Differential Effects of Tranylcypromine and Imidazole on Mammary Carcinogenesis in Rats Fed Low and High Fat Diets

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ABSTRACT

Neoplastic development in the rat mammary gland can be suppressed by inhibition of the activity of several enzymes involved in eicosanoid biosynthesis. In order to investigate the potential utility of prostacyclin and thromboxane synthetases as targets for mammary cancer chemoprevention, experiments were conducted to determine the influence of tranylcypromine (TCP), an inhibitor of prostacyclin synthetase, and imidazole (IMI), an inhibitor of thromboxane synthetase, on mammary carcinogenesis in rats by N-methyl-N-nitrosourea. Fifty-day-old female Sprague-Dawley [Hsd:SD(BR)] rats received a single s.c. dose of 0 or 40 mg of N-methyl-N-nitrosourea per kg of body weight. Beginning 7 days after carcinogen administration, groups of rats were fed isoenergetic, casein-based diets containing 3 or 20% corn oil (w/w), supplemented with (per kg of diet) 10 mg of TCP, 1000 mg of IMI, or sucrose carrier only. TCP reduced mammary carcinoma multiplicity in rats fed the 20% corn oil diet, but had no effect in rats fed the diet containing 3% fat. By contrast, supplementation with IMI increased mammary cancer incidence in the group fed the 20% fat diet and increased carcinoma multiplicity in the 3% fat group to the levels seen in rats fed the 20% fat diet. These data suggest that inhibition of prostacyclin synthetase, but not thromboxane synthetase, may present a useful mechanism for mammary cancer chemoprevention in animals consuming a diet high in fat. Furthermore, the differential effects of TCP and IMI in rats fed low and high fat diets suggest that the action of dietary fat in mammary cancer induction may involve influences on the arachidonic acid cascade.

INTRODUCTION

Chemical carcinogenesis in the rat mammary gland is subject to modulation through a variety of endocrine, pharmacological, and nutritional manipulations (1). Because of the close biological and histopathological correspondence between mammary neoplasia in rats and in humans, modification of mammary cancer response in the rat model may provide useful insights into the regulation of neoplastic development in the human breast. For example, the identification of factors which enhance mammary cancer induction in rats [e.g., dietary fat (2, 3)] can aid in the elucidation of risk factors for human breast cancer. Conversely, a number of factors which suppress neoplastic development in the rat mammary gland [e.g., early full-term pregnancy (4, 5)] appear to play a similar role in reducing the risk of breast cancer in humans (6). Finally, the experimental use of modifiers of carcinogenesis can provide data concerning the mechanisms through which neoplastic development in the mammary gland is regulated; such data can be applied to both cancer risk assessment and the identification of appropriate targets for the design of anticancerogenic drugs.

Modifiers of arachidonic acid metabolism are one class of agents with significant activity as inhibitors of rat mammary carcinogenesis. The initial mammary cancer chemoprevention studies with this class of compounds used inhibitors of the cyclooxygenase pathway of arachidonic acid catabolism; experiments performed in our laboratory and by Ip and coworkers demonstrated that the postcarcinogen phase of rat mammary carcinogenesis can be suppressed by dietary administration of indomethacin (7, 8) or flurbiprofen (9). However, although the anticarcinogenic activity of indomethacin is similar to that of more widely studied inhibitors of mammary carcinogenesis such as retinyl acetate (10) and selenium (11), the dose levels of indomethacin required for chemopreventive efficacy in rats are close to the threshold of lethal toxicity (12). For this reason, studies are ongoing to identify additional modifiers of arachidonic acid metabolism whose administration provides an effective means for the suppression of mammmary cancer induction, yet which possess improved therapeutic ratios, i.e., greater "margins of safety" between the dose levels required for anticarcinogenic efficacy and those which induce significant toxicity.

The oxidation of arachidonic acid by cyclooxygenase is an early step in the biosynthesis of a number of eicosanoids, including PGA₃, PGD₂, PGE₂, PGF₂α, PGL₂, and TXB₂. Because cyclooxygenase is involved in the synthesis of a large number of biologically active compounds, the possibility exists that the chemopreventive and toxic effects of cyclooxygenase inhibition are a result of alterations in the synthesis of different eicosanoids. In such a circumstance, the chemopreventive efficacy and toxicity of cyclooxygenase inhibition might be dissociable through the use of a more narrowly targeted approach designed to influence levels of fewer eicosanoids. The enzymes prostacyclin synthetase and TX synthetase utilize a common substrate in the post-cyclooxygenase portion of the arachidonic acid cascade; a negative interaction between the products of these two enzymes has been proposed (13, 14). The present study was designed to determine the efficacy of TCP, an inhibitor of prostacyclin synthetase (15, 16), and IMI, an inhibitor of TX synthetase (17, 18), as chemopreventive agents in the rat mammary gland, and to determine if the activity of these compounds as modifiers of mammary cancer induction is influenced by dietary fat intake.

MATERIALS AND METHODS

Experimental Animals. Virgin female Sprague-Dawley [Hsd:SD(BR)] rats were received at 28 days of age from Harlan/Sprague-Dawley, Indianapolis, IN. Rats were housed in groups of three in polycarbonate cages on hardwood bedding, and they were held in a temperature- and humidity-controlled room maintained on a daily cycle of 14 h of light and 10 h of dark. During the quarantine period, rats were allowed free access to a standard laboratory chow diet (Wayne Lab Chow; Allied Mills, Chicago, IL) and drinking water; 5 days prior to the initiation of the study, the chow diet was replaced with a semipurified, casein-based diet containing 3% fat. All food and bedding materials were changed twice weekly.

Experimental Diets. Basal diets used in the experiment were isoenergetic based diets containing 3 or 20% corn oil (w/w), supplemented with (per kg of diet) 10 mg of TCP, 1000 mg of IMI, or sucrose carrier only. TCP reduced mammary carcinoma multiplicity in rats fed the 20% corn oil diet, but had no effect in rats fed the diet containing 3% fat. By contrast, supplementation with IMI increased mammary cancer incidence in the group fed the 20% fat diet and increased carcinoma multiplicity in the 3% fat group to the levels seen in rats fed the 20% fat diet. These data suggest that inhibition of prostacyclin synthetase, but not thromboxane synthetase, may present a useful mechanism for mammary cancer chemoprevention in animals consuming a diet high in fat. Furthermore, the differential effects of TCP and IMI in rats fed low and high fat diets suggest that the action of dietary fat in mammary cancer induction may involve influences on the arachidonic acid cascade.
To facilitate homogeneous distribution of these agents in the diets, Young and Hallowes (19). Because benign mammary tumors were grossly abnormal tissues were fixed in 10% buffered formalin and complete necropsy. Sections of all mammary tumors and any other observed twice daily throughout the study to monitor their overall weekly to monitor mammary tumor appearance; the location and date experimental diets was begun.

Groups according to the protocol (Table 2), and administration of to administration. At 50 days of age, all rats received a single s.c. troit, MI) and was dissolved in sterile saline (pH 5.0) immediately prior with sucrose carrier only.

TCP and IMI were mixed into diets using a sucrose carrier (10 g/kg of were selected on the basis of preliminary subchronic toxicity studies.

by Teklad Test Diets, Madison, WI. The composition of the basal diets (w/w), as added corn oil. Diets were manufactured to our specifications with sucrose carrier only.

Experimental Protocol. MNU was purchased from Ash-Stevens (Detroit, MI) and was dissolved in sterile saline (pH 5.0) immediately prior to administration. At 50 days of age, all rats received a single s.c. injection of 0 or 40 mg of MNU per kg of body weight. Seven days after carcogenen administration, rats were randomized by weight into groups according to the protocol (Table 2), and administration of experimental diets was begun.

Beginning 4 wk after MNU administration, rats were palpated twice weekly to monitor mammary tumor appearance; the location and date of appearance of all palpable lesions were recorded. Animals were observed twice daily throughout the study to monitor their overall health status, and they were weighed weekly. All animals found dead during the experiment or killed at its termination were subjected to a complete necropsy. Sections of all mammary tumors and any other grossly abnormal tissues were fixed in 10% buffered formalin and processed by routine methods for histopathological classification. Mammary tumor pathology was defined according to the criteria of Young and Hallowes (19). Because benign mammary tumors were infrequent (<0.2 tumors/rat in all groups), only histologically confirmed mammary cancers were used in the data analysis.

Statistical Analysis. Values for mammary cancer incidence and multiplicity were calculated using life table analysis, and thus include corrections for intercurrent mortality. Comparisons of cancer latency curves were made using the logrank test (20). Animal survival and mammary cancer incidence at 180 days post-MNU were compared using χ² analysis; comparisons of T₅₀ were made using the median test (21). Group body weights were compared using analysis of variance.

**RESULTS**

TCP and IMI had opposite effects on mammary cancer induction by MNU. Significant differences between these two agents were found in comparisons of their influence on mammary carcinoma multiplicity and tumor latency and in the effects of dietary fat on their activity as modifiers of mammary carcinogenesis.

Administration of a supplement of 10 mg of TCP per kg of diet conferred significant protection against mammary carcinogenesis in rats fed the diet containing 20% corn oil (Table 2). As illustrated in Fig. 1, TCP decreased the mean number of mammary cancers per rat by approximately 40%, from 2.67 in controls to 1.64 (P < 0.01). The compound also increased T₅₀ from 98 days in the 20% fat control group to 130 days and decreased tumor-related mortality from 12% in controls to 0% (0.05 < P < 0.10). However, although exposure to TCP increased the median time to mammary cancer appearance, the compound had no effect on cancer incidence at the termination of the study.

In contrast to its chemopreventive activity in rats fed the 20% fat diet, TCP had no effect on mammary carcinogenesis in rats fed an isoenergetic diet containing 3% corn oil (Table 2). Terminal cancer incidence, tumor-related mortality, and median tumor induction time did not differ between the control and TCP groups fed the 3% fat diet; carcinoma multiplicity curves for the two groups were comparable throughout the experiment (Fig. 2).

The effects of dietary supplementation with 1000 mg of IMI per kg were generally opposite those of TCP. Whereas a significant reduction in carcinoma multiplicity was observed in rats fed the 20% fat diet supplemented with TCP, IMI had no effect on mammary cancer number in rats fed this level of fat. As indicated in Fig. 1, mammary cancer multiplicity in the IMI group fed the high fat diet was within 1% of control levels at the termination of the study. Similarly, comparisons of tumor-related mortality and median tumor latency demonstrated no differences between dietary control and IMI groups fed the 20% fat diet. However, primarily as a result of a late burst in carcinoma appearance, IMI did increase terminal cancer incidence to 96% from 78% in controls (P < 0.05).

In rats fed the 3% fat diet, administration of IMI increased mammary cancer number by approximately 30% from control levels (Fig. 2). This increase was not statistically significant

**Table 1 Composition of experimental diets**

<table>
<thead>
<tr>
<th>Dietary component</th>
<th>3% Corn oil diet (g/kg)</th>
<th>20% Corn oil diet (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein, high protein</td>
<td>200.0</td>
<td>200.0</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Corn oil</td>
<td>30.0</td>
<td>200.0</td>
</tr>
<tr>
<td>Expanded maltodextrin</td>
<td>360.0</td>
<td>150.0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>323.0</td>
<td>140.0</td>
</tr>
<tr>
<td>Cellulose</td>
<td>32.0</td>
<td>255.0</td>
</tr>
<tr>
<td>Mineral mix, AIN-76</td>
<td>35.0</td>
<td>35.0</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Vitamin mix, AIN-76A</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Total available energy* 3.7 kcal/g 3.7 kcal/g

* Calculated using the following nutrient values: protein, 4 kcal/g; carbohydrate, 4 kcal/g; fat, 9 kcal/g.

**Table 2 Influence of tranylcypromine and imidazole on mammary carcinogenesis induced by N-methyl-N-nitrosourea**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>MNU dose (mg/kg body wt)</th>
<th>Dietary fat level (%)</th>
<th>Chemopreventive agent (mg/kg diet)</th>
<th>Cancer incidence (%)</th>
<th>T₅₀ (days)</th>
<th>Cancers/rat</th>
<th>Body wt (g)</th>
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<tr>
<td>1</td>
<td>20</td>
<td>0</td>
<td>3</td>
<td>TCP (10)</td>
<td>0</td>
<td>298 ± 10²</td>
<td>298 ± 10²</td>
<td>298 ± 10²</td>
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<tr>
<td>2</td>
<td>10</td>
<td>0</td>
<td>3</td>
<td>TCP (10)</td>
<td>0</td>
<td>293 ± 6</td>
<td>293 ± 6</td>
<td>293 ± 6</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>0</td>
<td>3</td>
<td>TCP (10)</td>
<td>0</td>
<td>287 ± 10</td>
<td>287 ± 10</td>
<td>287 ± 10</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>0</td>
<td>20</td>
<td>TCP (10)</td>
<td>0</td>
<td>284 ± 6</td>
<td>284 ± 6</td>
<td>284 ± 6</td>
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<tr>
<td>5</td>
<td>10</td>
<td>0</td>
<td>20</td>
<td>TCP (10)</td>
<td>0</td>
<td>280 ± 7</td>
<td>280 ± 7</td>
<td>280 ± 7</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>0</td>
<td>20</td>
<td>TCP (10)</td>
<td>0</td>
<td>282 ± 5</td>
<td>282 ± 5</td>
<td>282 ± 5</td>
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<tr>
<td>7</td>
<td>10</td>
<td>0</td>
<td>20</td>
<td>TCP (10)</td>
<td>0</td>
<td>280 ± 7</td>
<td>280 ± 7</td>
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<tr>
<td>8</td>
<td>10</td>
<td>0</td>
<td>20</td>
<td>TCP (10)</td>
<td>0</td>
<td>277 ± 5</td>
<td>277 ± 5</td>
<td>277 ± 5</td>
</tr>
<tr>
<td>9</td>
<td>10</td>
<td>0</td>
<td>20</td>
<td>TCP (10)</td>
<td>0</td>
<td>272 ± 5</td>
<td>272 ± 5</td>
<td>272 ± 5</td>
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<tr>
<td>10</td>
<td>10</td>
<td>0</td>
<td>20</td>
<td>TCP (10)</td>
<td>0</td>
<td>272 ± 5</td>
<td>272 ± 5</td>
<td>272 ± 5</td>
</tr>
<tr>
<td>11</td>
<td>10</td>
<td>0</td>
<td>20</td>
<td>TCP (10)</td>
<td>0</td>
<td>269 ± 4</td>
<td>269 ± 4</td>
<td>269 ± 4</td>
</tr>
<tr>
<td>12</td>
<td>10</td>
<td>0</td>
<td>20</td>
<td>TCP (10)</td>
<td>0</td>
<td>269 ± 4</td>
<td>269 ± 4</td>
<td>269 ± 4</td>
</tr>
</tbody>
</table>

* Mean ± SE.

² P < 0.05 versus appropriate control group.

³ P < 0.01 versus appropriate control group.
TRANYLCYPROMINE AND IMIDAZOLE EFFECTS ON RAT MAMMARY CARCINOGENESIS

Fig. 1. Influence of tranylcypromine and imidazole on mammary carcinogenesis in rats fed a semipurified diet containing 20% corn oil. □, control; ○, tranylcypromine (10 mg/kg of diet); ▲, imidazole (1000 mg/kg of diet).

Fig. 2. Influence of tranylcypromine and imidazole on mammary carcinogenesis in rats fed a semipurified diet containing 3% corn oil. □, control; ○, tranylcypromine (10 mg/kg of diet); ▲, imidazole (1000 mg/kg of diet).

Fig. 3. Mammary cancer response in rats fed isenergetic diets containing 3% or 20% corn oil (w/w). □, 3% corn oil; ■, 20% corn oil.

DISCUSSION

Inhibitors of the generation of arachidonic acid, as well as its catabolism by cyclooxygenase and lipoxygenase, can all confer protection against cancer induction when administered to rats following exposure to an organotropic mammary gland carcinogen (7, 8, 22, 23). The results of the present study demonstrate that two compounds with activity as inhibitors of enzymes in the post-cyclooxygenase portion of the arachidonic acid cascade can also modify mammary cancer induction. However, only one of the two agents suppressed mammary carcinogenesis, while the other increased cancer induction.

Dietary administration of TCP at a level of 10 mg per kg of diet (approximately 0.7 mg/kg of body weight/day) resulted in a significant suppression of mammary carcinogenesis in rats fed a 20% fat diet, but conferred no protection in rats fed a diet containing 3% fat. No systemic or organ-specific toxicity was observed in TCP-treated rats at any point during the experiment. Furthermore, the preliminary subchronic toxicity study indicated that the only adverse effect of exposure to TCP at doses up to 10 times higher than that used in the carcinogenesis study was a 15% suppression of body weight (data not shown). The wide range between anticarcinogenic and toxic doses of TCP differs significantly from the results of our previous studies with indomethacin (8, 12). These data indicate that TCP, an agent which inhibits prostacyclin synthetase in several experimental systems (15, 16), can confer significant protection against mammary carcinogenesis in animals consuming a high fat diet. Furthermore, these data suggest that the toxicity of cyclooxygenase inhibition may be dissociable from its chemopreventive activity through the use of agents which modify the activity of enzymes lower in the arachidonic acid cascade.

By contrast to the effects of TCP, dietary supplementation with IMI at a level of 1000 mg per kg of diet (approximately 70 mg/kg/day) did not suppress mammary cancer induction; in fact, exposure to IMI was associated with increased tumor response in several groups. We are aware of no other studies which have examined the influence of a thromboxane synthesis inhibitor on carcinogenesis; however, the results of the present study with IMI are similar to several recent reports which noted either no effect or increased growth of transplantable tumors in mice treated with the thromboxane synthesis inhibitor, dazmagrel (UK-38485; 24–26).

An antagonism between the products of the prostacyclin and
thromboxane pathways of arachidonic acid metabolism has been proposed (13, 14). The opposing effects of TCP and IMI on mammary cancer induction could be interpreted as supportive of this hypothetical negative interaction. In view of the technical problems inherent in quantitating the effects of pharmacological agents on tissue-specific eicosanoid biosynthesis in vivo, it is not possible at the present time to confirm or to deny the relevance of this hypothesis to the whole animal mammary carcinogenesis data generated in this study. However, the differential effects of TCP and IMI are clearly suggestive.

A negative interaction between these pathways of eicosanoid metabolism has also been proposed in the regulation of tumor metastasis (27). However, prostacyclin and thromboxanes appear to have much different effects on metastasis than on carcinogenesis and tumor growth. The data from the present study and from studies investigating the influence of thromboxane synthetase inhibitors on the growth of transplantable tumors (24–26) suggest that inhibition of prostacyclin synthesis is protective, while inhibition of thromboxane synthetase stimulates tumor growth. By contrast, Honn et al. (27) have reported protection against tumor metastasis by inhibition of thromboxane synthetase and by administration of PGI2. The specificity and mechanisms of action of the agents used in these studies should be addressed. IMI has been demonstrated to be a highly selective inhibitor of TX synthetase in several experimental systems (28–30), suggesting that the enhancement of mammary carcinogenesis observed in groups exposed to IMI was mediated by suppression of TX synthetase activity and its concomitant effects on eicosanoid biosynthesis. Although it is likely that TCP suppresses mammary cancer induction through its activity as an inhibitor of prostacyclin synthetase (15, 16), other possible mechanisms cannot be excluded at the present time. TCP inhibits monoamine oxidase activity (31, 32), and like most monoamine oxidase inhibitors, it can alter the activity of a number of hepatic enzymes involved in xenobiotic metabolism (33). It is conceivable that TCP could inhibit mammary carcinogenesis through a mechanism related to this activity. It should be noted in this regard, however, that TCP administration was begun 1 wk after a single dose of a carcinogen (MNU) which requires no enzymatic activation. The use of MNU with this schedule of TCP administration precludes interference with carcinogen metabolism as a mechanism of action for TCP chemoprevention in the present study. However, possible influences of TCP on arachidonate release (16) or on the generation of PGD2 (34) cannot be ruled out.

Although the mechanisms of dietary fat action in normal and neoplastic development in the mammary gland remain undefined, several lines of evidence suggest that metabolites of arachidonic acid may be involved. Increased eicosanoid biosynthesis in response to linoleic acid has been demonstrated in several in vitro systems, including mammary gland organ culture (35). These data, when considered with the enhancing effects of linoleate on mammary cancer induction in vivo (36, 37), suggest a link between eicosanoids and dietary fat action in mammary carcinogenesis. The data from the present study may also support such a role and can be used to develop a model for the role of eicosanoids in dietary fat action. In such a model, the presence of one or more products of the cyclooxygenase pathway would be required at concentrations above a minimum level for neoplastic development; the specific role of the eicosanoid could be either directly stimulatory or permissive. When present only at low levels, as in animals fed a low fat diet, the relative paucity of this eicosanoid(s) would serve to limit mammary carcinogenesis. However, when the dietary fat level is increased to 20%, synthesis of the limiting eicosanoid(s) is increased, thereby stimulating neoplastic growth. A parallel mechanism would be operative when IMI is administered to rats fed the low fat diet; through its reorientation of arachidonic acid metabolism away from TX production and into other pathways, the biosynthesis of the eicosanoid(s) which stimulates mammary carcinogenesis is increased. Although these stimulatory compounds remain undefined, the data from the present study exclude TX from this role.

A converse mechanism could explain the influence of dietary fat level on the anticarcinogenic activity of TCP. Because the low level of eicosanoid production in rats fed the low fat diet is already limiting, further decreases in the production of stimulatory eicosanoids through administration of TCP would provide no additional protection against mammary cancer induction. By contrast, administration of TCP would be protective in rats fed the high fat diet, as the drug would inhibit biosynthesis of stimulatory or permissive factors which serve to enhance mammary cancer induction. Investigations of the anticarcinogenic activity of modifiers of arachidonic acid metabolism coupled with in vitro studies of eicosanoid biosynthesis in mammary epithelial cells will be required to determine whether PGI2, PGE2, or other products of the arachidonic acid cascade serve as specific regulators of mammary neoplasia.

ACKNOWLEDGMENTS

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