Effect of the Prostaglandin Synthetase Inhibitor Indomethacin on 7,12-Dimethylbenz(a)anthracene-induced Mammary Tumorigenesis in Rats Fed Different Levels of Fat

Christopher A. Carter, Richard J. Miholland, Wendy Shea, and Margot M. Ip

Department of Experimental Therapeutics, Roswell Park Memorial Institute, Buffalo, New York 14263

ABSTRACT

The effect of the prostaglandin synthetase inhibitor indomethacin on the dietary fat enhancement of 7,12-dimethylbenz(a)anthracene-induced mammary tumorigenesis has been examined in female Sprague-Dawley rats. Rats were fed either a normal-fat or high-fat diet (5 or 18% corn oil, respectively) with or without 0.004% indomethacin, starting 3 days after a single intragastric intubation of 5 mg 7,12-dimethylbenz(a)anthracene. Results of this experiment demonstrated that indomethacin completely blocked the stimulatory effect of fat on tumorigenesis, as measured by a decreased tumor incidence, a decreased number of tumors per group, a decreased tumor size, and an increased latency. No effect of indomethacin was observed in rats fed the normal-fat diet. These data suggest that at least part of the stimulatory effect of polyunsaturated fat on 7,12-dimethylbenz(a)anthracene-induced mammary tumorigenesis may be mediated through an increased synthesis of prostaglandins.

INTRODUCTION

The stimulatory effect of a high-fat diet on both spontaneous and carcinogen-induced mammary tumorigenesis in rodents is by now well established (5, 7, 15, 19, 20, 29, 32, 33). The major effect of dietary fat appears to be at the promotional stage of carcinogenesis (4, 15, 16, 18); however, the exact mode of action remains to be established. Although an endocrinological effect has been postulated (8, 19), recent evidence suggests that the feeding of a high-fat diet can exert its effect independently of any change in estrogen (6, 17) or prolactin (19, 32). Moreover, when circulating prolactin was measured by a decreased tumor incidence, a decreased number of tumors per group, a decreased tumor size, and an increased latency. No effect of indomethacin was observed in rats fed the normal-fat diet. These data suggest that at least part of the stimulatory effect of polyunsaturated fat on 7,12-dimethylbenz(a)anthracene-induced mammary tumorigenesis may be mediated through an increased synthesis of prostaglandins.

MATERIALS AND METHODS

Animals and Treatment. Female Sprague-Dawley rats (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were housed in a temperature (22°C)- and light-controlled (12 hr of light and 12 hr of dark) room with food and water available ad libitum. At 50 days of age, each rat received a single intragastric intubation of 5 mg DMBA (Sigma Chemical Co., St. Louis, Mo.) to either a normal-fat (5% corn oil) or high-fat (18% corn oil) dietary group. The diet of one-half the rats in each group was supplemented with 0.004% (w/w) indomethacin (Sigma). These diets were continued for the duration of the experiment. The normal-fat diet contained, in percentage by weight: corn oil (Mazola corn oil; Best Foods, Englewood Cliffs, N. J.), 5.0; vitamin-free casein (Teklad), 20.0; dextrose (Federal Bakers' Supply, Buffalo, N. Y.), 68.8; AIN-76 vitamin mix with 1980 modification (ICN Pharmaceuticals, Cleveland, Ohio), 1.0; choline bitartrate (ICN), 0.2; and Rogers and Harper salt mix (Teklad), 5.0. The high-fat diet contained, in percentage by weight: corn oil, 18; vitamin-free casein, 23.0; dextrose, 51.7; AIN-76 vitamin mix with 1980 modification, 1.2; choline bitartrate, 0.24; and Rogers and Harper salt mix, 5.9. The diets are formulated on the assumption that rats will consume an equal number of calories. Since the caloric density is higher for the higher-fat diet, rats will eat less of it, and protein, salt, and vitamins are adjusted accordingly so that the intake of these components will be the same in all groups of rats. Indomethacin was added to the diets by premixing with the vitamins, minerals, and choline bitartrate, followed by addition of this whole mixture to the bulk of the diet ingredients. Although the indomethacin content of the final diet preparation was not assayed, we assume that it was distributed in the diet as well as were the vitamins and minerals, since it was added together with these nutrients and since all the animals grew normally.

Rats were weighed and palpated weekly for the presence of mammary tumors. 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. At autopsy, the

1 This work was supported by Grants CA 13038 and CA 24538 from the National Cancer Institute.
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3 To whom requests for reprints should be addressed, at Department of Experimental Therapeutics, Roswell Park Memorial Institute, Buffalo, N. Y. 14263.
4 The abbreviations used are: DMBA, 7,12-dimethylbenz(a)anthracene; CMI, cell-mediated immune response.

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AUGUST 1983

3559

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tumors were excised and weighed, fixed in buffered formalin, and sectioned for histological examination.

Corticosterone Assay. Blood for corticosterone assay was obtained by orbital sinus puncture of rats lightly anesthetized with ether, between 9 and 11 a.m. Serum was separated by centrifugation and stored at −20° until assayed for corticosterone by the corticosteroid-binding procedure of Henning (13). Blood sampling was done 19 to 21 weeks after DMBA administration.

Statistical Analysis. Differences in the palpable tumor incidence curves were analyzed using Computer Program BMDP1L (1). This program is a survival analysis and is set up to perform a 2 × 2 contingency table for each week of the experiment. The Mantel-Cox statistic generated at the end is a generalized Savage test which compares observed events with expected events. Tumor incidence at the final time point (consisting of palpable plus nonpalpable tumors) was analyzed by χ² analysis, with Yates' correction (11). All other data were analyzed using the Kruskal-Wallis and Mann-Whitney nonparametric tests. These analyses were performed on the Roswell Park computer using the Statistical Package for the Social Sciences (28).

RESULTS

This experiment was designed to determine whether the administration of indomethacin would inhibit the stimulatory effect of fat on tumor development. In this study, rats were fed either normal or high levels of dietary fat, with or without indomethacin, starting 3 days after the administration of DMBA. Chart 1 shows the cumulative palpable tumor incidence in each group of rats at each week after administration of DMBA. It can be observed that the feeding of a high-fat diet significantly (p < 0.02) stimulated tumor incidence when rats fed this diet were compared to those on a normal-fat diet. Importantly, however, the inclusion of 0.004% indomethacin in the diet of the high-fat-fed rats was able to completely block this effect. Chart 1 also shows that indomethacin had no effect in rats fed the normal-fat diet. Similar observations on tumor incidence were made when nonpalpable tumors, discovered at autopsy, were included in the analysis (Table 1), although it should be noted that the final tumor incidences did not differ statistically.

Table 1 further expands on the tumorigenesis data. Specifically, it can be observed that total tumor number was twice as great in the high-fat-fed control group as in the normal-fat control. The addition of indomethacin to the high-fat diet, however, resulted in a reduction of the total number of tumors to a level similar to that observed in the normal-fat groups. The number of tumors per tumor-bearing rat was not affected by indomethacin; the observed increase in this parameter in the high-fat control rats was not statistically significant. Of great interest was the effect of indomethacin on tumor size. Tumors from rats fed the high-fat diet were significantly larger than those from the normal-fat controls (p < 0.01). The presence of indomethacin in the diet appeared to suppress tumor growth; tumors from high-fat-indomethacin-treated rats were only one-third the size of the high-fat controls (p < 0.01). A decrease in tumor size was also observed when the 2 normal-fat groups were compared; however, the smaller tumor weight of the indomethacin-treated group was not statistically significant.

These effects of indomethacin on tumor load are further illustrated in Chart 2, which shows the cumulative tumor diameter in each group at various times after administration of DMBA. These values were obtained by summing the average diameter for each tumor at each time point for all rats in the group; again, the striking inhibitory effect of indomethacin in the high-fat group is noteworthy. Also evident in Chart 2 is the fact that tumors appeared earlier in rats fed the high-fat diet (Table 1); this effect, however, was blocked by indomethacin. In rats fed a normal-fat diet, indomethacin did not affect the latent period.

It should be noted that in none of these experiments did indomethacin have any effect on the body weight of the rats (Chart 3), suggesting that its effect was selective and not due to weight loss. The latter was of some concern, since high doses of indomethacin are known to cause gastric ulceration. Final average carcass weights (body weight minus tumor weight) for the normal-fat control and indomethacin-treated and for the high-fat control and indomethacin-treated groups were significantly different, with the former groups weighing significantly more than the latter.

Table 1
Effect of indomethacin on development of DMBA-induced mammary tumors in rats fed high or normal levels of dietary fat

<table>
<thead>
<tr>
<th>Diet</th>
<th>No. of rats</th>
<th>No. of tumors</th>
<th>Tumors/rat</th>
<th>Tumors/tumor-bearing rat</th>
<th>Mean tumor size (g)</th>
<th>Latency (wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal fat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>34</td>
<td>44.1</td>
<td>35</td>
<td>1.03 ± 0.25c</td>
<td>2.33 ± 0.33</td>
<td>1.67 ± 1.00</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>32</td>
<td>40.6</td>
<td>30</td>
<td>0.94 ± 0.31</td>
<td>2.31 ± 0.80</td>
<td>0.52 ± 0.13</td>
</tr>
<tr>
<td>High fat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>32</td>
<td>59.4</td>
<td>71</td>
<td>2.22 ± 0.62</td>
<td>3.74 ± 0.89</td>
<td>4.08 ± 1.90d</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>34</td>
<td>38.2</td>
<td>35</td>
<td>1.03 ± 0.40</td>
<td>2.69 ± 0.86</td>
<td>1.46 ± 0.54</td>
</tr>
</tbody>
</table>

*a* Includes palpable and nonpalpable adenocarcinomas discovered at autopsy.

*b* Mean time to appearance of first tumor in each rat.

*c* Mean ± S.E.

*d* Statistically different from each of the other 3 groups (p < 0.01).

*e* Statistically different from each of the other 3 groups (p < 0.03).
The mechanism by which the prostaglandins and related compounds affect tumorigenesis is not clearly understood at present; however, prostaglandins have been shown to play a role in the regulation of both humoral and cell-mediated immunity (12). Additionally, it has been shown that prostaglandins exert an inhibitory effect on natural killer cells, components of the host defense system which are thought to play a role in immunosurveillance (3). Of interest in the studies reported here was the fact that indomethacin blocks the cyclooxygenase component of prostaglandin synthetase, it could be concluded that one or more of the cyclooxygenase products is responsible for the observed reduction in tumorigenesis. However, an increase in lipoxygenase products as a result of diversion of substrate flow into this pathway cannot be ruled out. Further investigations are currently underway in this laboratory to determine whether compounds that block both pathways can also block the stimulatory effect of fat and also to determine whether structurally different cyclooxygenase inhibitors exert the same effect as does indomethacin.

Based on the data presented in this paper, it can be hypothesized that one mechanism by which the feeding of diets high in polyunsaturated fat could stimulate mammary tumorigenesis is via an increased synthesis of prostaglandins. Such a possibility has been suggested previously by Hopkins and West (14).

The synthesis and metabolism of the prostaglandins have been reviewed recently (12, 23). In brief, linoleic acid is converted to arachidonic acid which can be further metabolized to the prostaglandin 2 series of prostaglandins via prostaglandin synthetase or to a series of monohydroxy- and monohydroperoxyeicosatetraenoic acids via the lipoxygenase pathway. Since indomethacin blocks the cyclooxygenase component of prostaglandin synthetase, it could be concluded that one or more of the cyclooxygenase products is responsible for the observed reduction in tumorigenesis. However, an increase in lipoxygenase products as a result of diversion of substrate flow into this pathway cannot be ruled out. Further investigations are currently underway in this laboratory to determine whether compounds that block both pathways can also block the stimulatory effect of fat and also to determine whether structurally different cyclooxygenase inhibitors exert the same effect as does indomethacin.

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**DISCUSSION**

These experiments demonstrate that the chronic administration of indomethacin can block the stimulatory effect of fat on DMBA-induced mammary tumor development. The fat used in these experiments, corn oil, contains 60% linoleic acid, an essential fatty acid that is a precursor of prostaglandin synthesis. Based on the data presented in this paper, it can be hypothesized that one mechanism by which the feeding of diets high in polyunsaturated fat could stimulate mammary tumorigenesis is via an increased synthesis of prostaglandins. Such a possibility has been suggested previously by Hopkins and West (14).

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fact, was observed. In such animals, the administration of indomethacin would decrease prostaglandin levels with the result that CMI would no longer be inhibited, and tumorigenesis would be decreased. In contrast is the situation in the rats fed the normal-fat diet. Since low levels of prostaglandin E stimulate CMI, it is possible that indomethacin would either be without effect on tumorigenesis or could actually inhibit the induction of CMI and thus initially, at least, stimulate tumorigenesis. Increased circulating levels of corticosterone could enhance this effect even further by decreasing substrate flow into the entire arachidonic acid pathway by virtue of its inhibitory effect on phospholipase A

REFERENCES

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Note Added in Proof

The dose of indomethacin used in these experiments was that reported by L. A. Hillyard and S. Abraham (Effect of dietary polyunsaturated fatty acids on growth of mammary adenocarcinomas in mice and rats. Cancer Res., 39: 4430-4437, 1979), who demonstrated that the stimulatory effect of fat on growth of the transplantable mouse mammary tumor 3910-30 was inhibited by this concentration of indomethacin.
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