The Inhibitory Effect of Caffeine on Hormone-Induced Rat Breast Cancer

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Studies have associated coffee and/or caffeine with human fibrocystic breast disease. Two animal studies have implicated caffeine as a promoter in rat mammary cancer. The current investigation examines the effect of two caffeine doses in ACI rats with and without diethylstilbestrol (DES). Without DES, cancer did not develop in any of the rats receiving either of the two caffeine dosages. With DES, increasing caffeine dosage lengthened the time to first cancer, decreased the number of rats that developed cancers, and decreased the number of cancers overall. The presence or amount of caffeine did not cause detectable histologic differences in the breast cancers. The presence or amount of caffeine did not influence animal weight or mortality, although the rats without DES weighed more and survived better into old age. The presence or amount of caffeine did not influence pituitary weights and prolactin levels, although values of the DES groups were three times higher than the values for the group without DES ($P < 0.05$). In conclusion, chronic caffeine ingestion inhibits rat breast cancer, neither by interfering with the high prolactin levels—a necessary step in murine tumor development—nor by causing hypocaloric intake.

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WITH ITS ESTIMATED AVERAGE INTAKE of 240 mg per person per day, caffeine is one of the most frequently used drugs in this country. Epidemiologic and experimental studies have associated the intake of coffee and/or caffeine with cancers of the breast, pancreas, and lower urinary tract.[1,2,3] Given the properties of caffeine (1,3,7 trimethylxanthine) and other methylxanthines (MeX), such profound effects are possible. Three basic cellular actions of MeX[4] may explain the diverse effects: (1) those actions associated with translocation of intracellular calcium; (2) those mediated by increasing accumulation of cyclic nucleotides, particularly cyclic adenosine 3′,5′ monophosphate (cAMP); and (3) those mediated by blockage of adenosine receptors. However, caffeine's effect in putative carcinogenesis may be the result either of its direct binding to DNA[5] or of its ability to inhibit repair of chromosomal damage caused by other agents.[6]

At the same time, other studies show that caffeine inhibits cancer formation by delaying induction of chemical carcinogenesis of lung[7] and skin tumors[8] in mice. In a study with small numbers per treatment group, Minton *et al.* reported that caffeine delayed the appearance of first tumor compared with control patients, although the rats receiving both caffeine and unsaturated fat had a shorter interval to first tumor.[1] In another study, Welsch and associates found that caffeine had a significant promoting effect on tumor formation in the same strain of rat and with the same carcinogen.[9]

Also confusing is the association of caffeine with human breast cancer and with human fibrocystic disease, some forms of which are suspect as premalignant lesions. Elevated cAMP levels are present in human fibrocystic breast tissue as compared with control tissue and in human breast cancer as compared with fibrocystic disease.[10] Some reports show that women with fibrocystic disease consume more caffeine than women without fibrocystic disease[11]; other studies show an insignificant difference.[12,13] In one case-control study, caffeine consumption was associated with fibrocystic disease when defined specifically as microcysts and gross cysts found on biopsy slides.[14] Moreover, when women with fibrocystic disease rigorously decreased their ingestion of MeX, fibrocystic changes, according to one study, also decreased.[11] In another study there was no significant difference when women decreased MeX intake.[15]

The current study examined the effect of two caffeine doses on "normal" rats and on those given diethylstilbestrol (DES) for induction of breast cancer. The ACI (AxC, Irish) strain was chosen as the experimental animal

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because it has a high incidence of breast cancer in the presence of estrogens.[16]

Materials and Methods

One-month-old female ACI rats (Harlan/Sprague-Dawley, Inc., Indianapolis, IN) were maintained on Rat Chow (Ralston Purina Co., St. Louis, MO) and water *ad libitum*. One hundred thirty rats were used in the study. All animals were housed in the same room with a 12-hour light/dark cycle, six to a cage, and were cared for by the same technician. Unequal numbers of rats per group were assigned in order to place fewer rats in the control group and more in the treatment groups. At 4 months of age, the rats were randomized to six groups: (1) no caffeine/no DES (control group), 16 rats; (2) low-dose caffeine/no DES, 18 rats; (3) high-dose caffeine/no DES, 18 rats; (4) no caffeine/DES, 24 rats; (5) low-dose caffeine/DES, 24 rats; and (6) high-dose caffeine/DES, 30 rats. Each rat had been individually numbered by an ear-punch-hole system.

Caffeine

Caffeine (Eastman Kodak Co., Rochester, NY) was dissolved into drinking water at 1 mg/ml for low-dose and 2 mg/ml for high-dose amounts. Rats continued on the plain or caffeinated water regimen from 4 months of age until they were killed 10.5 months later. Regardless of the regimen, rats drank approximately 10 ml per day. The mean weight was 155 g ± 6.7 g at the start of the trials and 190 g at the end. The groups on low-dose caffeine received about 60 mg/kg per day, and the groups on high-dose caffeine received about 120 mg/kg per day.

DES and Inactive Pellets

A powder mixture of 5 mg diethylstilbestrol (Sigma Chemical Co., St. Louis, MO) and 15 mg cholesterol (Steraloids, Inc., St. Louis, MO) was compressed into pellets (3.5 mm in diameter × 3.0 mm) with a pellet press (Parr Instrument Co., Moline, IL). A powder mixture of 20 mg cholesterol was made into the same size pellets for the inactive pellet. According to a described technique,[16] a hormone pellet (25% DES, 75% cholesterol) or an inactive pellet (100% cholesterol) was implanted while the rat was under anesthesia. Implantation was subcutaneous at the dorsum of each rodent's neck at the age of 4 months.

General Protocol

At 4 months of age, the group was started on the specified amount of caffeine (0, 1, or 2 mg/ml). One week later—allowing time for acclimation before anesthesia—the DES or inactive pellet was implanted. Each rat was weighed monthly and palpated bimonthly for mammary tumors. Mammary tumors were identified using nipples as anatomic reference points and were surgically removed under light ether anesthesia when tumor diameter was approximately 1 cm. Rats that died before the end of the experiment underwent necropsy; pituitaries and any abnormal tissue were harvested, when possible, for histologic examination. All rats were killed 10.5 months after implantation (age 14.5 months) by decapitation; pituitaries were weighed immediately; pituitaries and abnormal tissue were preserved. Blood was collected for measurement of prolactin levels.

Pathology and Biochemistry

All mammary tumors and pituitaries were fixed, sectioned, stained with hematoxylin and eosin, and reviewed histologically. Blood prolactin levels were determined by the double antibody radioimmunoassay procedure[17] with reagents supplied by the Pituitary Hormone Distribution Program of NIADDK (National Institute of Arthritis, Diabetes, Digestive Diseases and Kidney). The standard used for rat prolactin was RP-1. All samples were run in the same assay and in duplicate.

Statistical Methods

The number of tumors, rat weights, and pituitary weights of the different groups were evaluated by the distribution free multiple comparison procedure based on the Kruskal-Wallis rank sums.[18] Differences in tumor-free survivals among the groups were analyzed by the Kaplan–Meier survival function and the generalized Wilcoxon test.[19]

Results

The zero-, low-, or high-dose caffeine/no DES groups had no breast tumors. The effect of caffeine dosage on DES-induced rat breast cancer was recorded by time to first cancer (Fig. 1), number of rats in which cancers developed (Fig. 2), and the total number of cancers overall (Table 1). Tumor formation in animals receiving DES was delayed to a greater degree as caffeine dose increased. In the no caffeine/DES group, there was a median time of 7.4 months after DES implantation to tumor development; in the low caffeine/DES group, 10.5 months; in the high caffeine/DES group, 87% were tumor-free at 10.5 months when the study ended, as shown in Figure 1. There were significant differences in the interval to tumor formation between the no and low caffeine groups (mean = 5.99; df = 1; $P = 0.014$) and between the low- and high-dose caffeine groups (mean = 5.78; df = 1; $P = 0.016$). The total number of cancers per group and the percentage of rats developing a cancer was lower (Table 1) in the low-dose caffeine and high-dose caffeine/DES group when compared with the no caffeine/DES group. The high-dose

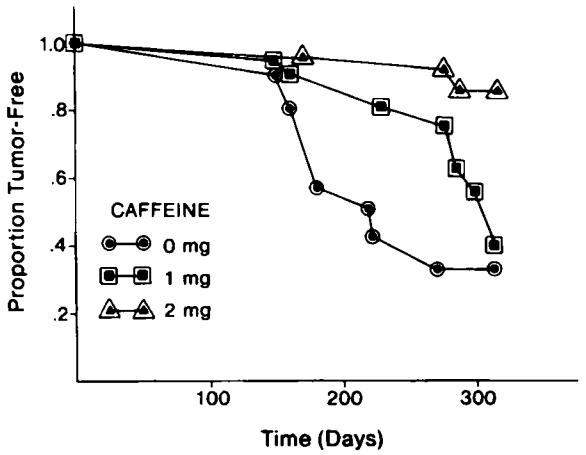

FIG. 1. The interval between date of DES implantation to first cancer among the three caffeine dosage groups (by the Kaplan-Meier survival function.)

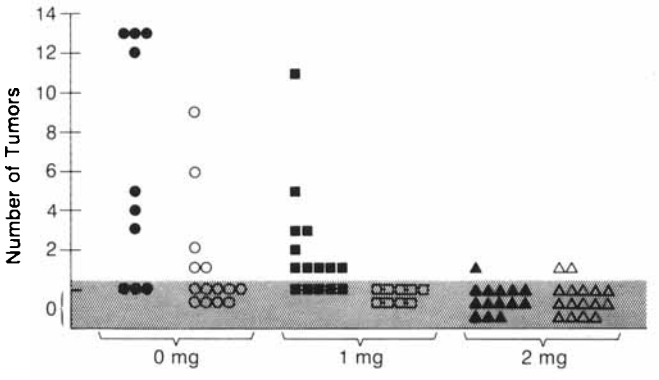

FIG. 2. Cancers developed and mortality by end of the study. The three groups shown all received DES and caffeine as indicated (circle, 0; square, 1 mg/ml; triangle, 2 mg/ml). Solid symbols represent those surviving in this aging colony at study's end.

caffeine/DES group had a significantly ($P < .05$) higher percentage of cancer-free rats (90%) than did the low-dose caffeine/DES group (58%) or the no caffeine/DES group (50%).

The presence or amount of caffeine did not change the histologic pattern of the breast tumors. At necropsy, gross inspection of abdominal and thoracic organs did not reveal any differences between rats treated with caffeine and those not treated with caffeine. Grossly normal mammary tissue was not reviewed histologically.

The presence or amount of caffeine did not affect the animal mortality rate. The groups treated with DES had a lower survival rate over the period of study. Specifically, one rat in each of the three groups (no, low, high caffeine) without DES died before the end of the 10.5-month study. In the DES groups, mortality was as follows: 14 of 24 in the no caffeine group, 9 of 24 in the low caffeine group, and 16 of 30 in the high caffeine group [mean = 2.30; df = 2; $P = 0.31$]. Because cancers were removed while the rats were under general anesthesia when the tumors were palpable at 1 cm, only rats in which breast cancer developed were exposed to the possibility of an anesthetic fatality. Thus, the greatest anesthetic risk occurred in the no caffeine/DES group, with 45 anesthetic exposures. There were 19 exposures in the low-dose caffeine/DES group and 3 exposures in the high-dose caffeine/DES group. Four of 24 rats in the highest tumor-producing group (no caffeine/DES) died while under anesthesia.

In the three groups without DES, the presence and amount of caffeine did not change body weight. For example, at sacrifice, the mean animal weight of the no caffeine/no DES group was 199.1 ± 9.2 g; of low caffeine/no, DES 215.1 ± 9.7 g. Likewise, in the three groups treated with DES, the presence and dosage of caffeine did not significantly change animal weight. The mean weight of the no caffeine/DES group was 179.2 ± 22.2 g; of the low caffeine/DES, 179.1 ± 25.4 g; of high caffeine/DES, 157.7 ± 22.9 g. The rats that received DES weighed significantly less ($P < 0.05$) than the animals that did not receive DES.

In the animals that did not receive DES, there was no significant effect on pituitary weight due to the presence or amount of caffeine. Likewise, in the animals that were given DES, there was no significant effect due to caffeine. The median pituitary weight at the end of the study of animals that did not receive DES was as follows: no caffeine, 13.9 mg (range, 10.5–70.9); low caffeine, 11.4 mg (range, 6.0–16.8); high caffeine, 12.3 (range, 3.7–17.4). The median pituitary weight of animals which received DES was: no caffeine, 107.3 (range, 33.1–222.2); low caffeine, 82.7 (range, 15.5–206.2); high caffeine, 81.0 (range, 13.9–327.3).

In animals that did not receive DES, the blood prolactin level was not altered significantly by the presence or amount of caffeine. Likewise, in animals that received

TABLE 1. Number of Rats with Various Numbers of Cancers

Treatment	No. of rats	Total cancers	No. of rats with n cancers		
			n = 0	n = 1	n ≧ 2
Cholesterol implant					
Water	16	0	16 (100%)	0	0
Low caffeine (1 mg/ml)	18	0	16 (100%)	0	0
High caffeine (2 mg/ml)	18	0	18 (100%)	0	0
DES implants					
Water	24	92	12 (50%)	2	10
Low caffeine (1 mg/ml)	24	29	14 (58%)	5	5
High caffeine (2 mg/ml)	30	3	27 (90%)	3	0

DES: diethylstilbestrol.

DES, the blood prolactin level showed no significant change due to caffeine. The median prolactin levels at the end of the study for animals that did not receive DES were as follows: no caffeine, 68 ng/ml (range, 32–2540); low-dose caffeine, 72 ng/ml (range, 34–5000); high-dose caffeine, 58 ng/ml (range, 32–174). The median prolactin levels for animals that received DES were: no caffeine, 2200 ng/ml (range, 1500–9100); low caffeine, 3450 ng/ml (range, 55–8600); high caffeine, 2200 ng/ml (range, 740–10,200).

Discussion

Reports concerning the association of breast cancer with caffeine in a murine model are confusing. Minton and co-workers[1] used a dimethylbenzanthracene (DMBA)-treated Sprague-Dawley rat model with caffeine administered by regular drinking water. Caffeine administration appears to have been started at the time of carcinogen administration and continued throughout the 9-month study. Minton's group analyzed only those rats in which at least one tumor developed and found that the time to first tumor was lengthened in the group that received caffeine alone, and was shortened in the group that received fat and caffeine. In addition, in each group, the number of rats in which at least one cancer developed did not appear to differ significantly, although statistical analysis was not performed: control group, 12/20; fat alone, 9/20; caffeine alone, 5/20; caffeine and fat, 11/20.

Welsch and associates[9] used the same rat model, carcinogen, and method of caffeine administration, but altered the caffeine interval to three periods: (1) before and during carcinogen administration; (2) immediately (3 days) after carcinogen administration for 20 weeks; and (3) starting 20 weeks after carcinogen administration. Contrary to Minton *et al.*, they found that caffeine had no effect on the latency period to first tumor development. The numbers of mammary cancers was slightly but significantly higher in rats that received caffeine only when the results for the three experiments were grouped together for statistical analysis: a method that may yield spurious results.

The current report used a different strain, the ACI rat, and a different carcinogen, DES, but the same method of caffeine administration in the drinking water supply. The caffeine dosage among the three studies was similar. A 10-mg dose per day in a 150-g rat, as occurs in the current study, is equivalent to a daily consumption of 3900 mg caffeine by a 60-kg woman on a body-weight basis. However, if one uses values corrected to metabolic body weight,[20] 10 mg of caffeine per rat is equivalent to 1700 mg of caffeine per 60-kg woman. The mean caffeine content of a cup of coffee is 100 mg.[21] If a woman were to drink eight cups of coffee and ingest diet pills, perhaps a few cold pills, and several caffeinated soft drinks, she could reach this level.

In contrast to the reports of Minton and colleagues and Welsch *et al.*, the current report shows that caffeine inhibited hormone-induced rat breast cancer in three ways: (1) by lengthening the time to first tumor; (2) by decreasing the number of rats in which a cancer developed; (3) by decreasing the total number of cancers (Figs. 1 and 2). It is unknown whether carcinogenesis by estrogens, as in the current study, or by DMBA, as in the other two studies, is more applicable to human breast cancer. Tumors formed by either method are usually hormone-dependent.[22,23]

The number of rats surviving at the end of the study was not noted in the reports by Minton *et al.* and Welsch and co-workers. Although there was a moderate number of deaths in our study, the presence or amount of caffeine did not decrease it further. The administration of DES is known to decrease survival. Moreover, the ACI rat is not a particularly hardy strain; that it often has a solitary kidney may affect its survival into relative aging (rats were 14.5 months old at the end of the study).

Reports have noted the effect of dietary fat and body fat on murine breast tumorigenesis,[24] and some authors have associated this with hormonal change. Body weights were unnoted in the Minton group's report and were mentioned for only part of the Welsch experiments. Contrary to expectation, neither the presence nor the amount of caffeine significantly changed body weight in the ACI rat, although the rats in the three caffeine groups (0, 1, 2 mg/ml) with DES weighed less than the rats in the comparable groups without DES.

An early step in carcinogenesis by hormones in the rat is the formation of a pituitary adenoma with resultant elevation of blood prolactin level. The presence or amount of caffeine did not significantly affect either pituitary weight or, the more accurate measurement, plasma prolactin levels. In the Welsch study, caffeine (250 or 500 mg/l) dosage did not significantly affect either mean pituitary weight or mean plasma prolactin level.

Therefore, caffeine appears to inhibit rat breast cancer neither by interfering with high prolactin levels nor by causing a hypocaloric intake. As noted earlier, a basic cellular reaction caused by MeX includes translocation of intracellular calcium. Caffeine has been reported to raise calcium levels in mammalian tissues,[25] whereas intracellular calcium levels have been reported to be elevated in tumor cells.[26]

However, caffeine ingestion could inhibit rat breast cancer by increasing levels of putative cell growth regulators such as cAMP. It has been known for almost a decade that the intracellular accumulation of cAMP, either endogenously generated or exogenously applied, inhibits the growth of several normal and transformed cells

in vivo and *in vitro*,[27] and that there is an inverse correlation between rate of cell growth and cAMP levels. In studies with rat (nonmalignant) mammary gland explants in culture, cAMP inhibits the increase of enzymes associated with lipogenesis and depresses DNA and RNA values and fatty acid synthesis.[28]

An increased cAMP value is present in DMBA-induced rat mammary tumors during regression. This is true whether regression is caused by ovariectomy,[29] tamoxifen administration,[30] dibutyryl cAMP treatment,[31] streptozotocin-induced diabetes,[29] or inhibition of prolactin secretion.[29] The last two findings were confirmed by another laboratory.[32] In addition, cholera toxin, which causes long-lasting activation of adenylate cyclase and thereby increases cAMP, inhibits growth of rat mammary tumors *in vivo* and human breast cancer cells (MCF-7) in culture.[33]

Caffeine and theophylline in therapeutic concentrations are known to inhibit phosphodiesterase—the enzyme responsible for cAMP degradation—by about 10%.[34] MeX are distributed throughout all body compartments.[4] Therefore, although cAMP levels were not measured in this study, it is possible that a constant elevation of cAMP was present and may be the factor that caused inhibition of hormone-induced breast cancers in the caffeine-treated animals.

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