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Effects of the phytochemicals, curcumin and quercetin, upon azoxymethane-induced colon cancer and 7,12-dimethylbenz[*a*]anthracene-induced mammary cancer in rats

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Curcumin and quercetin were evaluated in rats for their ability to modulate the carcinogenic activity of azoxymethane (AOM) in the colon and 7,12-dimethylbenz[*a*]anthracene (DMBA) in the mammary gland. In the AOM-induced colon cancer model, male Fischer 344 rats at 8 weeks of age started to receive either curcumin (8 and 16 g/kg) or quercetin (16.8 and 33.6 g/kg) in the diet and 1 week later, were administered AOM (30 mg/kg body wt.) by subcutaneous injection. The animals continued to receive the two agents in the diet until sacrificed 45 weeks later. Curcumin mediated a dose-dependent inhibition of the incidence and multiplicity of adenomas from 47% and 0.58 ± 0.12 adenomas/rat in the AOM-treated control group to 19% and 0.22 ± 0.08 and 0.06% and 0.08 ± 0.06 adenomas/rat for the low and high dose groups, respectively. A low yield of adenocarcinomas (0.06 ± 0.04 adenocarcinomas/rat) was induced by AOM which was not significantly altered by curcumin. Treatment with quercetin caused a dose-dependent increase in the yield of AOM-induced tumors in the colon from 0.06 ± 0.04 adenocarcinoma/rat to 0.64 ± 0.12 and 1.14 ± 0.17 for the low and high dose groups, respectively. In the DMBA-induced mammary cancer model, curcumin or quercetin was administered at either 10 or 20 g/kg diet, beginning 7 days prior to DMBA and continually throughout the remainder of the experiment. Neither curcumin nor quercetin significantly altered the incidence of animals with tumors or the tumor multiplicity, while the high concentration of both agents significantly increased tumor latency. These results demonstrate different responses to these agents in the two models. While curcumin was highly effective as a chemopreventive agent in the colon model, it was only weakly effective in the mammary model. In contrast, quercetin which was also only weakly effective in the mammary model, caused a dose-dependent enhancement of tumors induced by AOM in the colon model.

Introduction

Colorectal and breast cancer are two of the most common causes of cancer related deaths in the United States of America. In 1993, there were 150 000 new cases of colon cancer and an estimated 52 000 deaths (1), and 183 000 new cases of breast cancer and an estimated 46 000 deaths. Recent surveys of chemoprevention documented a variety of phytochemicals that displayed activity in animals and may be candidates for chemopreventive studies in humans (2–4). The azoxymethane (AOM*)-induced colon and the 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced mammary cancer models in rats have been employed to identify substances with chemopreventive activity (5,6).

Curcumin and quercetin are two naturally occurring antioxidants that are constituents of the human diet. Curcumin, a plant phenolic with known antiinflammatory activity, is the major yellow pigment in turmeric and curry and is obtained from the plant *Curcum Zonga Linn-Curcume*. It has been shown in rats to prevent AOM-induced aberrant crypt foci, a putative precursor lesion in the colon (7) and in mice to prevent tumorigenesis induced by AOM in the colon and by *N*-ethyl *N*-nitrosoguanidine in the duodenum and forestomach (8). Quercetin is a plant bioflavonoid, found in most edible fruits and vegetables. Daily human consumption has been estimated to be ~25 mg including its glycoside, rutin (9,10). Quercetin has demonstrated chemopreventive activity in a variety of laboratory animal models, including tumorigenesis induced by AOM in the colon of mice (11) and by chemical carcinogens in the mammary gland of rats (12). Hence, curcumin and quercetin are found in the human diet without demonstrating excessive toxicity and appear to have chemopreventive activity supporting their further evaluation. The US National Cancer Institute has proposed models in laboratory animals for use in screening and evaluating potency of substances for chemopreventive activity (13,14). Two of these models in rats, i.e. AOM-induced colon cancer and DMBA-induced mammary gland cancer, were used in the studies reported here to further evaluate curcumin and quercetin for possible use as chemopreventive agents.

Materials and methods

AOM-induced colon tumors

Animals. Male Fischer 344, certified viral antibody-free rats were purchased from Sasco, Inc. at 6 weeks of age and maintained at the AAALAC-accredited laboratory of EHRT, Inc., in accordance with the Animal Welfare Act (Public Law 89–544, 94–279) and NIH Publication No. 86–23 revised 1985 entitled *Guide for the Care and Use of Laboratory Animals*. Upon arrival and periodically throughout the course of the study, serum was obtained from animals and determined to be serologically negative for *Mycoplasma pulmonic* and various viruses. The rats were housed up to three/cage in polycarbonate solid bottom shoebox cages with stainless steel wire-bar lids. The animals were fed AIN-76A modified diet (Harlan/Teklad, Madison, WS) containing: casein, 20%; DL-methionine, 0.3%; corn starch, 52%; dextrose, 13%; corn oil, 5%; cellulose fiber, 5%; AIN mineral mixture, 3.5%; AIN vitamin mixture, 1.0% and choline bitartrate, 0.2%. The diet and deionized NANOpure II-purified water were provided *ad libitum*.

AOM-induced colon tumor assay. When the rats were 8 weeks of age, the test agents were added to the diet at 0.4 and 0.8 of the maximum tolerated dose (MTD), i.e. 8.0 and 16 g/kg diet for curcumin and 16.8 and 33.6 g/kg

*Abbreviations: AOM, azoxymethane; DMBA, 7,12-dimethylbenz[*a*]-anthracene; MTD, maximum tolerated dose; NSAID, nonsteroidal anti-inflammatory drug.

diet for quercetin. Curcumin (97% purity) was supplied by the Division of Cancer Prevention and Control, NCI, Bethesda, MD. Quercetin dihydrate (99% purity) was purchased from Freeman Industries. The MTD for curcumin (20 gm/kg diet) was determined in a 6 week exposure study as the maximum dose that did not affect the body weight, feed consumption and organ to body weight ratios for the liver and kidney. The MTD for quercetin was estimated from the toxicity and chronic bioassays sponsored by the USA, National Toxicology Program of the USA, in which there was no toxicity other than a decrease in body weight, in the 40 g/kg diet group (15,16). Body weight and feed consumption were monitored throughout the study and the rats checked daily for signs of toxicity.

On day eight from the start of administering the test agents in the diet, the animals were administered by a subcutaneous injection either AOM (30 mg/kg body wt.; Sigma Chemical Co., St Louis, MO) or the saline vehicle (4 ml/kg body wt.). The animals continued to receive the test agents in the diet for an additional 45 weeks, at which time they were sacrificed by carbon dioxide asphyxiation. One day prior to sacrifice, feed was removed in an effort to reduce the fecal content of the colon. The entire intestinal tract was examined by palpation. The intestinal tract was then excised and cut along the longitudinal median axis and examined again for lesions. The number, size and location of all tumors/lesions were recorded. The lesions were excised, fixed in 10% phosphate neutral buffered formalin, embedded in paraffin and processed by routine hematoxylin and eosin staining prior to histopathological examination. Tumors induced by AOM, were also harvested and examined from the ear duct (zymbal gland) and preputial gland.

AOM-induced aberrant crypt assay. The assay contained the following four treatment groups of nine male rats each except for Group 4 which contained six rats. In Group 1, the rats were administered quercetin at a concentration of 30 g/kg in the diet and after eight and 15 days of exposure were administered 15 mg/kg AOM by subcutaneous injection. In Group 2, the rats were administered 15 mg/kg AOM on day 8 and 15 of the experiment. Group 3 contained rats administered quercetin at 30 g/kg diet starting at day zero of the experiment and Group 4 contained control animals. Quercetin was administered in the diet for 10 weeks and the animals then sacrificed by carbon dioxide asphyxiation. The procedure of Bird *et al.* (17,18) was used to stain and evaluate the colons for aberrant crypt foci. The colons were excised, cut open along the longitudinal axis, flushed with cold saline, and fixed in 0.1 M phosphate buffered-2% paraformaldehyde (pH 7.4, 4°C) for 2 h. The colons were stained in 0.1% methylene blue in Formalde-Fresh solution (10% formalin, Fisher Scientific, Pittsburgh, PA) for 10 min and then evaluated under a microscope at a magnification of 40 ×. Aberrant crypt foci were distinguished from surrounding non-involved crypts by their increased size, increased distance from luminal to basal surface of cells and enlarged pericryptal zone.

DMBA-induced mammary tumors

Chemicals. Curcumin was purchased from Pfaltz and Bauer Inc. (Westbury, CT), quercetin from Sigma Chemical Co., (St Louis, MO), and 7.12-dimethylbenz[*a*]anthracene from Aldrich Chemical Co., (Milwaukee, WI).

Animals. Female Sprague–Dawley rats were obtained from Harlan Sprague–Dawley Inc. (virus free colony number 202). The rats arrived at 34 days of age and were placed immediately on Teklad (4%) diet. Rats were housed in groups of 5 per cage in a room maintained at 22.2 ± 1°C and artificially lighted for 12 h per day. Animals were allowed free access to the diet and drinking water throughout the experiment.

DMBA-induced mammary tumor assay. Starting at 43 days of age, 25 rats per group were exposed continually to either quercetin or curcumin at 10 or 20 g/kg diet. Samples from the dietary formulations at Weeks 1 and 8 were determined to contain at least 92% of the nominal concentrations. Curcumin was extracted from the diet in acid-butanol according to the procedure of Wahlstrom and Blennow (19) and analyzed on a spectrophotofluorimeter with excitation at 435 nm and emission at 525 nm. Quercetin was extracted in 100% methanol and analyzed by HPLC (Hitachi model L6200) using a spherisorb ODS-2 3 micron column (150×4.6 mm) according to the procedure of Bankova *et al.* (20). The mobile phase was water:methanol:acetic acid (75:60:5) with a flow rate of 0.75 ml/min. The quercetin peak was monitored by absorbance at 371 nm and the retention time was 7.82 min.

One week after the start of treatment with the chemopreventive agents, the rats were given DMBA (12 mg) in sesame oil by gavage as previously described (6,21). Rats were weighed weekly, palpated for mammary tumors twice per week and checked daily for signs of toxicity. After 60 days of treatment, the effect of the chemopreventive agents on the estrus cycle of rats that did not receive DMBA, was evaluated for a 2 week period. The studies were terminated 100 days following DMBA administration. At sacrifice, tumors were removed, weighed, processed by routine hematoxylin and eosin staining, and examined histopathologically employing previously described criteria (22). Five animals from each treatment group, that received the sesame oil vehicle and the high concentration of a chemopreventive agent or the control diet, underwent a complete necropsy.

Statistical analysis. Statistical comparison of tumor multiplicity was determined employing the Armitage test (23), while the logrank test (24) was used to compare tumor incidence data. The use of these statistical procedures for this model has been previously described (25).

Results

AOM-induced colon tumors

The effect of curcumin and quercetin on the body weight of the animals is presented in Table I. Quercetin had limited effect on the body weight of the animals (<5%). In contrast, the high and low concentration of curcumin resulted in a 9.7% and 6.4% decrease in final body weight, respectively. Prior to 46 weeks of exposure, only the group that received the high concentration of curcumin demonstrated a statistically significant reduction in the body weight.

Curcumin at both dose levels reduced the incidence and multiplicity of adenomas observed in AOM-treated rats (Table II). The low yield of adenocarcinomas was not significantly altered by curcumin. The incidence of animals with tumors (adenomas plus adenocarcinomas) was decreased significantly ($P \leq 0.05$) from 53% in the control diet group to 36 and 23% and the multiplicity decreased from 0.64 ± 0.10 in controls to 0.39 ± 0.07 and 0.27 ± 0.07 tumors/animal, by the low and high concentrations of curcumin, respectively. Neither dose of curcumin significantly affected the incidence or multiplicity of tumors in the small intestine (Table III). AOM also induced tumors in the preputial and zymbal glands. The incidence of tumors in these sebaceous glands was <20% in all treatment groups that received AOM and was not significantly affected by curcumin.

Quercetin did not alter the incidence or multiplicity of AOM-induced adenomas in the colon, but did increase significantly in a dose-dependent manner, the incidence and multiplicity of adenocarcinomas (Table II). For example, multiplicity was increased from 0.06 ± 0.04 in control animals to 0.64 ± 0.12 and 1.14 ± 0.17 adenocarcinomas/rat in the groups fed the low and high concentrations of quercetin, respectively. In contrast to its strong enhancing effect in the colon, quercetin did not alter the incidence or multiplicity of benign and/or malignant tumors in the small intestine (Table III) or in the preputial and zymbal glands.

The yield of aberrant crypt foci was not altered by administering quercetin in the diet starting 1 week prior to the first of two doses of AOM. Nine weeks after administering the first dose of AOM (10 weeks of treatment with quercetin), the yield of aberrant crypt foci/colon were 145.0 ± 16.1 and 166.6 ± 18.1 (mean ± SE) in the absence and presence of quercetin (30 g/kg diet), respectively. The increase in aberrant crypt foci in the presence of quercetin was not statistically significant (P-value > 0.05). Thus, quercetin when administered in the diet at a concentration similar to the tumorigenesis study, did not significantly alter the response of aberrant crypt foci. No aberrant crypt foci were detected in the two treatment groups that did not receive AOM irrespective of exposure to quercetin.

DMBA-induced mammary tumors

None of the dose levels of curcumin or quercetin significantly altered weight gain in female Sprague–Dawley rats. The final

Table I. Effect of curcumin and quercetin upon body weight

Test agent–Dose level (g/kg diet)	AOM[a]	Body weight (g)				
		Week 1	Week 3	Week 26	Week 42	Week 46
Control diet	−	165.54 ± 1.78 (12)[b]	219.39 ± 2.76 (12)[c]	427.00 ± 6.17 (12)[c]	479.91 ± 7.35 (12)[c]	492.16 ± 7.86 (12)[c]
Control diet	+	165.08 ± 1.06 (36)	197.68 ± 1.31 (36)	402.34 ± 3.65 (35)	446.70 ± 4.04 (32)	456.74 ± 5.12 (32)
Curcumin 8	+	165.00 ± 1.13 (36)	199.50 ± 1.16 (36)	388.34 ± 3.16 (36)	419.28 ± 6.15 (32)	427.46 ± 5.61 (30)[c]
Curcumin 16	+	164.34 ± 1.10 (36)	193.83 ± 1.28 (36)	373.38 ± 4.04 (35)[c]	407.59 ± 5.56 (31)	412.51 ± 6.59 (31)[c]
Curcumin 16	−	164.26 ± 1.17 (12)	218.59 ± 1.93 (12)	387.65 ± 4.88 (12)[d]	433.62 ± 6.50 (12)[d]	443.66 ± 5.84 (12)[d]
Quercetin 16.8	+	164.26 ± 1.08 (36)	190.50 ± 1.38 (36)	406.72 ± 3.67 (36)	445.04 ± 4.25 (33)	449.44 ± 4.80 (31)
Quercetin 33.6	+	164.26 ± 1.02 (36)	187.77 ± 1.42 (36)[c]	398.98 ± 4.60 (36)	434.86 ± 5.85 (33)	434.28 ± 7.06 (31)
Quercetin 33.6	−	163.22 ± 2.31 (12)	215.31 ± 2.75 (12)	414.53 ± 9.98 (11)	461.13 ± 10.75 (11)	473.40 ± 10.67 (11)

[a]AOM, azoxymethane.
[b]Mean ± SE for the number of animals indicated in the parentheses.
[c]Significantly different ($P < 0.01$) from AOM + control diet.
[d]Significantly different ($P < 0.01$) from vehicle + control diet when comparing animals not administered AOM.

Table II. Effect of curcumin and quercetin upon AOM-induced colon tumors

Treatment	Dose g/kg diet	AOM[a]	N	Adenomas		Adenocarcinomas	
				Incidence (%)	ADEN/Rat	Incidence (%)	CA/Rat
Control diet	0	−	12	0 (00)[b]	0.00	0 (00)	0.00
Control diet	0	+	36	17 (47)	0.58 ± 0.12[c]	2 (06)	0.06 ± 0.04
Curcumin	8	+	36	7 (19)*[d]	0.22 ± 0.08*	6 (17)	0.17 ± 0.06
Curcumin	16	+	36	2 (06)**	0.08 ± 0.06**	6 (17)	0.19 ± 0.08
Curcumin	16	−	12	0 (00)	0.00	0 (00)	0.00
Quercetin	16.8	+	36	16 (44)	0.53 ± 0.11	18 (50)**	0.64 ± 0.12**
Quercetin	33.6	+	36	15 (42)	0.53 ± 0.12	24 (67)**	1.14 ± 0.17**
Quercetin	33.6	−	12	0 (00)	0.00	0 (00)	0.00

[a]AOM, azoxymethane; N, number of animals; ADEN, adenomas; CA, adenocarcinomas.
[b]Number of animals with tumors and the percentage of animals with tumors in parenthesis.
[c]Results are mean ± SE.
[d]Significantly different from AOM + control diet by Mann-Whitney *U* test for incidence and by ANOVA and Student *t*-test for tumors/rat data with $*P < 0.05$ and $**P < 0.01$.

Table III. Effect of curcumin and quercetin upon AOM-induced small intestinal tumors

Treatment	Dose g/kg diet	AOM[a]	N	Adenomas		Adenocarcinomas	
				Incidence (%)	ADEN/Rat	Incidence (%)	CA/Rat
Control diet	0	−	12	0 (00)[b]	0.00	0 (00)	0.00
Control diet	0	+	36	4 (11)	0.11 ± 0.05[c]	7 (19)	0.19 ± 0.07
Curcumin	8	+	36	4 (11)	0.11 ± 0.05	8 (22)	0.22 ± 0.07
Curcumin	16	+	36	1 (03)	0.03 ± 0.03	12 (33)	0.33 ± 0.08
Curcumin	16	−	12	0 (00)	0.00	0 (00)	0.00
Quercetin	16.8	+	36	3 (08)	0.11 ± 0.07	8 (22)	0.22 ± 0.07
Quercetin	33.6	+	36	0 (00)	0.00	8 (22)	0.25 ± 0.08
Quercetin	33.6	−	12	0 (00)	0.00	1 (03)	0.08 ± 0.05

[a]AOM, azoxymethane; N, number of animals; ADEN, adenomas; CA, adenocarcinomas.
[b]Number of animals with tumors and the percentage of animals with tumors in parenthesis.
[c]Results are mean ± SE.

mean body weights of the DMBA-controls and of the treatment groups that received DMBA plus a chemopreventive agent differed by no more than 4% (data not shown). Neither curcumin nor quercetin altered the estrus cycle of the rats. Histologic evaluation of animals that received the vehicle of sesame oil and the high concentration of a chemopreventive agent or the control diet, did not reveal any signs of toxicity.

Treatment with DMBA resulted in an incidence of 88% of the animals with mammary tumors and a tumor multiplicity of 4.2 tumors/rat (Table IV; Figure 1A and B). The high concentration of curcumin nonsignificantly decreased the tumor incidence to 76% and multiplicity to 3.2, while significantly increasing the tumor latency as determined by logrank analysis ($P < 0.05$). The low concentration of curcumin did not alter tumor incidence, multiplicity, or latency.

Both dose levels of quercetin nonsignificantly decreased the incidence of animals with mammary tumors from 100% to ~90% (Table IV and Figure 2A and B). The low and high

Table IV. Evaluation of quercetin and curcumin in the prevention of DMBA-induced mammary tumors

Group	No. of rats	Carcinogen[a]	Treatment[b]	Adenocarcinoma[c]		Benign tumors[c]	
				Percent Incidence	Multiplicity No./Rat	Percent Incidence	Multiplicity No./Rat
Experiment I							
1	23	DMBA	Quercetin, 20 g/kg diet	91	2.9	4	0.04
2	25	DMBA	Quercetin, 10 g/kg diet	88	3.4	0	0.00
3	25	DMBA	None	100	4.2	16	0.16
Experiment II							
1	25	DMBA	Curcumin, 20 g/kg diet	76	3.0	8	0.08
2	25	DMBA	Curcumin, 10 g/kg diet	88	4.4	12	0.12
3	25	DMBA	None	88	4.1	0	0.00

[a]DMBA (12 mg) was administered by gavage at 50 days of age.
[b]The administration of curcumin or quercetin was initiated at 43 days of age and continued until the end of the study. Basic diet was Teklad (4%).
[c]Adenomocarcinoma or benign tumor response observed in the mammary gland at 100 days after DMBA administration. The percent incidence of animals with the lesion were compared by the Logrank test and the multiplicity of lesions by the Armitage test. The responses in Groups 1 and 2 were not significantly different from Group 3 (P-value < 0.05).

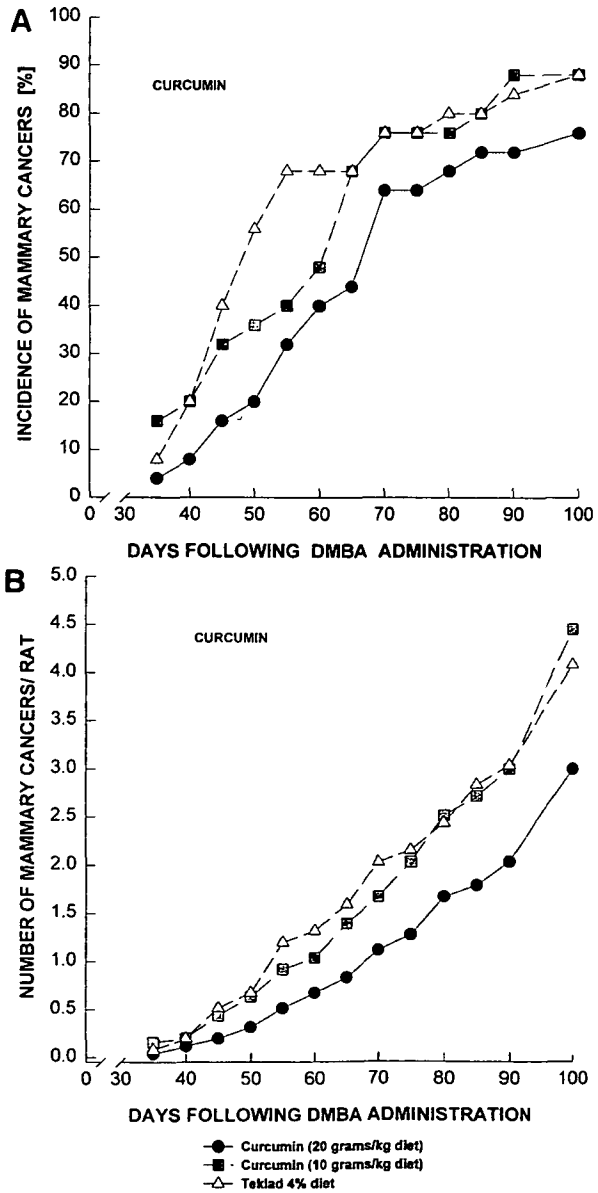

Fig. 1. Effect of curcumin on DMBA-induced mammary tumor (A) incidence; ● 20, g/kg diet; ■, 10 g/kg diet; ▲, Teklad 4% diet. (B) Multiplicity; ● 20, g/kg diet; ■, 10 g/kg diet; ▲, Teklad 4% diet.

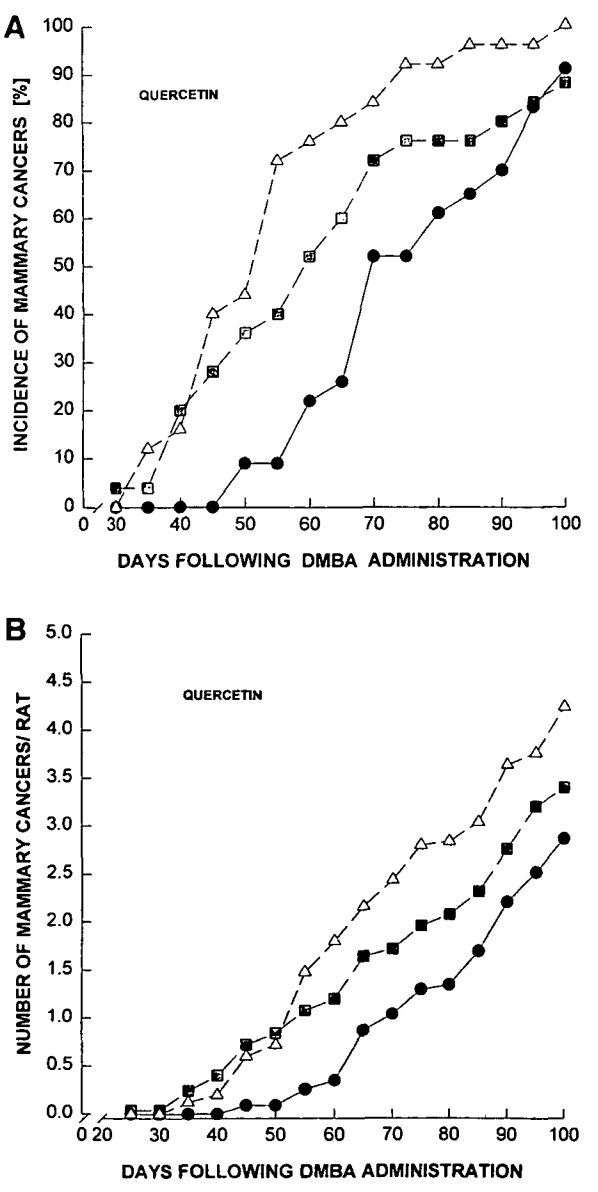

Fig. 2. Effect of quercetin on DMBA-induced mammary tumor (A) incidence; ● 20, g/kg diet; ■, 10 g/kg diet; ▲, Teklad 4% diet. (B) Multiplicity; ● 20, g/kg diet; ■, 10 g/kg diet; ▲, Teklad 4% diet.

concentration of quercetin decreased, albeit not significant by Armitage analysis, tumor multiplicity by 20 and 33%, respectively. In contrast, the higher concentration of quercetin significantly increased tumor latency employing logrank analysis ($P < 0.01$).

Discussion

Although the two phytochemicals examined have antioxidant activity, they have relatively broad and some non-overlapping biological effects which might explain their differing effects on chemical carcinogenesis. Curcumin, in addition to its antioxidant (26) and anti-mutagenic (27) effects, has demonstrated the ability to inhibit prostaglandin synthesis (28). In view of the demonstrated chemopreventive efficacy of various prostaglandin synthesis inhibitors e.g. non-steroidal anti-inflammatory drugs (NSAIDs), in the AOM-induced colon cancer model (29,30), the activity of curcumin could likely to be related to this effect. The chemopreventive activity demonstrated by curcumin is also consistent with its reported ability to prevent AOM-induced aberrant crypt foci in the colon of rats and colon tumorigenesis in mice (7,8). During the course of writing this paper, a recent publication by Reddy *et al.* reported that curcumin prevented AOM-induced colon cancer in rats (31). Their protocol was similar to ours and consisted of administering curcumin at 2 g/kg of diet starting 1 week prior to a total of two subcutaneous injections of 15 mg/kg AOM each administered weekly. The animals were sacrificed 52 weeks later. Curcumin decreased the incidence and multiplicity of adenocarcinomas. The longer duration (52 weeks) and treatment with two doses of 15 mg/kg AOM each of their study compared to our study of 45 weeks and a single 30 mg/kg AOM, probably explains why they observed adenocarcinomas while we observed adenomas. In any case, both studies demonstrated that curcumin prevented the induction of colon tumors by AOM. In mice, curcumin (5–40 g/kg diet) has also been reported to prevent colon cancer which was induced by AOM (8). Although, curcumin and various NSAIDs are effective inhibitors of colonic prostaglandin synthesis and of AOM-induced colon carcinogenesis, they typically as reported here for curcumin, have more limited effects on AOM-induced tumors of the small intestine, preputial gland and zymbal gland.

Curcumin was evaluated in the DMBA-induced rat mammary tumor model for chemopreventive activity because previous studies have shown that it (i) was effective in another DMBA-induced tumorigenesis model, i.e. mouse skin tumorigenesis (32) and (ii) had demonstrable anti-mutagenic properties (27). In contrast to its chemopreventive activity in the colon tumor model, curcumin at similar dose levels was only weakly effective in preventing DMBA-induced mammary tumors in rats. In this model, curcumin did not significantly alter the incidence of animals with DMBA-induced tumors or the multiplicity of tumors. However, the high but not the low, concentration of curcumin did significantly increase tumor latency.

The co-administering of quercetin with AOM and continuing until sacrifice of the animals, resulted in a very significant dose-dependent increase in the yield of adenocarcinomas in the colon. Furthermore, quercetin increased at an earlier time, albeit not statistically significant, the yield of AOM-induced precancerous lesions, i.e. aberrant crypt foci in the colon. The increased carcinogenic response to AOM by the co-administering of quercetin suggests co-carcinogenic activity for quercetin. The term co-carcinogen is used to describe an agent which is not carcinogenic by itself but increases the incidence of animals with tumors, tumor multiplicity and/or the stage of tumor progression (precancerous lesions to adenoma to adenocarcinoma), when administered concomitantly with a carcinogen, AOM. Hence, quercetin decreased the duration required for the occurrence of adenocarcinomas, so that they occurred at a time after AOM, i.e. 45 weeks, when adenomas were present in animals that did not receive quercetin.

The co-carcinogenic activity of quercetin in the AOM-induced colon tumor model in rats was unexpected. In chronic carcinogenicity bioassays including the 2 year study sponsored by the US National Toxicology Program, there had been no evidence of neoplasms related to the administration of quercetin (15,16,33,34). Quercetin also exhibited no indication of carcinogenic activity in short-term carcinogenesis bioassays including the strain A mouse bioassay (34) and the initiation-promotion bioassay in rat liver that used the preneoplastic lesion, glutathione S-transferase π-positive foci to indicate activity (36). Although not exhibiting carcinogenic activity, quercetin has been demonstrated *in vitro* to be one of the strongest mutagenic bioflavonoids (37,38). The genotoxic activity of quercetin *in vitro* includes mutagenicity in the Ames *Salmonella typhimurium* assay (38) and induction of chromosomal aberrations and sister chromatid exchange in cell culture (39).

The co-carcinogenic activity of quercetin, which we observed is especially surprising for the following reasons. First, quercetin demonstrates a number of biologic properties, e.g. inhibition of benzo[*a*]pyrene binding to DNA (40), antioxidant activity (41) and the ability to inhibit arachidonic acid metabolism (42), which are typically associated with chemoprevention. Second quercetin has been shown to be an effective chemopreventive agent in various rodent models including (i) carcinogen-induced mammary carcinogenesis in rats (12,43); (ii) 12-*O*-tetradecanoylphorbol-13-acetate-promotion of tumors on mouse skin (44) and perhaps most germanely (iii) AOM-induced colon tumors in mice (11). Furthermore, in cell culture, quercetin has been shown to inhibit the growth and proliferation of tumor cells derived from the colon (45). Despite these previous examples of chemoprevention by quercetin, co-carcinogenic activity was observed in the present study. Moreover, co-carcinogenic activity of quercetin has been reported previously by Zhu and Leihr (46). They reported that quercetin increased the yield of renal tumors, when administered concomitantly with estrogen in hamsters and proposed that the co-carcinogenic mechanism was an increase in the yield of the 4-OH estradiol metabolite of estradiol, which has been shown to react with DNA.

In contrast to its clear co-carcinogenic activity in the rat colon, quercetin at the highest concentration employed exhibited evidence for prevention of DMBA-induced mammary tumors. This was demonstrated by a significant increase in tumor latency and a decrease in tumor multiplicity by 32% ($P < 0.1$). These results are in agreement with the findings of Verma *et al.* showing that similar high dose levels of quercetin displayed chemopreventive activity in both the MNU-induced and DMBA-induced rat mammary tumor models (12). One might have expected even more striking results with quercetin, since it appears to be a relatively strong inhibitor of metabolic activation of a variety of carcinogens (40). Additionally there is evidence that quercetin may enhance the efflux of planar

carcinogens, such as DMBA from cells in culture (47). Nevertheless, the observed effects were relatively modest when compared with levels of inhibition of DMBA-induced mammary tumorigenesis obtained with other phytochemicals e.g. 3-indole carbinol (19).

The results reported here, demonstrate that potential chemopreventive agents can exhibit significant target organ specificity and range of activity. Thus, curcumin is an effective agent against AOM-induced colon tumorigenesis but minimally, if at all, effective against AOM-induced tumors of the small intestine, preputial gland or zymbal gland or against DMBA-induced mammary tumors. The presence of curcumin in turmeric and curry of some human diets, would suggest that the chronic exposure required for chemoprevention would not be unduly toxic. The only toxic effects of chronic exposure in humans has been respiratory symptoms and allergic dermatitis in spice factory workers (48). Therefore, curcumin would appear to warrant further evaluation for chemoprevention of colon cancer including clinical evaluation. On the other hand, our data suggest that quercetin is a strong co-carcinogen with respect to AOM-induced colon cancer in rats. Therefore, until this activity is better understood, its further evaluation as a chemopreventive agent should proceed with caution.

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