Inhibition of 7,12-Dimethylbenz(a)anthracene- and N-Nitrosomethyurea-induced Rat Mammary Cancer by Dietary Flavonol Quercetin

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ABSTRACT

The effects of dietary supplementation of flavonol quercetin on both 7,12-dimethylbenz(a)anthracene (DMBA)- and N-nitrosomethylurea-induced mammary cancer in female Sprague-Dawley rats were determined. Quercetin diet was started 1 wk before intragastric instillation of DMBA (65 mg/kg of body weight) or i.v. injection of N-nitrosomethylurea (50 mg/kg of body weight) and was continued during the entire period (20 wk) of the experiment. Dietary quercetin inhibited both the incidence and the number of palpable rat mammary tumors; rats fed on 2% quercetin had 25% less incidence of mammary cancer, while the average number of mammary tumors per rat was reduced by 39% at 20 wk post-DMBA administration compared to animals on a control diet. In a separate experiment, a 5% quercetin diet elicited a greater inhibitory effect on the induction of rat mammary tumors by DMBA than was observed with a 2% quercetin diet. The inhibitory effect of quercetin on mammary tumor incidence in rats on 2% and 5% diets and on tumor multiplicity in animals on a 5% diet was statistically significant (P < 0.05). In addition, the risk of the development of a palpable tumor (as determined by the nonparametric estimate of the hazard function) in the quercetin-fed group was lower than the group on control diet throughout the course of the experiment. Furthermore, 5% dietary quercetin significantly inhibited (P < 0.05) tumors in both experiments. The impact of dietary quercetin on the induction of rat mammary tumors by DMBA is found in the edible portion of the majority of dietary plants (e.g., citrus, berries, leafy vegetables, tubers and bulbs, herbs and spices, legumes, cereal grains, tea, and cocoa) (1).

INTRODUCTION

The flavonoids constitute an integral part of the human diet. It has been estimated that an average dietary intake of flavonoids is approximately 1 g per day (1, 2). Flavonoids are widespread among food plants including vegetables and fruits, and the flavonol quercetin (3,3',4',5,7-pentahydroxyflavone) (Fig. 1) is found in the edible portion of the majority of dietary plants (e.g., citrus, berries, leafy vegetables, roots, tubers and bulbs, herbs and spices, legumes, cereal grains, tea, and cocoa) (1). A number of flavonols of edible plants are mutagenic and genotoxic in in vitro assays, but the results of their carcinogenicity in a number of experimental animal models are inconsistent (3). Among flavonoids, mutagenicity is largely confined to flavonols, and quercetin is one of the most potent mutagens in the Ames test (4–9). Quercetin is directly mutagenic to both TA98 and TA100 strains of Salmonella typhimurium. Flavonol glycosides (e.g., rutin) are nonmutagenic unless they are hydrolyzed to the free aglycone (quercetin) (5). The mutagenicity of the flavonols and flavonol glycosides appears to be very dependent upon the absence of excision repair capability, as well as the presence of the pkM101 plasmid which enhances error-prone repair of DNA lesions (3). Elimination of either or both of these factors results in virtually complete loss of mutagenic activity (3). Two independent investigations using Chinese hamster ovary cells (10) and V79 cells (11) concluded that quercetin did not induce forward mutations at the HGPRT, APRT, and Na+/K+-ATPase loci. Furthermore, quercetin caused a marginal, but not significant, increase in sister chromatid exchange (10, 11). However, quercetin did induce significant increases in TK locus mutation in both Chinese hamster ovary and V79 cells (10, 11). The results were not affected by the addition of an exogenous enzyme preparation (11).

Additionally, investigators from the same laboratory, Erturk et al. (13), reported in an abstract that quercetin fed to female Sprague-Dawley and Fischer 344 rats was also a hepatocarcinogen but, to the contrary, quercetin in a short-term test for genotoxicity did not induce DNA repair in rat hepatocytes (14). In several independent studies by others (5, 15–20), quercetin or rutin in the diet, as high as 10%, was observed to be inactive in eliciting tumors in rodents.

Accumulating evidence lends support to the facts that quercetin and certain related flavonoids may be inhibitors of carcinogenesis (21–25). Thus, flavonoids were found to inhibit metabolism of carcinogens in vitro in isolated liver microsomes (26–28). Furthermore, quercetin fed, at the 4% level, to C57BL/6J mice inhibited benzo(a)pyrene-induced nuclear damage in colonic epithelial cells (29). It is also noteworthy that a number of hydroxylated flavonoids including quercetin were found to inhibit the mutagenic activity of bay-region diol-epoxides of benzo(a)pyrene (30). If topically applied in conjunction with the tumor promoter TPA3 to mouse skin, certain flavonoids (quercetin) inhibited skin tumor formation (21, 22, 24). However, little is known about the effects of dietary flavonoids on chemically induced tumors in experimental animal models, which are relevant to determining the relationships between diet and carcinogenesis.

The present study was designed to evaluate the effect of dietary quercetin on the induction of rat mammary tumors induced by DMBA or NMU. Data indicating that dietary quercetin inhibits both DMBA- and NMU-induced rat mammary cancer are summarized in this paper.

MATERIALS AND METHODS

Chemicals. DMBA was purchased from Aldrich Chemical Co., Milwaukee, WI, and also from Kodak, Rochester, NY. NMU was purchased from Sigma Chemical Co., St. Louis, MO. Quercetin dihydrate was obtained from Aldrich.

Animals and Diets. Virgin female Sprague-Dawley rats, 45 to 50 days old, were purchased from Harlan Sprague-Dawley, Madison, WI. All rats were housed in standard colony cages with bedding in a lighted on/off cycle of 12:12 h. Rats were given Purina Laboratory Chow and tap water ad libitum.

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rats were fed powdered Wayne Lab-Blox commercial diet and were housed in light-, humidity-, and temperature-controlled rooms. Food and water were available ad libitum. The rats were kept in a normal rhythm of 12-h-light and 12-h-dark periods. Quercetin was blended into the powdered Wayne Lab diet and stored in sealed containers at 4°C. The nutrient concentration of the experimental diet was diluted by the addition of quercetin. We do not believe that this dilution is of sufficient concern; thus the unmedicated diet was undiluted.

Tumor Induction Experiments. Mammary tumors in female Sprague-Dawley rats were induced by a single intragastric administration of DMBA (65 mg/kg of body weight) in 0.7 to 1.0 ml of sesame oil (31). Rat mammary tumors were also induced by i.v. injection of NMU (50 mg/kg) (32). NMU, in Isopac vials containing 1 g of NMU and approximately 350 mg of a 5% solution of acetic acid as a stabilizer, was dissolved in 0.9% sodium chloride solution. The rats were started on quercetin diet a week before carcinogen administration. Fresh diet was added to the protected feeders once a week with feed weigh-back being recorded. The difference between the weight of the feed added during the experimental period and that of the feed removed provided close estimates of feed consumption. The rats were weighed weekly for the first 8 to 10 wk of the experiment and every other week thereafter. The rats were palpated at least biweekly in the experiment using a 2% quercetin diet and weekly in the two 5% quercetin experiments. The tumor induction experiments were terminated at 20 wk post-carcinogen administration. At the end of the experiment, all rats received a complete postmortem examination. Tumors were fixed in 10% buffered formalin, and sections were stained for histopathological examination. The significance of the difference in the tumor multiplicity data obtained from the control and the quercetin-fed rats was determined with a one-sided t test (33).

RESULTS

The effect of 2% dietary quercetin on DMBA-induced rat mammary tumors is illustrated in Figs. 2 to 4. In this experiment, the quercetin diet was started 1 wk before carcinogen administration. The quercetin diet significantly (P < 0.05) inhibited the induction of palpable rat mammary tumors at 7, 9, 13, 14, 16, and 20 wk post-DMBA treatment. The number of tumors per rat at 20 wk was 2.3 ± 0.5 and 1.4 ± 0.3 in control and quercetin diet groups, respectively. This represents a 30% reduction in tumor yield for those animals fed a quercetin diet (P < 0.03, t test, one-sided). The data also indicate that feeding 2% quercetin to untreated (not given DMBA) rats for 20 wk did not induce palpable mammary tumors (Fig. 2A).

The time to first palpable tumor is presented in Fig. 2B. An analysis of the data via the log-rank test (33) demonstrated a significant difference between rats fed a control and 2% quercetin diet (P = 0.0025). Furthermore, the quercetin group had a lower percentage of rats with tumor than the control group during the entire period of the experiment (Fig. 2B). The

average consumptions of food by rats on control (18.2 ± 2.4 g/rat/day) and quercetin (15.2 ± 0.8 g/rat/day) diets were significantly different (P < 0.05, t test). However, both groups exhibited similar gains in body weight (Fig. 3).

We further investigated the effect of 5% dietary quercetin on induction of rat mammary cancer. Five % quercetin in the diet

\[ \text{Fig. 1. Chemical structure of quercetin (3,3',4',5,7-pentahydroxyflavone, CAS 117-39-5).} \]
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Fig. 3. Effect of dietary quercetin (2%) on the gain in weight. The experiment is described in the legend of Fig. 2. Points, percentage of weight gain (mean of group at Wk a - mean of group at Wk 0/mean of group at Wk 0) x 100.

significantly ($P < 0.0005$, t test) decreased the number of palpable mammary tumors per rat by 48% at 20 wk post-DMBA administration (Fig. 4A). Furthermore, there was a greater inhibitory effect on the time to first palpable tumor for animals fed a 5% quercetin diet compared to a 2% diet (Fig. 4B). The average consumptions of food by rats on control (17.6 ± 0.3 g/rat/day) and 5% quercetin (18.4 ± 0.6 g/rat/day) diets were not significantly ($P > 0.1$, t test) different, and both groups also demonstrated similar gains in body weight.

Dietary quercetin at a dose of 5% also inhibited the induction of rat mammary tumors by NMU. The extent of inhibition was less than that observed in DMBA-induced tumor formation. An analysis of the time to first palpable tumor indicated a somewhat weaker but significant difference between rats on quercetin and control diets ($P < 0.05$, log-rank test), and in a similar manner to the DMBA experiments, the incidence was reduced throughout the entire length of the experiment (Fig. 5B). Furthermore, a comparison of total palpable tumors per rat revealed a significant difference between the control and quercetin-fed rats ($P < 0.05$, t test) at 11, 13, and 14 wk post-NMU injection (Fig. 5A). Again, the average consumptions by rats on control (20.1 ± 0.6 g/rat/day) and 5% quercetin-fed (19.2 ± 0.7 g/rat/day) diets were not significantly ($P > 0.1$, t test) different, and both groups also exhibited similar gains in weight.

DISCUSSION

Quercetin, a component of edible plants, is mutagenic in bacterial assay systems (3, 4); however, in the presence of a competent excision repair system, the mutagenic activity is virtually eliminated (3). Generally, quercetin does not induce specific locus mutations in mammalian cells, but if forward mutations were statistically significant, the biological significance is questionable (10, 11). The carcinogenicity of quercetin is controversial and as of yet unresolved (5, 12–20). Quercetin has been shown to inhibit mouse skin carcinogenesis (21). Now, we present that dietary quercetin inhibits both DMBA- and NMU-initiated rat mammary tumor development.

Dietary quercetin, at both 2% and 5% levels, inhibited the incidence (Figs. 2B, 4B, and 5B) and the multiplicity of palpable mammary tumors (Figs. 2A, 4A, and 5A). The diet consumed on a weekly basis per rat was essentially similar in all experiments, and also the gain in body weight was not affected by the consumption of quercetin in the diet (Fig. 3). These results allude to the fact that the inhibition of the induction of rat mammary tumors by quercetin is not attributable to reduced caloric intake (34). The effect of quercetin in the diet on mammary tumor formation initiated by DMBA was dose de-
The mechanisms involved in the inhibition of the initiation of rat mammary cancer by quercetin are unclear. Quercetin inhibits epidermal cytochrome P-450-dependent monoxygenases (35) and DMBA-DNA adduct formation in SENCAR mice (36). Quercetin has also been shown to inhibit rat and human liver microsome cytochrome P-450-dependent enzymes (27, 28). In the results presented, quercetin diets were started 1 wk prior to direct (NMU) or indirect (DMBA) carcinogen administration. The increased effectiveness of quercetin at inhibiting indirect carcinogen-initiated mammary tumor formation suggests that inhibition of metabolic activation or interaction of the metabolite with DNA could be a possible mechanism of action. Further experimentation would be required to evaluate these hypotheses.

The development of DMBA- and NMU-initiated tumors appears to be hormone dependent (37). There are also limited data on the effects of flavonoids on hormone regulation (38). It is likely that quercetin may be inhibiting the synthesis of the hormone or interfering with hormone-receptor binding. Quercetin could also be blocking some primary events stimulated by the hormone-receptor interaction. Quercetin does appear to inhibit tyrosine protein kinase activity (39, 40) and phosphoinositide phosphorylation (40), both of which have been implicated as necessary for normal mammary growth and development (39, 40).

In summary, we report for the first time that dietary quercetin inhibits the induction of rat mammary cancer induced by either intragastric instillation of DMBA or i.v. injection of NMU. Dietary quercetin inhibited the incidence and multiplicity of rat mammary cancer. In addition, we hypothesize that the natural plant product quercetin may be an inhibitor of cancer induction in other systemic animal models for tissues such as colon, lung, and intestine.

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