Role of Prolactin in the Promotion of Dimethylbenz[a]anthracene-induced Mammary Tumors by Dietary Fat

Clement Ip, Philip Yip, and Lee L. Bernardis

Department of Breast Surgery and Breast Cancer Research Unit, Roswell Park Memorial Institute, Buffalo 14263. [C. I., P. Y.,] and Division of Endocrinology, Veterans Administration Medical Center, Buffalo, New York 14215 [L. L. B.]

ABSTRACT

Following 7,12-dimethylbenz[a]anthracene (DMBA) administration, increased tumor incidence and tumor yield were observed in intact rats fed a 20% corn oil diet compared to those fed a 0.5% corn oil diet. Elevated serum prolactin levels (determined at proestrus) were also found in the former group of animals. In order to delineate whether this was responsible for the promoting effect of dietary fat in DMBA-induced mammary carcinogenesis, rats fed these two diets were subjected to electrolytic lesion of the median eminence that resulted in higher circulating prolactin concentrations. Sham-operated animals were used as controls. Results showed that this endocrine manipulation increased the tumor incidence in the low-fat group nearly 3-fold, but it failed to elicit further enhancement in the high-fat rats. Although the serum prolactin level in the low-fat-lesioned rats was comparable to that in the high-fat-lesioned rats, the tumor incidence in the former group still lagged behind that in the latter (33.3 versus 70.4%). Thus, it can be concluded that, although increased circulating prolactin in rats fed the high-fat diet may be partly responsible for the higher tumor incidence, other factors besides prolactin may be involved in the promoting effect of dietary fat in mammary carcinogenesis.

INTRODUCTION

There is increasing evidence in the literature which shows that dietary fat may be important in the etiology and/or promotion of breast cancer. Carroll (5) pointed out that there is a strong positive correlation between the per capita consumption of fat and the age-adjusted mortality rate from breast cancer. This analysis has been confirmed by several investigators (1, 13–15). Results from animal experiments have added further support to the above conclusion. Diets rich in fat are known to enhance the development of both spontaneous and chemically induced mammary tumors in rodents. Although there is still no unifying concept as to how dietary fat stimulates carcinogenesis, suffice it to say that this phenomenon has been well documented with several tumor systems in different strains of rats and mice (5, 10).

Development and growth of DMBA3-induced mammary tumors in rats are profoundly affected when the endogenous levels of estrogen and prolactin are altered by either drug administration or endocrine ablation (4, 12, 22). In order to determine whether the effect of dietary fat in promoting DMBA-induced tumorigenesis is mediated by changes in estrogen or prolactin metabolism, Chan and Cohen (8) made use of the antiestrogen drug, nafloxidine, and the prolactin release inhibitor, 2-bromo-o-ergocryptine. Whereas mammary tumor development was decreased by chronic administration of the antiestrogen, the incidence of tumors in rats fed the high-fat diet still remained higher when compared to rats on the low-fat diet. On the other hand, treatment with the antiprolactin drug abolished the differential effect of high-fat and low-fat diets in tumorigenesis. Circulating prolactin level at proestrus-estrus in rats on the high-fat diet was found to be significantly higher than that of those on the low-fat diet (9). These authors thus postulated that the effect of dietary fat is probably mediated by prolactin via the hypothalamic-pituitary axis.

In this study, we also tried to investigate the role of prolactin in the promoting effect of dietary fat on carcinogenesis, but with a different approach. Sinha et al. (22) showed that increased release of prolactin from the pituitary as a result of electrolytic lesion of the median eminence greatly accelerated the growth of DMBA-induced mammary tumors. The rationale of the present study was that, if the effect of dietary fat were mediated by increased circulating prolactin, as was postulated by Chan et al. (8, 9), the differential effects of high-fat and low-fat diets in tumorigenesis would be eliminated in median-eminence-lesioned rats, since blood prolactin level would be elevated in both groups.

MATERIALS AND METHODS

Animals and Diets. Female Sprague-Dawley rats (Charles River Breeding Labs, Inc., Wilmington, Mass.) were fed from weaning (21 days old) either a synthetic high-fat diet or a low-fat diet. They were housed in a temperature- and light-controlled room with water and food available ad libitum. The high-fat diet contained, in percentage, by weight: Mazola corn oil, 20; vitamin-free casein (Teklad Test Diets, Madison, Wis.), 25; Alphacel salt mix (Teklad), 5; and vitamin diet fortification mixture (ICN Pharmaceuticals, Cleveland, Ohio), 5; Rogers and Harper dextrose (Federal Baker Supply, Buffalo, N. Y.), 43; Alphacel (ICN Pharmaceuticals, Cleveland, Ohio), 5; Rogers and Harper salt mix (Teklad), 5; and vitamin diet fortification mixture (ICN Corporation, Irvine, Calif.), 2. The low-fat diet had the same ingredients, with the exception that it contained 0.5% corn oil and 62.5% dextrose.

Mammary tumors were induced by i.g. administration of 5 mg of DMBA (dissolved in 1 ml of corn oil) when the animals were 50 days old. DMBA was purchased from Sigma Chemical Co., St. Louis, Mo. In order to minimize any possible effect of dietary fat on absorption of the carcinogen from the intestine, rats on the high-fat diet were switched to the low-fat diet 2 days...
prior to DMBA administration. They were returned to the high-fat regimen 1 day after DMBA had been given. Rats were palpated once a week, and tumors were identified by their location and size and were measured with a vernier caliper in 2 perpendicular diameters. At autopsy, tumors were excised, fixed in Bouin’s reagent, and sectioned for histological examination.

All intact rats and those that were sham-operated were sacrificed in the afternoon of proestrus in order to catch the peak level of circulating prolactin. The stages of the estrus cycle were determined by the vaginal smear technique. At least 2 normal cycles were followed before sacrifice. Blood was collected by Vacutainers without anticoagulant via heart puncture while the rats were under light ether anesthesia. RBC were removed by centrifugation to obtain serum. The samples were stored at $-70^\circ$ until ready for assay of prolactin. The pituitary was also removed at autopsy and was stored frozen for later assay of prolactin.

**Determination of Prolactin.** Prolactin in serum and pituitary was determined by the use of the double-antibody radioimmunoassay kit obtained from the Hormone Distribution Program, National Institute of Arthritis, Metabolism and Digestive Diseases. Their recommended procedure was followed with 2 modifications: the amount of $^{125}$I-labeled prolactin was increased to 60,000 cpm; and the incubation time with the first antibody was reduced to 24 hr. Samples were assayed at 2 dilutions each in triplicate.

**Electrolytic Lesion of the Median Eminence.** The animals received bilateral electrolytic lesions in the median eminence 10 days after DMBA administration, with the aid of a Baltimore Stereotaxic instrument (Model S). The operation was performed using stainless steel electrodes of 0.25-mm diameter that were coated with spar varnish and bored at the tips for approximately 0.2 mm. A lesion current of 1 ma was allowed to flow for 10 sec. Sham-operated animals (electrodes lowered to approximately 0.2 mm. A lesion current of 1 ma was allowed to flow for 10 sec. Sham-operated animals (electrodes lowered to approximately 0.2 mm) served as controls. The incisions were closed with stainless steel clips, and the rats were returned to their cages. Upon sacrifice, the brains were removed and fixed in 10% buffered formalin for subsequent histological verification of the lesion placement (3).

**RESULTS**

Chart 1 illustrates the cumulative incidence of rats with palpable tumors in both low-fat and high-fat groups following DMBA administration. In rats fed the high-fat diet, palpable tumors started to appear 8 weeks after administration of 5 mg of DMBA p.o. The incidence continued to rise for 9 more weeks before leveling off at 59.4% (19 of 32 rats). On the other hand, rats that were fed the low-fat diet started to develop tumors 10 weeks after administration of DMBA; and the incidence plateaued 3 weeks later at 8.8% (3 of 34 rats). The experiment was terminated 20 to 22 weeks after carcinogen administration. Results in Table 1 show the total tumor count (both palpable and nonpalpable) as well as the histological type of the tumors obtained in each dietary group. In the low-fat group, one more rat with a nonpalpable tumor was discovered at the time of sacrifice, thus raising the final tumor incidence to 11.8% (4 of 34 rats). Altogether, there were 5 palpable and 2 nonpalpable tumors, of which 6 were adenocarcinomas and 1 was a fibroadenoma. In the high-fat group, 2 more rats with nonpalpable tumors were included in the final tally of tumor incidence, thus increasing the score to 65.6% (21 of 32 rats). A total of 69 palpable and 7 nonpalpable tumors were credited to this group. Among these, 71 were adenocarcinomas and 5 were fibroadenomas. The number of tumors per tumor-bearing rat in the high-fat group was calculated to be twice that in the low-fat group (3.6 versus 1.8).

Table 2 details some of the physical and biochemical parameters in these rats at the time of sacrifice. Rats fed the high-fat diet were heavier than those that were fed the low-fat diet ($p < 0.001$). The increment was due to obesity rather than to an increase in body length. Rats fed the low-fat diet were, in fact, very similar to those that were fed laboratory chow in terms of their body weight and obesity index (results not shown). Thus, ingestion of the 0.5% fat diet did not seem to inhibit growth of the animals. The weights of the liver, spleen, and pituitary were comparable in the 2 groups of rats shown in Table 2, when the data were expressed on a per-100-g body weight basis. Circulating prolactin level was found to be elevated in the high-fat rats compared to the low-fat rats, although there was no
Table 2
Effect of dietary fat on certain physical and biochemical parameters of intact rats at time of sacrifice

<table>
<thead>
<tr>
<th>% fat</th>
<th>Final body wt (g)</th>
<th>Nasoanal length (mm)</th>
<th>Obesity index*</th>
<th>Organ wt/100 g body wt</th>
<th>Prolactin</th>
<th>Serum (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Liver (g)</td>
<td>Spleen (g)</td>
<td>Pituitary (μg/ mg wet wt)</td>
</tr>
<tr>
<td>0.5</td>
<td>278 ± 4.7</td>
<td>217 ± 1.2</td>
<td>306 ± 1.2</td>
<td>3.5 ± 0.06</td>
<td>0.19 ± 0.006</td>
<td>6.2 ± 0.20</td>
</tr>
<tr>
<td>20.0</td>
<td>303 ± 6.6</td>
<td>215 ± 1.3</td>
<td>317 ± 3.1</td>
<td>3.4 ± 0.09</td>
<td>0.19 ± 0.008</td>
<td>6.1 ± 0.21</td>
</tr>
</tbody>
</table>

*p Mean ± S.E.

Obesity index = \( \frac{\text{body wt (g)}}{\text{nasoanal length (mm)}} \times 10,000 \)

significant difference in the concentration of prolactin in their pituitaries.

In order to delineate the role of prolactin in the promotion of mammary tumorigenesis by dietary fat, a second experiment was initiated in which rats were lesioned in the median emience so as to increase their circulating prolactin level. Four groups of rats were included in this study: lesioned low- and high-fat rats; and their respective sham-operated controls. The operation was performed 10 days after DMBA administration. The cumulative palpable tumor incidences in these rats are shown in Chart 2. It should be noted that only confirmed cases of successful operation are reported here. Fig. 1 shows the localization of a median-emience lesion in a representative rat. Those animals showing damage to the ventromedial or dorsomedial hypothalamic nucleus were discarded. Electrolytic lesion of the median emience led to an earlier appearance of tumors as well as to a higher incidence of tumor-bearing rats in the low-fat group. At the time of sacrifice, the palpable tumor incidence was 12.5% (3 of 24 rats) in the sham-operated controls and 33.3% (9 of 27 rats) in the lesioned rats. This was in contrast to animals that were fed the high-fat diet, in which case the lesioned rats seemed to parallel very closely the sham-operated controls in terms of both the time course of tumor development and tumor incidence.

The final tumor count in these 4 groups is presented in Table 3. Based on χ² analysis, the following order of tumor incidence was established: high-fat lesion (70.4%) > high-fat sham (31.2%) > low-fat lesion (33.3%) > low-fat sham (12.5%). The difference between high-fat and low-fat groups was much greater in the sham-operated controls than in the lesioned animals. As can be seen in Table 4, lesions of the median emience resulted in an elevation in serum prolactin, irrespective of dietary fat consumption. This was probably due to increased secretion from the pituitary, since pituitary prolactin was slightly lower in the lesioned animals, although the reduc-

![Fig. 1. The photomicrograph shows the localization of a median-emience lesion in a representative rat. MEL, median-emience lesion; VMN, ventromedial hypothalamic nucleus; III, third ventricle. Cresyl violet, x 15.](image)

![Chart 2. Effect of median-emience lesion on the cumulative incidences of palpable mammary tumors in rats fed either a 0.5% or a 20% fat diet and given 5 mg of DMBA p.o. Rats received electrolytic lesions of the median emience 10 days after DMBA. Sham-operated rats served as controls. LF, low fat (0.5% fat); HF, high fat (20% fat). The number of rats used per group includes 24 in the low-fat-sham group, 27 in the low-fat-lesion group, 26 in the high-fat-sham group, and 27 in the high-fat-lesion group.](image)
Pituitary and serum prolactin levels in median-eminence-lesioned rats or sham-operated rats fed either a 0.5% or a 20% fat diet

All rats received 5 mg of DMBA p.o. at 50 days of age. Electrolytic lesion of the median eminence or sham operation was performed 10 days later. Animals were killed 20 to 22 weeks after administration of DMBA.

<table>
<thead>
<tr>
<th>Dietary group (% fat)</th>
<th>Operation</th>
<th>Pituitary prolactin (µg/mg)</th>
<th>Serum prolactin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>Sham</td>
<td>0.77 ± 0.09*</td>
<td>132 ± 15</td>
</tr>
<tr>
<td></td>
<td>Lesion</td>
<td>0.58 ± 0.07</td>
<td>406 ± 46*</td>
</tr>
<tr>
<td>20</td>
<td>Sham</td>
<td>0.86 ± 0.11</td>
<td>311 ± 35</td>
</tr>
<tr>
<td></td>
<td>Lesion</td>
<td>0.65 ± 0.08</td>
<td>488 ± 57</td>
</tr>
</tbody>
</table>

* Mean ± S.E.

DISCUSSION

In this study, we have confirmed previous reports from the laboratories of Carroll and Khor (6) and Chan and Cohen (8) that feeding a high-fat diet increased the DMBA-induced mammary tumor incidence of rats compared to those on a low-fat diet. It should be pointed out that the tumor incidence in these 4 groups of rats revealed that raising the prolactin level in the low-fat group increased the tumor incidence nearly 3-fold, but failed to elicit any further enhancement in the high-fat rats. It should be pointed out that rats with proper median-eminence lesions do not have estrus cycles. Thus, only the sham-operated control rats in this study were sacrificed during proestrus for blood prolactin determination.

The reason for using the 0.5% fat diet in this study instead of a 5% fat diet (which approximates the fat content in laboratory chow) is that tumor incidence produced by the former dietary regimen is very low. In our experience, the 5% corn oil diet resulted in a tumor incidence of about 40% in rats given 5 mg of DMBA. Thus, the difference in tumor incidence is magnified in 20% fat versus 0.5% fat, as opposed to 20% fat versus 5% fat. This manipulation would allow us to delineate more clearly factors that are responsible for further enhancement of tumorigenesis induced by dietary fat.

We were able to confirm earlier reports by Chan et al. (9, 10) that circulating prolactin at proestrus was elevated in rats fed a high fat diet versus those on a low-fat diet. Preliminary findings from our laboratory showed that the fluctuations in serum prolactin throughout the estrus cycle were similar in rats fed either diet, with the peak level attained in the afternoon of proestrus (results not shown). No significant difference in circulating prolactin was found between these 2 groups during metestrus and diestrus. Thus, only the proestrus values were reported in this study. Determination of pituitary prolactin, however, indicated that the concentration was comparable in rats fed either the high-fat or the low-fat diet, suggesting that the synthesis as well as the secretion processes were probably enhanced as a result of a high-fat intake. This is in agreement with a recent publication by Cave et al. (7) which maintains that feeding of a high-fat diet resulted in a slight increase in pituitary prolactin synthesis in vitro.

Perhaps the most important finding in this study is in elucidating the relevance of the elevated blood prolactin level in the enhancement of mammary carcinogenesis in rats fed a high-fat diet. The importance of prolactin in the development of DMBA-induced mammary tumors is well documented (19, 20, 24). We took advantage of the fact that lesion of the median eminence is qualitatively similar to that seen after stalk section or transplantation of the pituitary, resulting in increased secretion of prolactin (21). In rats fed the low-fat diet, we found that this endocrine manipulation led to an increased tumor incidence, presumably due to the stimulatory effect of the higher circulating prolactin. Although the serum prolactin level in the low-fat-lesioned rats was comparable to that in the high-fat-lesioned rats (Table 4), the tumor incidence in the former group still lagged behind that in the latter. In other words, elevation of prolactin in the periphery only narrows the gap but fails to obliterate the difference in tumor incidence in rats fed a low-fat diet versus those fed a high-fat diet. It is important to point out that placement of median-eminence lesions before DMBA administration has been found previously to inhibit mammary tumorigenesis in the rat (11). This is probably due to increased prolactin secretion and stimulation of mammary growth, thereby rendering the mammary glands refractory to the action of the carcinogen. In contrast, placement of the lesions after administration of DMBA and up to the time at which tumors first become palpable will enhance the development of the tumors (11, 22).

* Unpublished data, Clement Ip.
At the present time, very little is known about the mechanism by which dietary fat promotes tumorigenesis. Hopkins and West (17) have briefly reviewed changes induced by dietary fats in several biological systems that may have an influence in carcinogenesis. These include the effects of dietary lipid on the metabolism of chemical carcinogen, the structure and function of membranes, immunocompetence, DNA repair potential, and endocrine physiology. Our observations suggest that although increased prolactin output may be partly responsible for the promotion of DMBA-induced mammary tumorigenesis in rats fed the high-fat diet, it is by no means the only factor involved. This conclusion is in agreement with an earlier report by Tannenbaum (23) which maintains that high-fat diet stimulates the growth of spontaneous mammary adenocarcinomas that are hormone-independent.

REFERENCES

Role of Prolactin in the Promotion of Dimethylbenz[a]anthracene-induced Mammary Tumors by Dietary Fat

Clement Ip, Philip Yip and Lee L. Bernardis


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/40/2/374

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.