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Report

Caffeine inhibits development of benign mammary gland tumors in carcinogen-treated female Sprague-Dawley rats

David M. Wolfrom[1], A. Ramesha Rao[2] and Clifford W. Welsch[2]
[1] *Department of Anatomy and* [2] *Pharmacology/Toxicology, Michigan State University, East Lansing, MI 48824, USA*

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Summary

The purpose of this study was to assess the influence of caffeine on the incidence of benign mammary tumors in carcinogen (DMBA) treated female Sprague-Dawley rats. Four different animal models were used in these studies, i.e., the administration of DMBA to: [1] 55 day old virgin rats; [2] 53 day old ovariectomized, estrogen treated virgin rats; [3] 135 day old virgin rats and [4] 135 day old parous rats. A high incidence of benign mammary fibroadenomas was observed in each of the four animal models. In addition, in the estrogen treated ovariectomized animals, a high incidence of secretory mammary gland cysts was observed. Caffeine (500 mg/L drinking water) was administered daily throughout the study commencing 3–31 days after carcinogen treatment. Caffeine treatment significantly ($P < 0.05$ to $P < 0.001$) reduced the incidence of benign mammary fibroadenomas in the 55 day old virgin rat model ($P < 0.01$), in the 53 day old estrogen treated ovariectomized virgin rat model ($P < 0.05$ to $P < 0.001$) and in the 135 day old virgin rat model ($P < 0.05$). The number of benign mammary fibroadenomas was reduced by caffeine in the 135 day old parous rat model but this reduction was not significant ($P < 0.10$). In addition, in the estrogen treated ovariectomized virgin rat model, caffeine significantly ($P < 0.05$ to $P < 0.001$) reduced the incidence of mammary gland cysts. Caffeine treatment either increased or had no significant effect on body weight gains, depending upon the animal model. Thus, caffeine consumption can influence the development of benign mammary tumors (fibroadenomas and cysts) in carcinogen treated female Sprague-Dawley rats, an influence that was shown to be consistently inhibitory.

We have previously reported that chronic caffeine (1,3,7-trimethylxanthine) consumption via the drinking water (500 mg/L) can significantly influence the development and/or growth of mammary carcinomas in female rats treated with the chemical carcinogen DMBA [1, 2]. In the initiation stage, caffeine consumption was observed to significantly ($P < 0.05$) and consistently suppress mammary tumorigenesis while in the early promotion stage, caffeine consumption was observed to temperately and transiently stimulate the incidence of mammary carcinomas. However, prolonged consumption of caffeine during the promotion stage had no significant effect on the final number of mammary carcinomas.

While our previous experiments [1–3] and others [4, 5] have primarily examined the influence of chronic caffeine consumption on the development and/or growth of mammary carcinomas, the experiments reported in this communication investi-

Address for offprints: Clifford W. Welsch, Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824, USA

gated the heretofore unexamined influence of chronic caffeine consumption on DMBA-induced benign mammary gland tumorigenesis. Our results provide evidence that chronic caffeine consumption can significantly inhibit the development of benign mammary gland tumors in female rats treated with DMBA. Considering the substantial concern over the role of caffeine in the development of benign breast disease in humans (reviewed in 6), the results of our experiments are potentially of substantial importance in furthering our understanding of the role of caffeine in this disease process.

Materials and methods

Chemicals

DMBA (7, 12-dimethylbenzanthracene, Eastman Kodak Co., Rochester, NY) was administered once, either i.g. or i.v. For i.g. administration, DMBA was dissolved in corn oil, while for i.v. administration, DMBA was prepared as a lipid emulsion by the Upjohn Co. (Kalamazoo, MI). Caffeine (U.S. Biochemical Corp., Cleveland, OH) drinking water solutions (500 mg/L) were prepared 3 ×/week (MWF) using distilled water for all 3 experiments. Estrogen (17β-estradiol; U.S. Biochemical Corp., Cleveland, OH) solutions were prepared by mixing the steroid with a minimum amount of powdered gum arabic and adding this mixture to distilled water.

Animals

Nulliparous and parous female Sprague-Dawley rats were obtained from Harlan Sprague-Dawley, Inc. (Indianapolis, IN). Parous rats were mated between 55 and 70 days of age, gave birth to only one litter, and completed 20 to 21 days of lactation (to weaning). All animals were maintained in a temperature (24° C) and light (14 hr. light/day) controlled room and were given standard commercial rat chow (Teklad; Harlan Sprague-Dawley, Inc., Winfield, IA) *ad libitum* throughout the experiments.

Caffeine consumption and the development of benign and carcinomatous mammary gland tumors in ovariectomized estrogen/DMBA-treated Sprague-Dawley rats

DMBA (10 mg/ml corn oil) was administered once i.g. to each rat at 53 days of age. All rats were bilaterally ovariectomized at 72 days of age. Estrogen (17β-estradiol, 1, 10, or 100 μg/kg body weight) was administered s.c. daily from 84 days of age until termination of the experiment. Caffeine was administered via the drinking water (500 mg/L) from 84 days of age until termination of the experiment. All rats were sacrificed at 183 days of age. Administration of high doses of estrogens to DMBA treated, ovariectomized Sprague Dawley rats results in a high incidence of benign mammary tumors; many of these tumors possess cystic histopathological features.

Caffeine consumption and the development of benign and carcinomatous mammary gland tumors in DMBA-treated old parous and virgin Sprague-Dawley rats

DMBA (20 mg/ml corn oil) was administered once i.g. to each rat at 135 days of age. In parous rats, the carcinogen was administered between 23 and 38 days after cessation of lactation. Caffeine was administered via the drinking water (500 mg/L) from 150 days of age until termination of the experiment. All rats were sacrificed at 300 days of age. Administration of DMBA to old virgin or parous Sprague-Dawley rats results in a high incidence of benign mammary tumors.

Caffeine consumption and the development of benign and carcinomatous mammary gland tumors in DMBA-treated young virgin Sprague-Dawley rats

DMBA (2 mg/100 g B. wt.) was administered once i.v. to each rat at 55 days of age. Caffeine was administered via the drinking water (500 mg/L) from 58 days of age until termination of the experiment. All rats were sacrificed at 56 weeks after DMBA administration. Maintaining female Sprague-Dawley rats on experiment for a long period after DMBA treatment (56 weeks) results in a high incidence of benign mammary tumors.

Assessment of mammary gland tumorigenesis

All rats were palpated bi-weekly 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, and the animal was placed back on the experiment. At experiment termination, all 6 pairs of mammary glands were spread flat with the underlying integument attached and examined. All mammary tumors (palpable and non-palpable) were excised from these glands from all rats. All tumors that were excised during the experiment and at experiment termination were fixed in Bouin's solution, stained with hematoxylin and eosin, and examined histopathologically. All mammary tumors were classified histopathologically as either carcinomatous, benign (fibroadenomas), or cystic. The mammary tumors that were classified as cystic were benign fibroadenomas that contained definitive histological areas of dilated ducts with secretion (cysts). These cysts were most often quite large (macrocysts) while a few were small (microcysts).

Statistics

Number (%) of mammary tumor bearing rats and number of mammary tumors were evaluated by chi-square analysis. Body weights were evaluated by unpaired students 't' test. Significance was set at $P < 0.05$.

Results

Effect of caffeine on the development of benign and carcinomatous mammary gland tumors in ovariectomized estrogen/DMBA-treated Sprague-Dawley rats

In DMBA-treated female Sprague-Dawley rats that were subsequently ovariectomized and treated with either 1.0 μg or 10 μg estrogen, caffeine was observed to significantly ($P < 0.001$ and $P < 0.05$, respectively) decrease the total number of benign mammary fibroadenomas and the percentage of animals bearing benign mammary fibroadenomas (Table 1). Caffeine was also observed to significantly ($P < 0.001$ and $P < 0.05$, respectively) decrease both the total number of benign mammary cysts and the percentage of animals with benign mammary cysts in animals treated with the 100 μg dose of estrogen (Table 1). In animals treated with estrogen, either the 1.0, 10, or 100 μg dose levels, caffeine was observed to have no significant effect on the total number of mammary carcinomas or the percentage of animals bearing mammary carcinomas. Mean body weights were significantly ($P < 0.001$, $P < 0.01$) increased in the caffeine-treated animals at all three dose levels of estrogen treatment (Table 1).

Effect of caffeine on the development of benign and carcinomatous mammary gland tumors in DMBA-treated old parous and virgin Sprague-Dawley rats

In both parous and virgin rats, caffeine decreased both the total number of benign mammary fibroadenomas and the percentage of animals bearing benign mammary fibroadenomas (Table 2). The decrease in number of benign mammary tumors by caffeine was significant in the virgin rats ($P < 0.05$), and just missed significance ($P < 0.10$) in the parous rats. In both parous and virgin rats, caffeine

significantly ($P < 0.05$, $P < 0.01$) decreased the total number of mammary carcinomas (Table 2). Mean body weights were significantly ($P < 0.001$, $P < 0.05$) increased in both parous and virgin animals treated with caffeine (Table 2).

Effect of caffeine on development of benign and carcinomatous mammary gland tumors in DMBA-treated young virgin Sprague-Dawley rats

Caffeine administration for 56 weeks after DMBA treatment was found to significantly ($P < 0.01$) decrease the total number of benign mammary fibroadenomas; the percent of animals bearing these tumors was also decreased by caffeine but this decrease was not significant ($P < 0.10$) (Table 3). In contrast, caffeine treatment had no significant effect on either the total number of mammary carcinomas or the percentage of animals bearing mammary carcinomas (Table 3). A slight stimulatory effect of caffeine on mammary carcinoma multiplicity was observed in the early stages of mammary carcinoma development; this effect was transitory and temperate ($P = 0.056$) (data not shown). Caffeine treatment did not significantly affect body weight gains (Table 3).

Discussion

As a naturally occurring plant alkaloid, caffeine is found in coffee, tea, cocoa, and as an additive in many different soft drinks, candies, and medications (both prescription and non-prescription). Through these various sources, caffeine consumption can be enormous and, in fact, caffeine is one of the most widely consumed drugs in many parts of the world today [7].

The role of caffeine consumption in the genesis of human breast disease is unclear at the present time (reviewed in 6). While studies have reported a positive association between caffeine consumption and benign breast disease in humans [8, 9], other studies have not supported this association]10, 11]. With regards to human breast cancer, caffeine consumption has been reported to increase [12, 13], to

Table 1. Effect of caffeine consumption on the development of benign and carcinomatous mammary gland tumors in ovariectomized estrogen/DMBA-treated Sprague-Dawley rats

Treatment[a]	No. of animals	Mean B.wt. (g ± SE)		Total no. of mammary fibroadenomas	Percent of rats with mammary fibroadenomas	Total no. of mammary cysts	Percent of rats with mammary cysts	Total no. of mammary carcinomas	Percent of rats with mammary carcinomas
		Initial	Final						
Estrogen (1.0 μg)	41	223 ± 2	271 ± 3[b]	38[b]	53.7[b]	2	4.9	47	63.4
Estrogen (1.0 μg plus caffeine)	42	222 ± 2	299 ± 4[c]	8[c]	14.3[c]	2	4.8	64	59.5
Estrogen (10 μg)	42	223 ± 2	250 ± 2[d]	120[f]	81.0[f]	24	35.7	38	47.6
Estrogen (10 μg plus caffeine)	42	222 ± 2	259 ± 3[e]	63[g]	57.1[g]	31	33.3	42	42.9
Estrogen (100 μg)	42	222 ± 2	241 ± 2[b]	4	9.5	40[b]	52.4[f]	5	11.9
Estrogen (100 μg plus caffeine)	41	223 ± 2	253 ± 2[c]	1	2.4	23[c]	26.8[g]	4	9.8

a: DMBA (10 mg/ml corn oil) was administered once i.g. at 53 days of age. Ovariectomy was performed at 72 days of age. Estrogen (μg/kg B.wt) was administered daily starting at 84 days of age until termination of the experiment. Caffeine was administered via the drinking water (500 mg/L) from 84 days of age until termination of the experiment, at 183 days of age.
b/c: $p < 0.001$
d/e: $p < 0.01$
f/g: $p < 0.05$

Table 2. Effect of caffeine consumption on the development of benign and carcinomatous mammary gland tumors in DMBA-treated old parous and virgin Sprague-Dawley rats

Treatment[a]	No. of animals	Mean B.wt. (g ± SE)		Total no. of mammary fibroadenomas	Percent of rats with mammary fibroadenomas	Total no. of mammary cysts	Percent of rats with mammary cysts	Total no. of mammary carcinomas	Percent of rats with mammary carcinomas
		Initial	Final						
Parous – controls	34	245 ± 2	263 ± 3[b]	36	61.8	3	5.9	10[f]	20.6[a]
Parous – caffeine	33	247 ± 3	281 ± 4[c]	20	39.7	0	0	1[g]	3.0[e]
Virgin – controls	29	254 ± 2	282 ± 4[d]	69[d]	72.4[d]	2	6.9	34[d]	44.6[d]
Virgin – caffeine	30	251 ± 2	292 ± 3[e]	25[e]	43.3[e]	3	10.0	7[e]	20.0[e]

a: DMBA (20 mg/ml corn oil) was administered once i.g. at 135 days of age. Caffeine was administered via the drinking water (500 mg/L) from 150 days of age until termination of the experiment, at 300 days of age.
b/c: $p < 0.001$
d/e: $p < 0.05$
f/g: $p < 0.01$

have no effect [14, 15], or to reduce [16, 17] the risk of developing breast cancer. Thus, to date, no consistent association between caffeine consumption and the development of breast disease (either benign or malignant) in human populations has been provided.

Caffeine consumption has been reported to stimulate [3, 5], to have no effect [18], and to inhibit [4] the genesis and/or growth of mammary tumors in experimental animals depending upon the species of laboratory animal used and the experimental conditions. One of the most popular experimental animal models in the study of human breast cancer is the DMBA-induced rat mammary carcinoma model [19]. Various morphological (i.e. tumors of ductal origin) and physiological (i.e., hormone/growth factor responsiveness) features that closely parallel human breast cancer account, in part, for the popularity of this experimental model. We have previously reported that this model is developmentally growth responsive to a relevant and reasonable dose level of caffeine, administered via the drinking water (500 mg/L) [1, 2]. Given the responsiveness of this model to caffeine and the substantial concern about the potential role of caffeine consumption in the development of benign breast disease in human populations, we felt it important to extend our initial observations on DMBA-induced rat mammary carcinomas [1, 2] to the heretofore unexamined role of caffeine in DMBA-induced benign mammary gland tumorigenesis.

Table 3. Effect of caffeine consumption on the development of benign and carcinomatous mammary gland tumors in DMBA treated young virgin Sprague-Dawley rats

Treatment[a]	No. of animals	Mean B.wt. (g ± SE)		Total no. of mammary fibroadenomas	Percent of rats with mammary fibroadenomas	Total no. of mammary carcinomas	Percent of rats with mammary carcinomas
		Initial	Final				
Controls	22	168 ± 2	312 ± 5	183[b]	86.0	126	95
Caffeine	22	170 ± 2	305 ± 7	68[c]	68.0	124	100

a: DMBA (2 mg/100 g. B.wt.) was administered once i.v. at 55 days of age. Caffeine was administered via the drinking water (500 mg/L) from 3 days after DMBA treatment until termination of the experiment, at 56 weeks after DMBA treatment.
b/c: $p < 0.01$

Benign mammary tumors can be induced with high frequency in female Sprague-Dawley rats by using a chemical carcinogen such as DMBA. There are three ways in which this can be accomplished. Firstly, one can administer a standard dose level of DMBA to 2 month old intact female rats, excise the early developing mammary tumors as they arise (almost all of which are carcinomas), and monitor the incidence of the later developing mammary tumors, most of which are benign mammary fibroadenomas. Secondly, one can modify the above procedure by using ovariectomized rats (DMBA-treated) and treat them with relatively high doses of estrogens (which suppress the development of mammary carcinomas). If the dose of estrogen = 10 μg/kg body weight, the vast majority of the mammary tumors that develop will be benign mammary fibroadenomas. If the dose of estrogen is increased 10 fold (100 μg/kg), most of the mammary tumors will be benign mammary cysts (secretory). Such cysts closely resemble, morphologically, human fibrocystic disease [20]. Thirdly, one can administer a standard dose of DMBA to older female rats (virgin or parous); the vast majority of the mammary tumors that occur are benign mammary fibroadenomas. In the present study, we used each of these techniques to induce benign mammary neoplasms. Importantly, chronic caffeine consumption commencing after DMBA treatment (promotion stage) consistently reduced the incidence of these benign mammary tumors, both fibroadenomas and cysts. This reduction by caffeine of these benign mammary neoplasms occurred in the absence of any observable toxicity. Indeed, the caffeine consuming animals often had body weight gains that were greater than controls.

In contrast, chronic caffeine administration generally did not significantly affect the incidence of mammary carcinomas. This lack of an effect of chronic and prolonged caffeine consumption during the promotion stage of DMBA-induced mammary carcinoma development in female Sprague-Dawley rats has been previously reported [1, 2]. However, and in contrast, in older parous and virgin rats treated with DMBA, chronic caffeine administration was observed to significantly suppress the development of mammary carcinomas. This is the first time that we have ever observed an inhibition by caffeine of the development of mammary carcinomas when the drug is administered during the promotion stage of this tumorigenic process. It is known that the developmental stage of the rodent mammary gland (as influenced by age and parity) does have a profound effect on the initiation stage of mammary gland carcinogenesis and the subsequent growth responsiveness of these tumors to a hormonal and/or growth factor stimulus [21]. The significant difference in incidence of mammary carcinomas in the DMBA treated virgin and parous animals supports this contention and confirms previous reports [22, 23].

The mechanism by which caffeine influences the development of DMBA-induced benign or carcinomatous mammary gland tumors is unclear. Caffeine has been reported to inhibit phosphodiesterase activity [24], to increase serum free fatty acid levels [25], to alter neurotransmitter secretion and/or metabolism [26], to increase intracellular calcium [27], to alter insulin and anterior pituitary secretion [28, 29], and to block adenosine receptors [30]. Although the adenosine receptor antagonism mechanism is currently receiving the most attention, each of these mechanisms, alone or in combination, could influence this tumorigenic process. Our study, which demonstrates a profound inhibitory effect of caffeine on the incidence of benign mammary fibroadenomas and benign mammary cysts and a differential effect on mammary carcinoma development (depending upon the animal model used) should facilitate our understanding of the potential modulating effect of this drug in the etiology of human breast diseases.

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