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Inhibition by caffeine of ovarian hormone-induced mammary gland tumorigenesis in female GR mice

L.C. VanderPloeg and C.W. Welsch

Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824 (U.S.A.)

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Summary

The purpose of this study was to determine whether or not caffeine could influence the development of ovarian hormone dependent mammary tumors in GR mice. Virgin female GR mice were treated daily for 24 weeks with 17β-estradiol and progesterone, commencing at 8—10 weeks of age. One week after the onset of hormone treatment, caffeine (500 mg/l drinking water) was administered daily until experiment termination to one-half of the hormone-treated mice. Hormone treatment induced mammary tumors in 95—100% of the mice. Caffeine treatment significantly ($P < 0.05$) reduced the mean number of mammary tumors per mouse and significantly ($P < 0.05$) increased the mean latency period of mammary tumor appearance.

Keywords: caffeine; mammary tumors, GR mice

Introduction

Caffeine (1,3,7-trimethylxanthine) is one of the three most commonly used psychoactive drugs in the world today, the other two being alcohol and nicotine [1]. Because of its widespread use, a number of epidemiological studies have sought to determine whether or not there is a relationship between caffeine consumption and neoplastic diseases. The results have been inconsistent and conflicting [2,7,10,23]. With regard to breast cancer, such studies have also been inconsistent, as consumption of the drug has been shown to have no effect, to have a slight stimulatory effect or to have an inhibitory effect on the development of this disease (reviewed in Refs. 6 and 22).

In experimental animal studies (rodents), where most of the confounding variables that beset and beleaguer epidemiological studies can be eliminated, caffeine consumption has been shown to stimulate, to have no effect or to inhibit the development and/or growth of mammary tumors, a phenomenon dependent upon the animal model examined and the time span of caffeine treatment (reviewed in Ref. 22). For example, caffeine consumption has been reported to stimulate the development of spontaneous mammary tumors in female C3H mice [12,13,21] and in carcinogen treated female $BD2F_1$ mice [21]. The mammary tumors that develop in female C3H mice are MTV positive and are alveolar in origin while the mammary tumors that occur in carcinogen treated $BD2F_1$ mice are MTV negative and are ductal in origin

Correspondence to: C.W. Welsch, Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824, U.S.A.

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[3—5,8,9,11,16]. A third type of mouse model, the female GR mouse, is very different from the C3H or $BD2F_1$ mouse models [3—5,14,17]. The effect of caffeine consumption on mammary tumor development in this model has not been reported. The GR mouse mammary tumors contain MTV which is transmitted not only by milk but also by the male and female gametes [11]. Morphologically, these tumors consist of carcinomatous tubular elements interspersed among large areas of scirrhous connective tissues, thus, differing substantially from the mammary tumors that occur spontaneously in the C3H mouse and those that are induced by carcinogens in the $BD2F_1$ mouse. Most importantly, however, the GR mouse mammary tumors are extremely hormone dependent, i.e., their development and growth are dependent upon the hormones of pregnancy [4,5,14,17]. Thus, pregnancy or the exogenous administration of estrogen and progesterone results in a rapid onset of tumor development; such tumors require the continued presence of these hormones for continued growth processes. The C3H and $BD2F_1$ mouse mammary tumors, while dependent upon these hormones for their early development, often do not require these hormones upon reaching an advanced stage (palpable) therefore become hormone independent [4,5,9,18]. The purpose of the study reported in this communication is to determine whether or not caffeine consumption can influence the development of the GR mouse mammary tumors as has been amply demonstrated in the C3H and $BD2F_1$ mouse mammary tumor models.

Materials and Methods

Two strains of GR mice, both of which carry MTV and develop mammary tumors that are hormone dependent, were used in this study. The breeding stock of the strain GRS/SN were obtained from Dr. W. Heston of the NIH (Bethesda, MD) and the breeding stock of the strain GR/A were obtained from Dr. H. Nagasawa of Meiji University, Experimental Animal Research Laboratory, Tama-Ku, Kawasaki, Kanagawa 214, Japan. A total of 202 mice were used in this study, 110 of the strain GR/A and 92 of the strain GRS/SN.

The mice were housed in a temperature-controlled (24°C) and light-controlled (14 h/day) room. All mice were fed ad libitum a standard diet of commercial mouse chow (Wayne Lab Blox, Allied Mills Inc., Chicago, IL). The mice were weaned at 21 days of age and separated by sex. When the virgin female mice were 8—10 weeks old, hormone treatment was initiated. Progesterone (United States Biochemical Corp., Cleveland, OH) was administered in the form of a pellet prepared in our laboratory containing 30 mg progesterone and 10 mg cholesterol (Sigma Chemical, St. Louis, MO). The pellets were implanted s.c. and renewed every 30 days. Estrogen (17β-estradiol, United States Biochemical Corp., Cleveland, OH) was supplied in the drinking water (0.5 mg/l). One week after hormone treatment was begun, caffeine (500 mg/l of distilled drinking water, ICN pharmaceuticals, Inc., Cleveland, OH) was administered daily to 55 GR/A mice and 45 GRS/SN mice. A control group of 55 GR/A mice and 47 GRS/SN mice continued to receive distilled water. Caffeine was prepared fresh twice weekly. Mice were placed in control and caffeine treated groups to assure equal mean group body weights at the onset of caffeine treatment. Beginning 6 weeks after hormone treatment all of the mice were palpated for the presence of mammary tumors. When the tumors reached a diameter of > 1.5 cm the mouse was killed and examined for the presence of non-palpable mammary tumors. All of the animals were killed after 24 weeks of hormone treatment (23 weeks of caffeine treatment) and examined for palpable and non-palpable mammary tumors. All mice were weighed weekly throughout the study. Mean number of mammary tumors per mouse and mean latency period of mammary tumor appearance were analyzed using Student's t-test. The number of animals bearing mammary tumors was analyzed by χ^2-analysis. Significance was set at $P < 0.05$.

Results

Chronic treatment of female GR/A mice or GRS/SN mice with 17β-estradiol and progesterone for 24 weeks resulted in high incidence of mammary tumors (95—100%) (Table I). The daily administration of caffeine to these mice, via the drinking water, resulted in a significant reduction in number of mammary tumors per mouse (GR/A mice, $P < 0.05$, GRS/SN mice, $P < 0.01$) and a significant increase in the mean latency period of first mammary tumor appearance (GR/A mice, $P < 0.05$, GRS/SN mice, $P < 0.01$) (Table I, Fig. 1). Mean latency period of the appearance of all mammary tumors was significantly increased ($P < 0.01$) in GRS/SN mice by caffeine; in GR/A mice, a numerical increase in mean latency period of all mammary tumors was observed in the caffeine treated mice but this difference did not reach the 5% level of statistical probability ($P = 0.15$). Caffeine treatment of GR/A and GRS/SN mice resulted in a reduction in mean final body weights of 3.4% and 7.7%, respectively. Final mean body weights (g ± S.E.) in the GR/A mice were 26.6 ± 0.5 (controls) and 25.7 ± 0.4 (caffeine); in the GRS/SN mice, final mean body weights in the control and caffeine treated mice were 26.2 ± 0.7 and 24.2 ± 0.3, respectively. Caffeine treatment did not significantly effect body weight gains in the GR/A mice ($P = 0.21$); body weight gains were significantly reduced by caffeine treatment in the GRS/SN mice ($P < 0.01$). No apparent ill or adverse effects of caffeine treatment were observed in either the GR/A or GRS/SN mice during the course of this study.

Discussion

Caffeine, when administered via the drinking water, significantly suppressed the development of ovarian hormone-induced mammary tumorigenesis in both GR mouse strains. This is the first time that we have observed an antitumor activity of caffeine in mouse mammary

Table I. Influence of caffeine on mammary tumor development in female GR mice treated with estrogen and progesterone.

Treatment*	No.of mice	No. of mice with mammary tumors (%)	Mean no. of mammary tumors/ mouse (±S.E.)	Mean latency period of mammary tumor development	
				1st mammary tumor	All mammary tumors
GR/A mice					
Control	55	55(100)	3.0 ± 0.2[a]	12.3 ± 0.6[a]	15.5 ± 0.4
Caffeine	55	52(95)	2.4 ± 0.3[b]	14.1 ± 0.6[b]	16.2 ± 0.4
GRS/SN mice					
Control	47	46(98)	2.9 ± 0.2[c]	11.1 ± 0.5[c]	13.9 ± 0.3[c]
Caffeine	45	44(98)	2.0 ± 0.1[d]	15.1 ± 0.7[d]	16.9 ± 0.5[d]
GR/A plus GRS/SN mice					
Control	102	101(99)	3.0 ± 0.1[c]	11.7 ± 0.4[c]	14.7 ± 0.3[c]
Caffeine	100	96(96)	2.2 ± 0.1[d]	14.5 ± 0.5[d]	16.5 ± 0.3[d]

*17β-Estradiol (0.5 mg/l drinking water) and progesterone (monthly s.c. pellets) were administered to GR mice commencing at 8—10 weeks of age and continuously for 24 weeks (experiment termination).

[a/b] $P < 0.05$.

[c/d] $P < 0.01$.

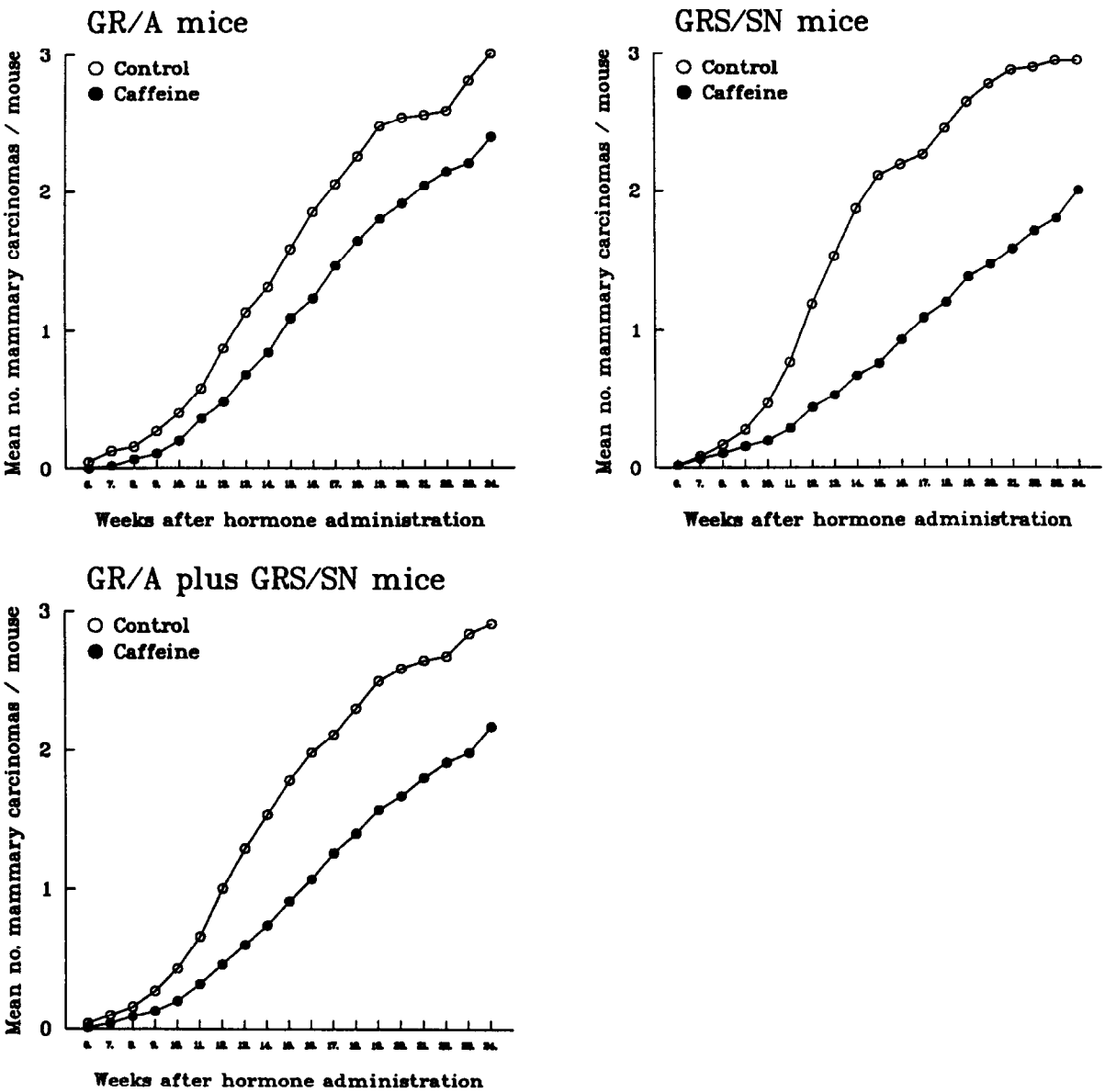

Fig. 1. Influence of caffeine on mammary tumor development in female GR mice treated with estrogen and progesterone. 17β-Estradiol (0.5 mg/l drinking water) and progesterone (monthly s.c. pellets) were administered to GR mice commencing at 8—10 weeks of age and continuously for 24 weeks (experiment termination). Caffeine (500 mg/l drinking water) was administered daily throughout the entire study, commencing 1 week after the onset of hormone treatment. GR/A mice, controls vs. caffeine, $P < 0.05$. GRS/SN mice, controls vs. caffeine, $P < 0.01$. GR/A + GRS/SN mice, controls vs. caffeine, $P < 0.01$.

tumor models. Indeed, we and others have shown that caffeine consumption can enhance the development of mammary tumors in C3H mice [12,13,21] and in carcinogen (DMBA) treated $BD2F_1$ mice [21]. The only consistent and profound inhibitory activity of caffeine in the development of mammary carcinomas in rodents, that we have observed to date, prior

to the present observations, is an inhibition of the initiation stage of DMBA-induced mammary gland tumorigenesis in female Sprague—Dawley rats [VanderPloeg, L.C. et al., unpublished data,19,20]. In the rat mammary tumor studies, we postulated that caffeine inhibits the initiation stage of this tumorigenic process by modifying carcinogen (DMBA) metabolism (VanderPloeg, L.C. et al., unpublished data).

How caffeine inhibits mammary tumor development in ovarian hormone treated GR mice is not known. The dose of caffeine used in these studies (500 mg/l of drinking water) is identical to the dose used in our C3H and $BD2F_1$ mouse studies; such a dose significantly stimulated mammary tumor development in these mouse strains. Caffeine did significantly decrease body weight gains in the GRS/SN mice which could account, at least in part, for the suppression of mammary tumor development. However, caffeine inhibited mammary tumor development in GR/A mice without a significant effect on body weight gains; such an observation would tend to rule out body weight gain reduction as an important factor in this inhibitory process. Caffeine, at considerably higher doses than that utilized in our study (doses that caused a significant reduction in body weight gains) has been reported by Petrek et al. [15] to suppress the development of mammary tumors in estrogen treated female ACI rats. Our results are consistent with the observation of Petrek et al. [15] with but one germane exception, i.e., we observed caffeine induced inhibition of hormone-induced mammary gland tumorigenesis in the absence of significant alterations in body weight gains (GR/A mice).

That caffeine can stimulate the developmental growth of alveolar mammary tumors in C3H mice [12,13,21] and ductal mammary tumors in carcinogen-treated $BD2F_1$ mice [21], yet inhibit the development of mammary tumors in hormone-treated GR mice (same dose levels of caffeine) is an interesting series of observations that may shed light on the mechanism by which caffeine influences mammary tumorigenic processes. In C3H mice and in carcinogen-treated $BD2F_1$ mice, mammary tumors arise from alveolar hyperplasias (hyperplastic alveolar nodules) and in ductal hyperplasias (hyperplastic ductal nodules), respectively [3—5,8,9,11,16]. In contrast, mammary tumors that arise in estrogen/progesterone-treated GR mice, often referred to as pregnancy responsive mammary tumors, arise from plaques [3—5,14,16]. The morphological distinction between nodules and plaques are that nodules develop from small alveolar or ductal foci while plaques develop from a substantial length of the duct [4,5]. Plaques are much larger than nodules and nodules do not grow into plaques. Thus, hyperplastic nodules (alveolar or ductal) and plaques are alternative stages in mammary neoplastic development, not consecutive ones [4,5]. The palpable mammary tumors that arise in hormone (estrogen/progesterone) treated GR mice are morphologically classified as a Type P carcinoma (large foci of irregular shaped tubules lined by a single layer of cuboidal cells) according to the well recognized and utilized classification system of Dunn [3,16]. In contrast, mammary tumors that occur spontaneously in C3H mice are classified as Type A (uniform acinar structure, consisting of cuboidal cells in single rows surrounding circular acini cavities) and those that arise in carcinogen treated $BD2F_1$ mice are classified as Type B (highly varied in morphology, single layer of cuboidal cells arranged in cords or tubules, often with papillary projections). That caffeine can influence the development of these types of mammary tumors, differentially, is a potentially important observation, one that will hopefully facilitate our understanding of the role of this drug in mammary tumorigenesis processes.

How caffeine might affect mammary tumor development and/or growth in rodents is at this time entirely speculative (reviewed in Ref. 22). Caffeine has been reported to alter phosphodiesterase activities, to affect anterior pituitary gland secretion, to alter insulin secretion, to affect CNS neurotransmitter activities, to modify serum fatty acid levels, to alter intracellular calcium transport and to block adenosine receptors. Any number of these physiological/biochemical events, singly or in

combination, could influence mammary tumor development and/or growth processes.

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