1.28 ± 0.11d</td>
<td>1.40 ± 0.38d</td>
</tr>
</tbody>
</table>

* Mean ± SEM (N = 7-25). The values for number of tumors and tumor burden per tumor bearing rat are shown after square root transformation and compared among dietary groups using one-way analysis of variance.

* CO, corn oil; INDO, 0.004% w/w indomethacin; DFMO, 0.5% (w/w) D.L-2-difluoromethylornithine; MO, menhaden oil.

* Tumors were diagnosed as tubulopapillatory carcinomas (TPC), cystic TPC, solid tubular carcinomas and tubular carcinomas.

* Tumor incidences were compared using Chi-square procedure without continuity correction. Incidences which are significantly (P < 0.001-0.05) different are followed by different superscripts.

* Comparison among the dietary groups was made using one-way analysis of variance. Means which are significantly (P < 0.05) different are followed by different superscripts.

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DISCUSSION

These data suggest that eicosanoid and polyamine syntheses are involved in tumor promotion by high fat diets. When compared to feeding a 20% corn oil diet: (a) Feeding a diet containing a n-3/n-6 fatty acid ratio of 1.2 (15% menhaden oil and 5% corn oil) reduced tumorigenesis, PGE, and LTB4 synthases, and ODC activity by 31%, 88%, and 43%, respectively, (b) Feeding a 20% CO diet containing 0.5% DFMO reduced tumorigenesis, PGE, LTB4, and ODC by 72%, 91%, 52%, and 25% respectively, (c) Feeding a diet containing an n-3/n-6 fatty acid ratio of 1.2 plus 0.5% DFMO (MO + CO + DFMO) reduced synthesis. (J) Feeding a diet containing an n-3/n-6 fatty acid ratio of 1.2 and 0.5% DFMO led to a reduction in body weight after 13 weeks of feeding high fat diets.

Although feeding 0.004% indomethacin did not inhibit tumor induction, it inhibited PGE synthesis and ODC activity by 45% and 36%, respectively, and shunted eicosanoid synthesis toward lipoxygenase products as shown by a 60% increase in LTB4 synthesis. (d) Feeding a diet containing a n-3/n-6 fatty acid ratio of 1.2 plus 0.5% DFMO (MO + CO + DFMO) reduced tumorigenesis, PGE, LTB4, and ODC by 72%, 91%, 52%, and 96% respectively. Although feeding diets that contained 0.5% DFMO in a 20% fat diet led to a reduction in body weight than rats fed CO + DFMO, tumorigenesis was not less in the CO + DFMO group compared to the CO + DFMO fed group. No significant differences in food intake between the CO fed, CO + DFMO fed or CO + INDO + DFMO fed groups were observed. The failure to gain weight may have resulted from an enhanced systemic toxicity of DFMO associated with the level of fat in the diet. Since caloric density determines the quantity of food consumed by rats and since food consumption was unchanged when diets containing DFMO were fed, it appears that the absorption of nutrients and calories was not affected by DFMO. Nevertheless, feeding 0.5% DFMO in a 20% fat diet led to a reduction in body weight not observed when DFMO is given in the drinking water to rats fed a lab chow diet. Thomson et al. (37) reported that higher levels of DFMO administered in drinking water reduced body weights but they concluded that the inhibitory effect on mammary carcinosogenesis could not be accounted for on the basis of effect on somatic growth. Ongoing studies in our laboratory are being conducted to determine if the decreased tumorigenesis was a specific effect of the treatment or secondary to failure to gain weight.

Feldman et al. (16) studied the effect of 0.004% indomethacin in a 20% corn oil diet on R3230AC mammary tumor growth. They found an 89% reduction in both tumor and plasma PGE levels. However, feeding indomethacin did not reduce tumor growth when indomethacin was started 3 days prior to tumor implantation. In the present study, DMBA was used to induce mammary cancer, and indomethacin was started 3 weeks post-DMBA administration. No effect by indomethacin on tumor incidence was observed although tumor PGE production was reduced by 45%. These observations are similar to those seen by Feldman et al. (16) despite differences in the tumor model and the experimental protocol.

Carter et al. (12), however, reported that feeding 0.004% indomethacin in a 20% corn oil diet inhibited DMBA-induced mammary tumor incidence. Several differences in protocol between their work and the present study may explain the apparent discrepancy in findings. In their study, a 5-mg dose of DMBA was used in contrast to the present study which used 10 mg to induce mammary tumors. It is possible that indomethacin is a less effective inhibitor of mammary tumor tumorigenesis when a high carcinogen dose is used. An additional protocol difference concerns the optimal time at which indomethacin treatment should be started. Carter et al. (12) started indomethacin 3 days post-DMBA while in the present study indomethacin was started 3 weeks post-DMBA. The significant protective activity of indomethacin against chemically induced mammary tumors may be best achieved when indomethacin is started shortly after carcinogen administration, during the initiation period while DMBA is being metabolized (12). Moreover, it appears that indomethacin is a less effective inhibitor of mammary tumorigenesis when a high response mammary tumor model is used, i.e., a high dose of chemical carcinogen combined with promotion by a high fat diet.

Leukotriene LTB4 was measured as a marker for lipoxygenase activity since it is of special significance in inflammatory responses. It is also a mediator of T-lymphocyte function, regulating the balance between helper and suppressor T-lymphocytes, tipping response toward the suppressor side (47). In

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