Evidence That the Effectiveness of Antioxidants as Inhibitors of 7,12-dimethylbenz(a)anthracene-induced Mammary Tumors Is a Function of Dietary Fat Composition

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Abstract

A study of tumor incidence and tumor growth rates in 7,12-dimethylbenz(a)anthracene-treated female Sprague-Dawley rats fed different types and amounts of dietary fat indicates that the difference in tumor incidence may be a reflection of marked differences in the growth of neoplastic clones to a palpable size within the time frame of the study. In addition, the observation is made that some antioxidants which inhibit tumor development in animals fed commercial rations are not effective when given in purified diets.

Introduction

Enhancement of tumor incidence in laboratory rats and mice by dietary fat is now well established (1–8, 10, 12), but the mechanism of this effect is unknown. Unsaturated fat results in a higher tumor incidence from a given dose of carcinogen as compared to the same dietary level of saturated fat (6). The influence of dietary fat on tumor incidence appears to be most notable when fed from the time of carcinogen exposure (3).

The relationship between the incidence of breast and colon carcinomas in humans shows a high degree of positive correlation with the per capita consumption of fat (14). The importance of caloric density in the effect of dietary fat on carcinogenesis is not clear. In comparing 2 diets which have the same composition except that one contains unsaturated fat while the other contains saturated fat, significantly different tumor incidence rates are observed when the 2 groups are given the same dose of carcinogen (10). This would suggest that unsaturation per se is involved since the caloric densities are identical. Our studies indicate that dietary fat, particularly unsaturated fat, increased the growth rate of mammary carcinomas initiated by DMBA. This suggests that the increased incidence of tumors observed in animals fed diets containing significant amounts of fat may be the result of some stimulatory effect on initiated cells. All available information indicates that the frequency of breast cancer in the United States might be lower if our diet were modified to diminish fat intake. Dietary habits, however, tend to be very ingrained, and such an approach is probably not feasible. Another approach with the potential for reducing the enhanced breast cancer because of high fat consumption may be the use of tumor inhibitors. A number of antioxidant compounds have been shown to inhibit carcinogenesis in various tissues that is produced by a variety of carcinogens (13). We have recently completed a series of studies which demonstrates that certain antioxidant compounds are good inhibitors of breast tumor incidence in female Sprague-Dawley rats (10) and the incidence of hepatoma in male rats fed 2-acetylaminofluorene (11). However, the most important result of these studies, in our view, is that the composition of the diet which is being fed appears to be a major factor in determining if a particular antioxidant will inhibit tumorigenesis. The studies described in this report demonstrate that while some antioxidants are effective inhibitors of carcinogenesis in rats and mice fed commercial rations, they do not influence the incidence of DMBA-induced mammary tumors in female Sprague-Dawley rats fed purified diets.

Materials and Methods

Animals. Weanling female Sprague-Dawley rats (30 per experimental group except where indicated) were housed in stainless steel cages under controlled environmental conditions in the Laboratory Animal Resources Center of this institution. Diets were fed ad libitum.

Diet Composition. The components, which were varied, were the content of fat, carbohydrate, and antioxidant, as shown in Table 1. Antioxidants, when added, were supplemented as follows: BHT, 0.3%; BHA, 0.3%; propyl gallate, 0.3%; and a-tocopherol, 0.2%.

Carcinogen Administration. Female rats (fasted 24 hr) were given a single dose of 10 mg DMBA in 1 ml of corn oil by stomach tube.

Tumor Incidence. The rats were palpated weekly, between 77 and 230 days of age, and the size and location of each tumor was recorded. Only tumors histologically identified as mammary parenchymal cell carcinomas were included in the incidence values.

Results

Chart 1 shows that DMBA induced mammary tumor incidence in female rats when fed one of the 3 diets. Because of the large number of animals in each dietary group in this particular study (180 rats each), the difference in tumor incidence observed as a function of diet is highly significant. Only the 20% polyunsaturated fat-containing dietary group reached 100% incidence within the period of observation (32 weeks). The 20% saturated fat dietary group had a significantly lower incidence (70%) while the low-fat dietary group had the...
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lowest incidence (=30%). Measurement of tumor growth indicated that the tumors in the low-fat dietary group developed very slowly and occasionally regressed. Tumors in the 20% saturated fat dietary group grew rapidly but not as much as did those in the 20% polyunsaturated fat dietary group. The growth rate ratio in the 3 dietary groups was 1:20:36. Results of studies performed in this laboratory on the effect of BHT on mammary tumor incidence caused by DMBA in female rats fed the various diets have been published (10). Incidence data have been reproduced in this report so that comparisons with the other antioxidants tested can be made. Chart 2 shows that BHT supplementation at a level of 0.3% is an effective tumor inhibitor in all 3 dietary groups but was more effective in the animals fed the 20% saturated fat diet than in the group fed the polyunsaturated fat diet. An identical experiment was performed in which 0.3% BHA was supplemented in each of the 3 diets. Chart 3 shows that BHA had no significant effect on tumor incidence in any of the dietary groups. On the other

Table 1

<table>
<thead>
<tr>
<th></th>
<th>High-polyunsaturated-fat diet (%)</th>
<th>High-saturated-fat diet (%)</th>
<th>Low-fat diet (%)</th>
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<tr>
<td>Casein</td>
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<td>46</td>
<td>64</td>
</tr>
<tr>
<td>Alphacel (nonnutrient bulk)</td>
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<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Vitamin mixture</td>
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<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

* Stripped corn oil.

* Stripped, hydrogenated coconut oil, 16%, and linoleic acid, 2%.

C Linoleic acid.

d For composition of salt mixture, modified to contain 0.03% zinc chloride, see the report by Hubbell et al. (9).

a Vitamin mixture (Vitamin Fortification Mixture of ICN Life Sciences Co., Cleveland, Ohio 44128).

Chart 1. Percentage of rats with mammary tumors versus age.

Chart 2. Comparisons of the relative rates of mammary tumor development in the 3 dietary groups with and without BHT supplementation. Animals received a single 10-mg dose of DMBA in 1 ml stripped corn oil at 50 days of age. All groups had a total of 30 animals. Abscissa, age of rats in days.

Chart 3. BHA study. A, polyunsaturated fat diet; Δ, polyunsaturated fat diet plus BHA; O, high-saturated-fat diet; C, high-saturated-fat diet plus BHA; □, low-fat diet; ▪, low-fat diet plus BHA.

Chart 4. Propyl gallate (PG) study.

Chart 5. Tocopherol study. A, unsaturated (UNSAT.) fat diet; Δ, unsaturated fat diet plus α-tocopheryl acetate (0.2%); O, saturated (SAT.) fat diet; C, saturated fat diet plus α-tocopheryl acetate (0.2%); □, low-fat (LF) diet; ▪, low fat diet plus α-tocopheryl acetate (0.2%).
hand, supplementation of the diets with 0.3% propyl gallate resulted in inhibition of tumorigenesis in all 3 dietary groups but was least effective in animals fed the polyunsaturated fat diet (Chart 4). Inclusion of 0.2% α-tocopheryl acetate in the various diets had no influence on the rate of tumor development in the different dietary groups (Chart 5).

Discussion

The effect of the different types and amounts of dietary fat was consistent in that the animals fed the 20% polyunsaturated fat diet always developed 100% tumor incidence within 30 to 32 weeks of age after having been given DMBA when 7 weeks old. Neither the 20% saturated fat nor, especially, the 2% linoleic acid dietary groups reached that level. Our results agree with those of Carroll et al. (4, 6, 12), in that diets containing significant levels of unsaturated dietary fat facilitated tumor formation. A diet containing an equal amount of mostly saturated fat resulted in a considerably lower rate of tumor incidence. Animals fed a diet containing only 2% linoleic acid resulted in the lowest tumor yield and the longest latent period. The differences in rate of tumor development may be a reflection of tumor growth rate (the 1:20:36 ratio described under “Results”). The difference in tumor incidence and latent periods may be the result of differential growth rates which enable palpation of more tumor cell masses as a function of time in animals in which clones of initiated cells grow most rapidly. The mechanism through which dietary fat enhances tumor growth is unknown. We are currently determining whether or not rapidly growing mammary tumors in animals on high-fat diets slow their growth or regress when the animals are placed on a low-fat diet. If this occurs, the possibility that low-fat diets may be a useful adjunct in the therapy of human mammary carcinoma might be considered.

The most unusual aspect of these studies was the nature of the results on the influence of different antioxidants on tumor incidence. All of those tested in this investigation have been reported to be tumor inhibitors. BHA, as mentioned above, has been demonstrated to be particularly effective in reducing tumor incidence by a number of carcinogens, including DMBA-induced mammary carcinomas (13). However, this antioxidant had no effect on mammary tumor incidence when added to any of the 3 diets. The dietary level of BHA was used the same as the level of BHT (0.3%), which was very effective. The primary difference between our studies and those of others is the use of purified diets in the investigations described in this report, whereas commercial rat rations have been used in the studies by other investigators. The lack of effectiveness of BHA and α-tocopherol in our studies may be the result of some factor or factors in the purified diets which render them ineffective. The purified diets are composed primarily of products derived from natural sources, and factors that can decrease or abolish the effectiveness of some tumor inhibitors may be present in these diets. The possibility also exists that commercial rat rations, also compounded from plant and other natural products, contain factors which are essential for the protective action of BHA and α-tocopherol to be exerted. Identification of factors in natural food products that modify the effectiveness of a tumor inhibitor would be of importance because of the potential such modifiers might have in cancer prevention.

References

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