Ability of Dietary Fat to Overcome the Resistance of Mature Female Rats to 7,12-Dimethylbenz(a)anthracene-induced Mammary Tumorigenesis

Clement Ip

Department of Breast Surgery and Breast Cancer Research Unit, Roswell Park Memorial Institute, Buffalo, New York 14263

ABSTRACT

The present study is designed to delineate the action of dietary fat on the development of 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary tumors in female Sprague-Dawley rats and to investigate the interdependence of fat intake and age as modifiers of mammary oncogenesis. Compared to rats given DMBA at 50 days of age (immature rats), those that were exposed to DMBA at 150 days of age (mature rats) were more resistant to the carcinogen irrespective of fat intake, although the incidence of tumors in rats fed a high-fat diet (20% corn oil) still remained substantially higher than in those rats on a low-fat diet (0.5% corn oil) at different doses of DMBA tested. In this experiment, both diets were fed from weaning until termination of the experiment. When adult rats (150 days old) were given 5 mg of DMBA each week for 4 consecutive weeks, tumors appeared earlier, and the proportion of adenocarcinomas to fibroadenomas was higher in rats fed the 20% fat diet. Using the adult rats as the model for tumor induction, the contribution of a high dietary fat intake before and after DMBA administration in the promotion of mammary tumorigenesis was assessed. The types of diet fed before and after DMBA were designated LF-LF, LF-HF, HF-LF, and HF-HF, in which LF and HF represent 0.5% and 20% fat diets, respectively. Results of this experiment established the following order of tumor incidence: HF-HF (84.0%) > LF-HF (60.9%) > HF-LF (36.0%) > LF-LF (20.8%). It appears from this study that the level of fat intake after DMBA treatment is more important in determining the subsequent development of mammary cancer than is fat intake prior to DMBA administration, thus confirming earlier observations reported by Carroll and Khor and by Hopkins, Hard, and West. In addition, it was also found that in rats that were fed a 0.5% fat diet from weaning and given a single dose of 5 mg of DMBA at 50 days of age, a transfer to a 20% fat diet as late as 20 weeks after DMBA was able to stimulate a significant increase in the number of rats bearing tumors. It can be concluded from these findings that dietary fat acts preferentially, although by no means exclusively, on the promotion phase of carcinogenesis and that the differential effect of high-fat and low-fat diets on tumorigenesis can be demonstrated regardless of the age at which the carcinogen is given.

INTRODUCTION

Age is an important factor in the induction of mammary cancer by polycyclic hydrocarbons in female rats. Huggins et al. (13) treated Sprague-Dawley rats of various ages with a single dose of 3-methylcholanthrene and reported that rats between 50 and 65 days of age were most susceptible to tumorigenesis. Similar results were obtained by Dao (7) with DMBA2 in Wistar-Furth rats. The tumor incidence varied from 80% in rats given DMBA at 50 days of age to less than 5% in rats given DMBA at 150 days of age. There are other reports that indicate that age at carcinogen administration also affects the proportion of carcinomas versus fibroadenomas (18) as well as the frequency of spontaneous tumor regression (8).

An enhancement of mammary tumorigenesis by dietary fat in rats treated with DMBA has been confirmed by several investigators (3, 5, 12, 15) as well as by our laboratory (14). In view of the observation that adult rats are more resistant to the development of DMBA-induced mammary tumors, it would be of great interest to determine if the promoting effect of a high-fat diet is manifest in rats that are fed the diet since weaning and given DMBA at 150 days of age. The temporal relationship between the introduction of a high-fat diet and DMBA administration should also be explored so as to better delineate the action of dietary fat on the carcinogenic process. Experiments described in the present study are thus designed to provide some insight on the interdependence of dietary fat and age as modifiers of mammary oncogenesis.

MATERIALS AND METHODS

Female Sprague-Dawley rats (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were fed from weaning (21 days old) either a synthetic high-fat diet or a low-fat diet. In certain experiments, rats on a high-fat diet were switched to a low-fat diet or vice versa during the course of the study, as indicated in the text. The high-fat and low-fat diets contained 20% and 0.5% respectively, of Mazola corn oil (donated generously by Best Foods, Englewood Cliffs, N. J.) by weight. The composition of the other ingredients was described in a previous publication (14). Since Mazola corn oil contains approximately 0.02% of α-tocopherol (analysis provided by the supplier), the low-fat diet was also supplemented with 40 mg of α-tocopherol (ICN Pharmaceuticals, Cleveland, Ohio) per kg of diet so that the total α-tocopherol content would be similar in both the high-fat and the low-fat diets. Mammary tumors were induced by i.g. administration of DMBA (Sigma Chemical Co., St. Louis, Mo.) that was dissolved in corn oil at a concentration of 5 mg/ml. A 5-ml syringe and a 5-French stomach tube were used for this purpose, and 5 mg of DMBA were given to each rat. When multiple doses were used, rats were fed 5 mg/week for no more than 4 weeks; the

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2 The abbreviations used are: DMBA, 7,12-dimethylbenz(a)anthracene; i.g., intragastric.

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first dose was given when the rats were either 50 or 150 days old. The total dosage is denoted in the text as \( n \times 5 \) mg, in which \( n \) represents the number of weekly administrations. In order to minimize any possible effect of dietary fat on absorption of the carcinogen from the intestine, rats were fasted 12 hr before and after the p.o. feeding of DMBA. Tumor palpation was carried out as described previously (14).

RESULTS

Since a number of factors such as rat strain, age, and carcinogen dosage, the schedule of administration, and fat intake are known to affect mammary tumorigenesis, our first experiment was designed to study the effect of multiple doses of DMBA, given initially at 50 or 150 days of age, on mammary tumor development in female Sprague-Dawley rats that were fed either a high-fat diet (20% corn oil) or a low-fat diet (0.5% corn oil) starting from weaning. The experiment was terminated 24 weeks after the first intubation of DMBA. Table 1 shows the final tumor incidence as a function of the DMBA dosage and the time of treatment in each dietary group. Tumor incidence is expressed as the percentage of rats with tumors.

When DMBA was given initially at 50 days of age to rats fed the low-fat diet, the resulting tumor incidence increased in proportion to the dose of DMBA. The incidence was 12.5% with a single dose of 5 mg of DMBA and rose to 92.0% with a total dose of 4 \( \times \) 5 mg. An enhancement of mammary tumorigenesis was observed in rats that were fed the high-fat diet compared to those on the low-fat diet and given the same amount of DMBA. Thus ingestion of a high-fat regimen produced an incidence of 66.7% (a 5-fold increase over the low-fat group) with a dose of 5 mg of DMBA. All of the animals (100%) in the high-fat group developed tumors after being treated with 10 mg of DMBA, in contrast to an incidence of 40% in the low-fat group.

Table 1 also shows that if rats were exposed to DMBA at 150 days of age, they became more resistant to the carcinogen irrespective of fat intake, although the incidence of tumors in rats fed the high-fat diet still remained higher than in those rats on the low-fat diet at all levels of DMBA tested. Thus, when these adult rats were given 5, 10, 15, or 20 mg of DMBA, the corresponding incidences were 0, 4.2, 8.7, and 16.7% in the low-fat group, as compared to incidences of 8.3, 25.0, 52.0, and 78.3% in the high-fat group.

<table>
<thead>
<tr>
<th>Dietary group (%) fat</th>
<th>1 ( \times ) 5 mg</th>
<th>2 ( \times ) 5 mg</th>
<th>3 ( \times ) 5 mg</th>
<th>4 ( \times ) 5 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial dose of DMBA at 50 days of age</td>
<td>0.5</td>
<td>12.5</td>
<td>40.0</td>
<td>78.3</td>
</tr>
<tr>
<td>Initial dose of DMBA at 150 days of age</td>
<td>0.5</td>
<td>0</td>
<td>4.2</td>
<td>8.7</td>
</tr>
</tbody>
</table>

* Mammary tumor incidence is expressed as the percentage of rats with tumors.

Compared to rats that were fed the 0.5% fat throughout the experiment, a change of the diet from 0.5 to 20% fat after DMBA treatment was able to increase the incidence of tumor-bearing rats from 20.8 to 60.9% (Group 1 versus Group 2). As a matter of fact, feeding of a high-fat diet after DMBA was only slightly less effective as starting the diet from weaning (Group 2 versus Group 4). On the other hand, a regimen of a high-fat diet before the final DMBA treatment followed by a low-fat diet (HF-LF) resulted in a smaller increase in tumorigenesis when...
Effect of Dietary Fat and Age on Mammary Tumorigenesis

Table 2
Mammary tumor incidence following DMBA administration at 150 days of age to rats fed either a 0.5% or a 20% fat diet

<table>
<thead>
<tr>
<th>Dietary group (% fat)</th>
<th>No. of rats</th>
<th>Rats with tumors</th>
<th>Palpable</th>
<th>Non-palpable</th>
<th>Adeno-carcinomas</th>
<th>Fibroadenomas</th>
<th>Total tumor load (g)</th>
<th>Tumors/tumor-bearing rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>24</td>
<td>4 (16.7)</td>
<td>10</td>
<td>1</td>
<td>6</td>
<td>5</td>
<td>5.6</td>
<td>2.8 ± 0.42</td>
</tr>
<tr>
<td>20</td>
<td>23</td>
<td>18 (78.3)</td>
<td>50</td>
<td>3</td>
<td>35</td>
<td>18</td>
<td>59.9</td>
<td>2.9 ± 0.33</td>
</tr>
</tbody>
</table>

a Numbers in parentheses, percentage of rats with tumors.
b Mean ± S.E.

Table 3
Relation of dietary fat feeding and DMBA administration on mammary tumorigenesis in adult female Sprague-Dawley rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Diet treatment</th>
<th>No. of rats</th>
<th>Body wt at 150 days of age (g)</th>
<th>Final body wt (g)</th>
<th>Rats with tumors</th>
<th>Total no. of rats</th>
<th>Tumors/tumor-bearing rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LF-LF</td>
<td>24</td>
<td>304 ± 6.1</td>
<td>328 ± 7.1</td>
<td>5 (20.8)</td>
<td>9</td>
<td>1.8 ± 0.34</td>
</tr>
<tr>
<td>2</td>
<td>LF-HF</td>
<td>23</td>
<td>296 ± 7.5</td>
<td>321 ± 7.3</td>
<td>14 (60.9)</td>
<td>37</td>
<td>2.6 ± 0.32</td>
</tr>
<tr>
<td>3</td>
<td>HF-HF</td>
<td>25</td>
<td>308 ± 8.3</td>
<td>341 ± 6.6</td>
<td>9 (36.0)</td>
<td>20</td>
<td>2.2 ± 0.33</td>
</tr>
<tr>
<td>4</td>
<td>HF-LF</td>
<td>25</td>
<td>308 ± 7.2</td>
<td>336 ± 7.9</td>
<td>21 (84.0)</td>
<td>55</td>
<td>2.6 ± 0.30</td>
</tr>
</tbody>
</table>

a LF, 0.5% fat diet; HF, 20% fat diet. Groups LF-LF and HF-HF were fed 0.5 and 20% fat diets, respectively, from weaning to termination of the experiment. Groups LF-HF and HF-LF were switched from 0.5 to 20% fat and vice versa at the time the last dose of DMBA was given.
b Mean ± S.E.

c Numbers in parentheses, the percentage of rats with tumors.

d DISCUSSION

There are 3 notable findings in this study concerning the relationship between dietary fat and age at carcinogen administration on mammary carcinogenesis. First, a high-fat intake is able to produce a substantial increase in tumorigenesis in rats that are given DMBA at 150 days of age (adult rats), even compared to those rats that were maintained on the LF-HF schedule (Group 3 versus Group 2). Thus, it can be seen that the time of introduction of a high-fat diet in relation to DMBA administration is important in determining the subsequent tumor incidence. Based on the present experiment, the following order of efficacy was established: HF-HF > LF-HF > HF-LF > LF-LF.

As pointed out in Table 1, when 50-day-old rats fed a 0.5% fat diet were given a single 5-mg dose of DMBA, only a small percentage (12.5%) of the rats developed tumors. In our experience with this particular regimen and this dose of DMBA, tumors started to appear 10 to 12 weeks after DMBA administration and continued to do so for about 4 to 6 weeks (results not shown). We decided to investigate whether a change in the diet from 0.5% to 20% fat 20 weeks after DMBA would further stimulate tumor development in these animals. A third experiment was then initiated involving 60 rats that were fed 0.5% fat from weaning and given a single dose of 5 mg of DMBA at 50 days of age. During the next 20 weeks after DMBA, 6 rats developed tumors and 2 died (with no tumor). At this point, the remaining 52 rats were divided into 2 groups; the first group continued to receive the 0.5% fat diet, and the second group was fed the 20% fat diet. The experiment was terminated 20 weeks later. Results of this study are shown in Table 4.

In rats that were switched from the 0.5% to the 20% fat diet, tumors started to appear 7 weeks after the transfer, with more developing in the next 7 weeks. When the experiment was terminated 20 weeks after the change of diet, 6 of 26 rats developed a total of 9 tumors. During the same period, one rat developed a tumor in the group that received the 0.5% fat diet.

There are 3 notable findings in this study concerning the relationship between dietary fat and age at carcinogen administration on mammary carcinogenesis. First, a high-fat intake is able to produce a substantial increase in tumorigenesis in rats that are given DMBA at 150 days of age (adult rats), even
though these animals are more resistant to the carcinogen than
are those that are treated at 50 days of age (immature rats). In
other words, the differential effect of high-fat and low-fat diets
in tumorigenesis can be demonstrated regardless of age at
which the carcinogen is given. Second, with the adult rats as
the model for tumor induction, our experiment shows that the
level of fat intake after DMBA treatment is more important in
determining the subsequent development of mammary cancer.
Third, a change of the diet from 0.5 to 20% fat 20 weeks after
DMBA administration (at 50 days of age) results in a further
stimulation of tumor appearance compared to those rats that
are fed the 0.5% fat diet throughout the experiment. Thus, it
may be concluded from these observations that dietary fat acts
preferentially, although by no means exclusively, on the pro-
pharmacokinetics and metabolism of the carcinogen may also
be mediated
The prevailing environment that would enhance DMBA-in-
duced tumor development as a result of a high-fat intake may
include elevated circulating prolactin (6, 14) and estrogens.\(^3\)
since the growth of these tumors are dependent on hormones.
The effect of dietary fat may also conceivably be mediated
through the maintenance and induction of prolactin receptors
(16) or the cellular immune system with the possible involve-
ment of prostaglandins (9) or lymphocyte dysfunction (17).

\(^3\) C. Ip and M. Ip, unpublished data.

There is one other interesting point in Table 2 that deserves
some attention, specifically, the distribution of adenocarcino-
mas and fibroadenomas. In rats that are fed either a high-fat or
a low-fat diet and are given DMBA when they are 50 days old,
over 90% of the tumors obtained are adenocarcinomas. This
observation has been confirmed by several investigators (2, 5,
15), including this author (14). However, when rats were
treated at 150 days of age, we found that the proportion of
adenocarcinomas to fibroadenomas in the high-fat group is a
coincidence or due to the increased stimulation by estrogens
and prolactin as discussed above.

The 0.5% corn oil used in the low-fat diet, although only
marginal in the adequacy of essential fatty acids, did not seem
to inhibit the growth of the animals (refer to body weights of
Groups 1 and 4 in Table 3). They did not manifest any signs of
fatty acid deficiency such as rough hair coat, scaly feet, necro-
sis of tail, or hyperemia of the renal tissues even after 50 weeks
on this diet. 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 because of the very low tumor incidence
produced by the former dietary regimen, thus enabling us to
magnify the difference in tumor incidence between the 20% fat
group and the 0.5% fat group.

As pointed out in a recent report by Newberne et al. (19),
the formulations of high-fat and low-fat diets used in this study
may lead to small changes in the consumption of protein,
vitamins, and minerals in addition to changes in fat and car-
bohydrate intake, since the diets are not isocaloric in nature.
In their opinion, this actually results in the comparison of the
effects of 2 different diets rather than of the effects of dietary
fat. We have measured the food intake of rats fed the high-fat
and low-fat rations in our experiment. The average amounts of
food consumption daily per rat in the high-fat and low-fat
groups were about 13 and 16 g, respectively. This represented
a very similar caloric intake between these 2 groups. Although
there was a 20% difference in the ingestion of the other
nutrients besides fat and carbohydrate, the total lipid con-
sumption in the high-fat (20%) group was approximately 30 to
35 times that of the low-fat (0.5%) group. We therefore justifi-
ably believe that our diets were adequate to reflect a prepon-
derant change in fat intake.

In conclusion, it should be put in perspective that the impli-
cation of this study is not without its parallel in human mammary
cancer. Descendants of Japanese immigrants to the United
States show an increase in breast cancer rate, but this increase
is not seen among the immigrants themselves (1). Adaptation
to a western diet (presumably a higher fat intake) may be
partially responsible for this disease. Thus, the influence of a
dietary factor operating either early in life or continuously over
a longer life span may be critical in determining the risk of a
particular generation to breast cancer.

REFERENCES

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C. Ip

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