Influence of Caffeine on Development of Benign and Carcinomatous Mammary Gland Tumors in Female Rats Treated with the Carcinogens 7,12-Dimethylbenz(a)anthracene and N-Methyl-N-nitrosourea

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ABSTRACT

The effect of chronic caffeine consumption (500 mg/liter of drinking water) on the initiation and promotion stages of 7,12-dimethylbenz(a)anthracene (DMBA) (a low dose, 0.5 mg/100 g body weight, i.v.) and N-methyl-N-nitrosourea (MNU) (a standard dose, 2.5 mg/100 g body weight, i.v.) induced mammary gland tumorigenesis in female Sprague-Dawley rats was determined. In the initiation studies, caffeine was administered for 30 days prior to and for 3-4 days after carcinogen treatment (carcinogens administered at 55-57 days of age); in the promotion studies, caffeine was administered beginning 3-4 days after carcinogen treatment and until experiment termination (DMBA study and MNU study, 48 and 26 weeks after carcinogen treatment, respectively). In the DMBA study, there were 62-73 rats/group, in the MNU study, 40 rats/group. Eighty-nine percent of the mammary tumors induced by DMBA were benign (adenomas, fibroadenomas, often with cystic secretory activity), 11% were carcinomas (intraductal and invasive); virtually all of the MNU-induced mammary tumors were carcinomas (≥99%). Caffeine consumption during the initiation stage in the DMBA-treated rats resulted in a significant decrease in the mean number of mammary carcinomas per rat (50% reduction, P < 0.01) and mean number of benign mammary tumors per rat (28% reduction, P < 0.05); caffeine consumption during the promotion stage significantly decreased the mean number of benign mammary tumors per rat (57% reduction, P < 0.001) while not significantly influencing mammary carcinoma number. In contrast, caffeine consumption during either the initiation or promotion stages of MNU-treated rats did not significantly influence this tumorigenic process. The influence of caffeine on urinary and fecal excretion of tritiated DMBA on rat mammary gland development at the time of carcinogen treatment also was determined. Slightly reduced levels of tritium in 24-h urinary samples were observed in caffeine-treated animals (P = 0.06). No significant effect of caffeine on 24- to 96-h fecal or 48- to 96-h urinary excretion of the isotope was observed. No apparent effect of caffeine on rat mammary gland development (number of ducts, degree of lobuloalveolar development) was observed. That caffeine significantly suppresses the initiation stage of DMBA-induced rat mammary gland tumorigenesis, while not influencing this stage when MNU is used as a carcinogen, suggests that caffeine acts via an alteration in carcinogen (DMBA) activation. The lack of a pronounced effect of caffeine on tritiated DMBA excretion, however, does cast some doubt on this mechanism. How caffeine inhibits the development of benign mammary tumors, when administered during the promotion stage, is not known and is currently under investigation.

INTRODUCTION

In previous reports we have shown evidence of a significant relationship between chronic caffeine consumption and the development of mammary carcinomas in female rats treated with a chemical carcinogen (1, 2). When caffeine was administered via the drinking water prior to and during treatment with the carcinogen DMBA (initiation stage), mammary tumorigenesis was consistently suppressed. However, when caffeine was administered commencing 3 days after carcinogen treatment and until experiment termination (promotion stage), there was a temperate and transitory early increase in the incidence of mammary carcinomas, but the final number of mammary carcinomas was not significantly different from that of the control animals.

In the experiments reported in this communication, we further examined the role of caffeine in DMBA-induced rat mammary gland tumorigenesis by using a low dose of the carcinogen instead of the standard dose levels used in previous studies (1, 2). This low dose level of the carcinogen is known to produce a low incidence of mammary carcinomas and a high incidence of benign mammary tumors in female Sprague-Dawley rats. This provides an opportunity to examine the influence of caffeine on the development of a spectrum of mammary tumors which are more akin, with respect to incidence and histopathological features, to that observed in human populations.

The mechanisms involved in the previously observed inhibitory activities of caffeine on the initiation stage of DMBA-induced mammary gland tumorigenesis were also investigated. Three questions were addressed. (a) Can caffeine inhibit the initiation stage of rat mammary gland tumorigenesis when a carcinogen is used, such as MNU, that (unlike DMBA) does not require metabolic activation? (b) Can caffeine influence the urinary and fecal excretion pattern of administered tritiated DMBA? (c) Can caffeine influence the developmental growth of the rat mammary gland, thus altering the sensitivity of the gland to a carcinogenic stimulus?

MATERIALS AND METHODS

All of the animals used in these studies were female Sprague-Dawley rats obtained at 21 days of age from Harlan Sprague-Dawley, Indianapolis, IN. The animals were housed in a temperature (24°C) and light controlled (14 h/day) room. The animals were fed ad libitum a standard commercial rat chow (Wayne Lab Blox, Allied Mills, Inc., Chicago, IL). Caffeine (ICN Pharmaceuticals, Inc., Cleveland, OH) was prepared fresh 3 times a week and added to the drinking water (distilled water) at a concentration of 500 mg/liter.

Caffeine Consumption and Mammary Tumorigenesis Using a Low-Dose Level of Chemical Carcinogen DMBA. Twenty-seven-day-old female rats were divided into 4 groups, a control and a caffeine-treated group for both the initiation and promotion studies. DMBA (0.5 mg/100 g body weight), in the form of a fat emulsion (Upjohn Corp., Kalamazoo, MI), was administered to all rats once at 55 days of age by tail vein injection.

Initiation Study. Seventy rats were given caffeine in their drinking water commencing at 27 days of age and continuing until 59 days of age. At 59 days of age, their drinking water was changed to distilled water and continued as such until termination of the experiment. The control group of 73 animals was given distilled drinking water throughout the experiment.

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2 Research supported by NIH Research Grant CA-37613.

3 To whom requests for reprints should be addressed, at Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824.

The abbreviations used are: DMBA, 7,12-dimethylbenz(a)anthracene; MNU, N-methyl-N-nitrosourea.
Promotion Study. Sixty-two rats were given distilled drinking water from 27 to 59 days of age. At 59 days of age caffeine was added to their drinking water and caffeine treatment was continued until termination of the experiment. The control group of 72 animals was given distilled drinking water throughout the experiment.

Beginning 8 weeks after injection of the carcinogen and until the termination of the experiment, all rats were palpated biweekly for the presence of mammary tumors. All tumors were recorded as to location and time of first appearance. Tumors were excised when they were observed to be larger than 1 cm in diameter.

All rats were sacrificed 48 weeks after DMBA injection. The internal organs were examined for any evidence of metastasis or other abnormalities, and the pituitary gland, uterus, ovaries, spleen, and liver were removed and weighed (promotion study only). All tumors throughout the study and at experiment termination (mammary and nonmammary, palpable and nonpalpable) were excised, fixed in Bouin’s solution, stained with hematoxylin and eosin, and examined histologically.

Caffeine Consumption and Mammary Tumorigenesis Using the Chemical Carcinogen MNU. One hundred and twenty female rats at 29 days of age were divided into three groups. The chemical carcinogen MNU (Ash Stevens, Detroit, MI) was administered to all rats once by tail vein injection at 57 days of age (2.5 mg/100 g body weight). A single control group was comprised of 40 animals that were given distilled drinking water for the duration of the experiment.

Initiation Study. Forty rats were given caffeine in their drinking water from 29 until 60 days of age. At 60 days of age, they were given distilled drinking water until the termination of the experiment.

Promotion Study. Forty rats were given distilled drinking water from 29 until 60 days of age. At 60 days of age they were given caffeine in their drinking water and caffeine treatment was continued until termination of the experiment.

Beginning 8 weeks after carcinogen injection, all rats were palpated on a biweekly basis for the presence of mammary tumors. Mammary tumor location, number, and histological characteristics were assessed as described in the DMBA study. The study was terminated 6 months after carcinogen treatment.

Caffeine Consumption and Excretion of the Chemical Carcinogen DMBA. Forty-two female rats at 27 days of age were divided into 2 groups of 21 animals each. The experimental group was given caffeine in their distilled drinking water until termination of the experiment at 59 days. The rats in the control group were given distilled drinking water. Two weeks before the injection of tritiated DMBA (Amersham Corp., Arlington Heights, IL), the animals were housed singly in wire mesh metabolic cages. At 55 days of age all of the animals were given injections via the tail vein of the carcinogen at a concentration of 0.5 mg nonlabeled DMBA/100 g body weight and 0.043 mCi tritiated DMBA/100 g body weight.

The urine and feces were collected at 24-h intervals for 96 h. The urine samples were adjusted to volume and a 1-ml aliquot was dissolved directly in an aqueous scintillation cocktail. Fecal samples were weighed and a 100-ml aliquot was solubilized with perchloric acid and hydrogen peroxide before adding a scintillation cocktail. Samples were prepared in duplicate and reported as total cpm for 24 h. At 96 h after carcinogen injection the animals were sacrificed by CO2 inhalation. The liver was excised and weighed.

Caffeine Consumption and Mammary Gland Developmental Growth. Mammary gland developmental growth was assessed on the animals in the above described study (caffeine/tritiated DMBA excretion). Ninety-six h after carcinogen injection (59 days of age), the No. 4 mammary glands (inguinal) were excised, spread flat between a glass slide and nylon fabric, fixed in glacial acetic acid/ethanol, and stained with alum carmine for whole-mount evaluation. Mammary gland developmental growth was assessed by a standard procedure (3) and according to the following rating system. 1, few ducts, few or no end duct buds; 2, moderate number of ducts, moderate number of end duct buds; 3, extensive number of ducts, extensive number of end duct buds; 4, extensive number of ducts, minimal lobuloalveolar development; 5, extensive number of ducts, moderate lobuloalveolar development; 6, extensive number of ducts, extensive lobuloalveolar development as in late pregnancy.

Statistics. The percentage of mammary tumor-bearing rats was evaluated by χ2 analysis. Mammary tumor multiplicity, urine and fecal tritium levels, and organ and body weights were evaluated by unpaired Student’s t test. Mammary gland development was evaluated by the Mann-Whitney rank test.

RESULTS

Effect of Caffeine on Initiation Stage of DMBA (Low Dose)-induced Mammary Tumorigenesis in Female Sprague-Dawley Rats. When DMBA was administered to 55-day-old rats that had been treated with caffeine from 27 until 59 days of age, a 50% decrease in percentage of animals bearing mammary carcinomas (P < 0.001) and mammary carcinoma multiplicity (P < 0.01) was observed (Table 1; Fig. 1). A 28% decrease in benign mammary tumor multiplicity also was observed in the caffeine-treated animals (P < 0.05) (Table 1; Fig. 1). A difference (97 versus 93%) in the percentage of animals bearing benign mammary tumors was observed between control and caffeine-treated animals but this difference was not significant (Table 1). There was no significant difference between body weight gains in control and caffeine-treated animals (Table 1).

Effect of Caffeine on Promotion Stage of DMBA (Low Dose)-induced Mammary Tumorigenesis in Female Sprague-Dawley Rats. When DMBA was administered to 55-day-old rats that were treated with caffeine commencing 4 days later and continuing until termination of the experiment (for 48 weeks), there was a decrease of only 10% in mammary carcinoma multiplicity and only a 4% decrease in percentage of animals bearing mammary carcinomas; such differences were not significant (Table 1; Fig. 1). There was a decrease of 57% in benign mammary tumor multiplicity (P < 0.001) and decrease of 8% in the percentage of animals bearing these tumors (P < 0.05) in the caffeine-treated animals (Table 1; Fig. 1). There was no signif-

Table 1 Effect of caffeine on incidence of palpable and nonpalpable carcinomatous and benign mammary tumors in female Sprague-Dawley rats treated with a low-dose level of DMBA

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rats</th>
<th>Mean body wt. onset of study (g ± SE)</th>
<th>Mean body wt. termination of study (g ± SE)</th>
<th>Total no. of mammary carcinomas</th>
<th>No. of rats with mammary carcinomas (%)</th>
<th>Mean no. of mammary carcinomas/rat (± SE)</th>
<th>Total no. of benign mammary tumors</th>
<th>No. of rats with benign mammary tumors (%)</th>
<th>Mean no. of benign mammary tumors/ rat (± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initiation stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>73</td>
<td>42 ± 0.6</td>
<td>300 ± 4</td>
<td>69 (A)</td>
<td>42 (58) (C)</td>
<td>1.0 ± 0.1 (A)</td>
<td>703 (E)</td>
<td>71 (97)</td>
<td>9.6 ± 0.8 (E)</td>
</tr>
<tr>
<td>Caffeine</td>
<td>70</td>
<td>42 ± 0.6</td>
<td>289 ± 4</td>
<td>32 (B)</td>
<td>20 (29) (D)</td>
<td>0.5 ± 0.1 (B)</td>
<td>484 (F)</td>
<td>65 (93)</td>
<td>6.9 ± 0.7 (F)</td>
</tr>
<tr>
<td>Promotion stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>72</td>
<td>43 ± 0.7</td>
<td>294 ± 4</td>
<td>75</td>
<td>39 (54) (E)</td>
<td>1.0 ± 0.1 (E)</td>
<td>668 (C)</td>
<td>70 (97)</td>
<td>9.3 ± 0.7 (C)</td>
</tr>
<tr>
<td>Caffeine</td>
<td>62</td>
<td>42 ± 0.6</td>
<td>295 ± 3</td>
<td>56</td>
<td>31 (50) (F)</td>
<td>0.9 ± 0.2 (F)</td>
<td>251 (D)</td>
<td>55 (89)</td>
<td>4.0 ± 0.4 (D)</td>
</tr>
</tbody>
</table>

* Caffeine was added to the drinking water (500 mg/liter) from 27 to 59 days of age. DMBA (0.5 mg/100 g body weight) was administered i.v. at 55 days of age. The study was terminated 48 weeks after carcinogen treatment.

A/B, P < 0.01; C/D, P < 0.001; E/F, P < 0.05.

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Fig. 1. Effect of caffeine on the incidence of palpable carcinomatosus and benign mammary tumors in female Sprague-Dawley rats treated with a low-dose level of DMBA. Initiation stage: caffeine was added to the drinking water (500 mg/liter) from 27 to 59 days of age. DMBA (0.5 mg/100 g body weight) was administered i.v. at 55 days of age. Promotion stage: caffeine was added to the drinking water (500 mg/liter) from 59 days of age until the study was terminated. DMBA (0.5 mg/100 g body weight) was administered i.v. at 55 days of age. Initiation stage: controls versus caffeine, carcinomas, P< 0.01. benign tumors, P< 0.05. Promotion stage: controls versus caffeine, carcinomas, no significant difference, benign tumors, P< 0.001.

### Table 2 Effect of caffeine on incidence of palpable and nonpalpable tumors in female Sprague-Dawley rats treated with MNU

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rats</th>
<th>Mean body wt on study (g ± SE)</th>
<th>Mean body wt termination of study (g ± SE)</th>
<th>Total no. of mammary carcinomas</th>
<th>No. of rats with mammary carcinomas (%)</th>
<th>Mean no. of mammary carcinomas/rat (± SE)</th>
<th>Total no. of benign mammary tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>40</td>
<td>66 ± 0.7</td>
<td>278 ± 4</td>
<td>111</td>
<td>34 (85)</td>
<td>2.8 ± 0.4</td>
<td>2</td>
</tr>
<tr>
<td>Initiation stage&lt;br&gt;Caffeine</td>
<td>40</td>
<td>64 ± 0.7</td>
<td>281 ± 4</td>
<td>107</td>
<td>32 (80)</td>
<td>2.7 ± 0.4</td>
<td>1</td>
</tr>
<tr>
<td>Promotion stage&lt;br&gt;Caffeine</td>
<td>40</td>
<td>66 ± 0.7</td>
<td>289 ± 4</td>
<td>102</td>
<td>31 (78)</td>
<td>2.6 ± 0.4</td>
<td>1</td>
</tr>
</tbody>
</table>

* Caffeine was added to the drinking water (500 mg/liter) from 29 to 60 days of age. MNU (2.5 mg/100 g body weight) was administered i.v. at 57 days of age. The study was terminated 26 weeks after carcinogen treatment.

* Caffeine was added to the drinking water (500 mg/liter) from 60 days of age until the study was terminated. 26 weeks after carcinogen treatment. MNU (2.5 mg/100 g body weight) was administered i.v. at 57 days of age.

The significant difference in body weight gains between the control and the caffeine-treated animals (Table 1). Furthermore, caffeine consumption did not significantly affect the weight of the pituitary, uterus, ovaries, spleen, or liver (data not shown).

Effect of Caffeine on the Incidence of Palpable Mammary Carcinomas and Benign Tumors in Female Sprague-Dawley Rats. When MNU was administered at 57 days of age to rats that had received caffeine from 27 until 59 days of age (initiation stage) there was no significant difference in mammary carcinoma multiplicity or in the percentage of animals bearing mammary carcinomas (Table 2; Fig. 2). When MNU was administered at 57 days of age to animals who received caffeine commencing 3 days later and continuing until termination of experiment (for 26 weeks) (promotion stage), there was no significant difference in mammary carcinoma multiplicity or in the percentage of animals bearing mammary carcinomas (Table 2; Fig. 2). The body weight gains of the animals in both groups were also not significantly affected by caffeine treatment (Table 2). The number of benign mammary tumors was low in all three animal groups, too low for a meaningful statistical analysis (Table 2).

Effect of Caffeine on Urinary and Fecal Excretion of Tritiated DMBA in Female Sprague-Dawley Rats. When tritiated DMBA was injected i.v. into 55-day-old rats that had been treated with caffeine from 27 until 59 days of age, there was a reduction (P = 0.06) in the amount of tritium in the urine after 24 h in the caffeine-treated group compared to controls (Table 3). No difference in urinary levels of tritium between control and caffeine-treated rats at 48, 72, and 96 h was observed (data not shown). Caffeine consumption did not affect fecal levels of tritium at 24 (Table 3), 48, 72, or 96 h (data not shown). Liver weights were significantly increased in the caffeine-treated rats (P < 0.002) (Table 3).

Effect of Caffeine on Mammary Gland Development in Female Sprague-Dawley Rats. When rats were treated with caffeine from 27 to 59 days of age and whole-mount preparations were made of the number 4 (inguinal) mammary glands (at 59 days of age), there were no apparent morphological differences in mammary gland development (degree of ductal branching and lobuloalveolar development) between the control and the caffeine-treated animals (Table 3). In both groups, an extensive...
Caffeine was added to the drinking water (500 mg/liter) from 29 to 60 days of age. MNU (2.5 mg/100 g body weight) was administered i.v. at 57 days of age. Promotion stage: caffeine was added to the drinking water (500 mg/liter) from 60 days of age until the study was terminated. MNU (2.5 mg/100 g body weight) was administered i.v. at 57 days of age. Initiation and promotion stages: controls versus caffeine, carcinomas, no significant difference.

Number of ducts and a minimal amount of lobuloalveolar development was observed.

Histopathological Analysis of Mammary Tumors. In these studies, 2662 mammary tumors were observed. Each tumor was examined for its histopathological characteristics by using the classification system of Russo et al. (4). In the study using MNU as the carcinogen, a total of 324 mammary tumors were observed and examined; 99% of these tumors were mammary carcinomas, the remaining tumors were benign mammary fibroadenomas (Table 2). Of the carcinomas, 96% were invasive carcinomas and 4% were intraductal carcinomas. Caffeine treatment did not significantly influence the proportion of carcinomas classified as invasive or intraductal. In the study using DMBA (low dose) as the carcinogen, a total of 2338 mammary tumors were observed and examined; 11% of these tumors were mammary carcinomas and 89% were benign mammary tumors. Of the carcinomas, 39% were invasive carcinomas and 61% were intraductal carcinomas. Caffeine treatment did not significantly influence the proportion of carcinomas classified as invasive or intraductal. Of the 2106 benign mammary tumors, <1% were fibromas, 61% were fibroadenomas, and 39% were adenomas. Caffeine treatment did not affect the percentage of tumors classified as fibromas, fibroadenomas, or adenomas. Fifty percent of the fibroadenomas and adenomas showed histological evidence of cystic secretory activity (large and small ducts with secretion). Caffeine treatment during the initiation stage did not affect the percentage of benign mammary tumors with cystic secretory activity. However, when caffeine was administered during the promotion stage, 66% of the benign mammary tumors were cystic (with secretion), while in the controls, 53% of the tumors showed evidence of cystic secretion (P < 0.05).

Calculated Amount of Ingested Caffeine. The amount of caffeine ingested, based on the volume of drinking water consumed by each rat each day, was ≈15 mg caffeine/rat/day. This is equivalent, on a body weight basis, to a daily consumption of 3600 mg caffeine by a 60-kg woman. If one uses values corrected according to metabolic body weight (5), 15 mg of caffeine/rat/day is equivalent to 1600 mg of caffeine/60-kg woman/day. For a point of reference, the mean caffeine content of a cup of coffee is ≈100 mg (6). In these studies, caffeine consumption did not alter body weight gains nor adversely affect the health of the animals.

DISCUSSION

In the studies involving the use of DMBA as a mammary gland carcinogen, we used a very low dose of the carcinogen for the purpose of inducing a spectrum of mammary tumors which are more akin, with respect to incidence and histopathological features, to that observed in female human populations. We succeeded, as approximately 11% of the mammary tumors that were observed in control rats were carcinomatous and remaining mammary tumors were benign tumors of varying histopathological features. Importantly, caffeine treatment during the initiation stage of this tumorigenic process resulted in a striking reduction (≈50%) in the incidence of both carcinomatous and benign mammary tumors. While it has been previously reported that caffeine can inhibit the initiation stage of DMBA-induced rat mammary gland tumorigenesis, when the carcinogen is administered at standard dose levels (1, 2), this is the first report that demonstrates that caffeine can suppress this tumorigenic process when relatively low and more relevant levels of the carcinogen are used. That caffeine consumption can inhibit the initiation stage of DMBA-induced benign mammary gland tumorigenesis has heretofore not been reported.

In contrast, when caffeine was administered during the initiation stage of MNU-induced rat mammary gland tumorigenesis, no significant effect of caffeine on this tumorigenic process was observed; mammary carcinoma incidence was virtually identical in control and caffeine-treated animals. Since MNU is a direct acting carcinogen, unlike DMBA which requires metabolic activation, one would immediately conclude that the action of caffeine in the suppression of the initiation stage of DMBA-induced rat mammary gland tumorigenesis was via an alteration of DMBA metabolism. To test this hypothesis we administered tritiated DMBA to caffeine-treated rats. At 24 h after tritiated DMBA treatment, a slight reduction (P = 0.06) in the amount of urinary tritium in the caffeine-treated rats was observed. However, no significant effect of caffeine on 48- to 96-h urinary or 24- to 96-h fecal excretion of the isotope was observed.

Table 3 Effect of caffeine on urinary and fecal excretion of [3H]DMBA and mammary gland development in female Sprague-Dawley rats treated with a low-dose level of DMBA

Caffeine was added to the drinking water (500 mg/liter) from 27 to 59 days of age. DMBA (0.5 mg/100 g body weight) and 3H-DMBA (0.043 mCi/100 g body weight) were administered i.v. at 55 days of age. All rats were housed singly, in metabolic cages: urine and feces were collected daily for 4 days and analyzed for tritium levels.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rats</th>
<th>Mean body wt, onset of study (g ± SE)</th>
<th>Mean body wt, termination of study (g ± SE)</th>
<th>Urine, 24-h [3H]-DMBA metabolites excreted (cpm ± SE)</th>
<th>Feces, 24-h [3H]-DMBA metabolites excreted (cpm ± SE)</th>
<th>Mean liver wt (g ± SE)</th>
<th>Mean mammary gland development scores (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>21</td>
<td>48 ± 0.9</td>
<td>186 ± 2</td>
<td>228,184 ± 7,756 (A)*</td>
<td>157,909 ± 13,778</td>
<td>10.9 ± 0.2 (C)</td>
<td>4.2 (3–6)</td>
</tr>
<tr>
<td>Caffeine</td>
<td>21</td>
<td>48 ± 1.0</td>
<td>189 ± 3</td>
<td>204,865 ± 9,324 (B)</td>
<td>165,353 ± 12,048</td>
<td>12.0 ± 0.3 (D)</td>
<td>4.3 (3–6)</td>
</tr>
</tbody>
</table>

* A/B, P = 0.06; C/D, P < 0.002.

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observed. Thus, unequivocal evidence for an effect of caffeine on DMBA metabolism has not been provided in our study. Caffeine can affect, however, enzyme systems that influence polycyclic aromatic hydrocarbon metabolism. For example, it has been reported that caffeine can enhance or inhibit the induction of P-450 mixed function oxidase activities, a phenomenon that is influenced by the dose and duration of caffeine treatment (7–9). Furthermore, caffeine has been reported to inhibit the in vitro binding of DMBA to mouse epidermal cells (10). Since the development stage of the rodent mammary gland can have a profound effect on the initiation stage of DMBA-induced rat mammary gland tumorigenesis (11), and that chronic caffeine administration can affect mammary gland growth processes in female mice (enhances hormone-induced lobuloalveolar development) (12), we felt it important to examine the influence of caffeine treatment on mammary gland development in the rats of this study. The degree of mammary gland development (number of ducts and end duct buds and lobuloalveolar development) at the time of carcinogen treatment was found to be virtually identical in controls and caffeine-treated rats. The lack of an effect of caffeine on rat mammary development was not surprising, for if such an effect of caffeine was indeed present, one would expect a difference in the incidence of mammary tumors among controls and caffeine-treated animals in the MNU study; clearly this was not the case. Thus, the mechanism by which caffeine significantly inhibits the initiation stage of DMBA-induced rat mammary gland tumorigenesis appears to be via an alteration in DMBA metabolism. The lack of an effect of caffeine on the initiation stage of MNU-induced rat mammary gland tumorigenesis, an alteration in 24-h urinary excretion of tritiated DMBA by caffeine, a significant increase in liver size by caffeine, and a lack of caffeine-induced changes in mammae morphology would support this concept.

In the studies designed to evaluate the influence of caffeine consumption on the promotion stage of rat mammary gland tumorigenesis, no significant effect of caffeine on mammary carcinoma incidence or multiplicity was observed in DMBA- and MNU-treated rats. A lack of an effect of prolonged caffeine administration during the promotion stage on mammary carcinoma development in DMBA-treated rats has been reported previously (2). In these previously reported studies, an early temperate but transitory stimulation of mammary carcinoma development, on occasion, was observed; such a stimulatory effect of caffeine was not observed in the present studies. However, a very striking reduction (≈57%) in the number of benign mammary tumors was observed in rats treated with caffeine during the promotion stage. Furthermore, the percentage of benign mammary tumors in the caffeine-treated animals that had histological features of cystic ductal secretion was significantly greater than the percentage of cystic (secretory) benign mammary tumors in the control animals. Such observations would suggest that caffeine consumption inhibits mammary epithelial cell proliferative processes (sharp decrease in number of benign mammary tumors) and stimulates a differentiation type of mammary epithelial cell phenotype (increased secretory activity). While, in the present study, we were unable to observe any apparent morphological difference in mammae development in young rats treated with caffeine for a short time period (32 days), prolonged consumption of caffeine (332 days) by older rats could yield mammae growth patterns that are entirely different. Year-old female rats, compared to young rats, have profound differences in mammary gland growth responsiveness and endocrine secretory patterns (13). Furthermore, it has been reported that mature female mice, when treated chronically with caffeine, have a heightened mammae responsiveness to an administered hormonal milieu that promotes differentiation (lobuloalveolar) processes (12). It is important to point out that the sharp reduction in the development of benign mammary tumors by chronic and prolonged caffeine treatment (promotion stage) was observed without any evidence of toxicity; such animals were as healthy and had body weight gains identical to that of the control animals. How caffeine might affect mammary cell differentiation/proliferation processes is at this time entirely speculative. Caffeine has been reported to alter phosphodiesterase activities (14), to affect anterior pituitary gland secretion (15, 16), to alter insulin secretion (17), to affect central nervous system neurotransmitter activities (18–20), to modify serum fatty acid levels (21), to alter intracellular calcium transport (22), and to block adenosine receptors (23). Any number of these physiological/biochemical events, singly or in combination, could influence mammary gland differentiation/proliferation processes (24).

The histological characteristics of the benign mammary tumors in female Sprague-Dawley rats treated with low-dose levels of DMBA are varied. Typically, these tumors are classified as fibromas (consist almost entirely of connective tissue elements), fibroadenomas (near equal distribution of connective tissue and glandular elements), and adenomas (consist almost entirely of glandular elements). What is often overlooked in these tumors is that in older rats, a significant fraction of these tumors are secretory. Secretion occurs in alveolar units or in the lumen of ducts. Many of these ducts which contain intraluminal secretions are cystic and contain an abundance of secretion. Other ducts are of normal diameter but continue to contain intraluminal secretion. While these benign tumors are classified as either fibroadenomas or adenomas, microscopically they resemble, in many ways, the common benign breast tumors observed in women referred to as fibrocystic disease. Human fibrocystic disease is lesions characterized by cysts (ducts with secretion) (25). If the cysts are large enough to be easily visible, e.g., 2 or 3 mm or more in diameter, the lesion is referred to as gross cystic disease. If the cysts are seen only microscopically, such cysts are classified as microscopic cystic disease. The benign mammary tumors that occur in female Sprague-Dawley rats treated with a low dose of DMBA, tumors that occur relatively late in the life of the animal, morphologically resemble human fibrocystic disease, both gross cystic disease and microscopic cystic disease. That chronic caffeine consumption can significantly influence the development of these benign secretory lesions in female rats, as reported in this communication, may provide insight into the potential and often controversial relationship between caffeine and the development of fibrocystic disease in human populations.

Whether or not caffeine is important in the development of benign and carcinomatous breast lesions in human populations is not definitely known (reviewed in Ref. 26). Caffeine consumption has been reported to increase (27, 28), to have no effect (29, 30), and to reduce (31, 32) the risk of breast cancer development in human populations. The development of fibrocystic breast disease in human populations has been reported by several groups to be stimulated by caffeine consumption (33–35); others have not been able to demonstrate a relationship between caffeine consumption and the development of this disease (36–38). In rodent studies, it is clear that caffeine consumption, at relevant and reasonable dose levels, can significantly influence (inhibit or stimulate) the development of benign (this study) and carcinomatous (this study and in Refs. 1, 2, 12, and 39–43) mammary gland lesions, a phenomenon...
dependent upon the species and strain of rodent under study and the time span of caffeine administration. In view of the fact that caffeine is one of the three most widely consumed drugs in the world today (the others being nicotine and alcohol), it is important to examine pathological processes that are influenced by this drug, including diseases of the mammary gland. The major results of our study, i.e., the inhibitory activity of caffeine on the initiation stage of poly cyclic aromatic hydrocarbon (DMBA)-induced rat mammary gland tumorigenesis, the lack of such an effect when a direct-acting initiating agent is used (MNU), plus the profound inhibitory effect of the drug during the promotion stage on the development of benign mammary tumors, are potentially important observations that should facilitate our understanding of the relationship between caffeine consumption and this disease process.

**ADDENDUM**

We just recently completed an analysis of the level of [H]-DMBA in the mammary glands (paired No. 4, inguinal) of control and caffeine-treated rats. These glands were obtained from the animals in the caffeine/tritiated DMBA excretion study (Table 3). Mean (±SE) cpm/mg DNA in control animals (A = 37) and in caffeine-treated animals (p = 0.003). Thus, less than one-half the amount of [H]-DMBA was associated with DNA of the mammary glands of the caffeine-treated animals compared to the control animals. While the cpm are admittedly quite low, the difference in means is highly significant (Student's t test). These results support the concept proposed in this communication that caffeine suppresses the initiation stage of DMBA-induced rat mammary gland tumorigenesis by altering carcinogen (DMBA) metabolism.

**REFERENCES**

Influence of Caffeine on Development of Benign and Carcinomatous Mammary Gland Tumors in Female Rats Treated with the Carcinogens 7,12-Dimethylbenz(a)anthracene and N-Methyl-N-nitrosourea

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