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Chemopreventive Effect of Curcumin, a Naturally Occurring Anti-Inflammatory Agent, during the Promotion/Progression Stages of Colon Cancer[1]

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ABSTRACT

Curcumin, derived from the rhizome of *Curcuma longa* L. and having both antioxidant and anti-inflammatory properties, inhibits chemically induced carcinogenesis in the skin, forestomach, and colon when it is administered during initiation and/or postinitiation stages. This study was designed to investigate the chemopreventive action of curcumin when it is administered (late in the premalignant stage) during the promotion/progression stage of colon carcinogenesis in male F344 rats. We also studied the modulating effect of this agent on apoptosis in the tumors. At 5 weeks of age, groups of male F344 rats were fed a control diet containing no curcumin and an experimental AIN-76A diet with 0.2% synthetically derived curcumin (purity, 99.9%). At 7 and 8 weeks of age, rats intended for carcinogen treatment were given s.c. injections of azoxymethane (AOM) at a dose rate of 15 mg/kg body weight per week. Animals destined for the promotion/progression study received the AIN-76A control diet for 14 weeks after the second AOM treatment and were then switched to diets containing 0.2 and 0.6% curcumin. Premalignant lesions in the colon would have developed by week 14 following AOM treatment. They continued to receive their respective diets until 52 weeks after carcinogen treatment and were then sacrificed. The results confirmed our earlier study in that administration of 0.2% curcumin during both the initiation and postinitiation periods significantly inhibited colon tumorigenesis. In addition, administration of 0.2% and of 0.6% of the synthetic curcumin in the diet during the promotion/progression stage significantly suppressed the incidence and multiplicity of noninvasive adenocarcinomas and also strongly inhibited the multiplicity of invasive adenocarcinomas of the colon. The inhibition of adenocarcinomas of the colon was, in fact, dose dependent. Administration of curcumin to the rats during the initiation and postinitiation stages and throughout the promotion/progression stage increased apoptosis in the colon tumors as compared to colon tumors in the groups receiving AOM and the control diet. Thus, chemopreventive activity of curcumin is observed when it is administered prior to, during, and after carcinogen treatment as well as when it is given only during the promotion/progression phase (starting late in premalignant stage) of colon carcinogenesis.

INTRODUCTION

Colorectal cancer, one of the leading causes of cancer deaths in both men and women in the United States, accounts for ~56,000 deaths annually (1). Although several epidemiological and laboratory studies suggest a relationship between large bowel cancer risk and dietary factors (2–4), there is increasing evidence that a high consumption of fruits and vegetables and intake of certain nonnutrients that are present in foods reduce the risk of colon carcinogenesis (5). Although risk reduction by nutritional intervention may not be sufficient to protect high-risk individuals against colon cancer development, an alternative or complementary effective approach for secondary prevention has been to identify the agents with chemopreventive potency and to evaluate them in high-risk individuals in combination with nutritional intervention (6–8).

It is noteworthy that the use of medicinal plants or their crude extracts in the prevention and/or treatment of several chronic diseases has been traditionally practiced in various different ethnic societies worldwide. Turmeric, the powdered rhizome of *Curcuma longa* L., has been used to treat a variety of inflammatory conditions and chronic diseases (9, 10); it is also used as coloring and flavoring additive to foods. Curcumin [Fig. 1; diferuloylmethane; 1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], which has been identified as the major pigment in turmeric, possesses both anti-inflammatory (11–13) and antioxidant properties (14, 15). It has been demonstrated that topical application of curcumin inhibits benzo(*a*)pyrene-induced DNA adduct formation, and development of skin tumors as well as TPA[3]-induced epidermal DNA synthesis and tumor promotion in mouse skin (16–18). Curcumin has a strong inhibitory effect on cell proliferation in the HT-29 and HCT-15 human colon cancer cell lines (19). Importantly, dietary administration of curcumin during initiation and/or postinitiation periods significantly suppresses development of chemically induced forestomach, duodenal, and colon tumors in CF-1 mice (20); it also reduces formation of focal areas of dysplasia and aberrant crypt foci in the colon that are early preneoplastic lesions in rodents (21, 22). Pereira *et al.* (23) have reported that administration of 0.8 and 1.6% curcumin continuously during the initiation and postinitiation phases significantly inhibited development of AOM-induced colonic adenomas in rats. We have shown that continuous dietary administration of 0.2% curcumin during the initiation and postinitiation stages significantly inhibited the incidence and multiplicity of AOM-induced colon adenocarcinomas and the tumor burden in F344 rats (24). Although all of the above studies clearly demonstrate the potential chemopreventive activity of curcumin during the initiation and postinitiation periods of colon carcinogenesis, there were no studies on the efficacy of this agent during the promotion/progression stage when the premalignant lesions would have developed. We deemed it important to show that curcumin treatment can be delayed after the carcinogen administration in experimental carcinogenesis and still be effective, so as to provide baseline knowledge for possible clinical use of this agent in secondary prevention of colon cancer in high-risk individuals, such as patients with colonic polyps.

Curcumin was shown to inhibit colon carcinogenesis during the postinitiation stage through the modulation of COX activity in the tumor tissue (24). COXs are involved in the synthesis of PGs, which have been shown to affect tumor growth (24), suggesting that effects on the arachidonic acid cascade by curcumin may play a role in its tumor-inhibitory activity. We and others have shown previously that several inhibitors of PG synthesis, such as aspirin, ibuprofen, sulindac, and piroxicam suppress colon carcinogenesis in laboratory animal model assays (25–28). Inhibition of colon carcinogenesis was consistently associated with a decrease in the activity of COX in colon

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[3] The abbreviations used are: TPA, 12-*O*-tetradecanoylphorbol-13-acetate; AOM, azoxymethane; COX, cyclooxygenase; PG, prostaglandin; NSAID, nonsteroidal anti-inflammatory drug; LOX, lipoxygenase; HETE, hydroxyeicosatetraenoic acid.

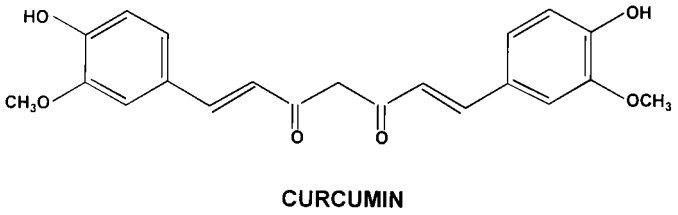

Fig. 1. Chemical structure of curcumin [1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione].

Table 1 *Percentage composition of experimental semipurified diets*

Ingredients	% composition	
	Control diet[a]	Experimental diets
Casein	2.0	20.0
DL-Methionine	0.3	0.3
Cornstarch	52.0	51.8 or 51.4
Dextrose	13.0	13.0
Corn oil	5.0	5.0
Alphacel	5.0	5.0
Mineral mix, AIN	3.5	3.5
Vitamin mix, AIN revised	1.0	1.0
Choline bitartrate	0.2	0.2
Curcumin[b]	0	0.2 or 0.6

[a] Adopted from the AIN reference diet (AIN-76A), with modification of the source of carbohydrate.
[b] Curcumin was added to the diets instead of cornstarch.

tumors (25, 26, 28). Evidence also suggests that curcumin acts on pathways that may inhibit cell proliferation (19) and enhance apoptosis (29). *In vitro* studies by Hanif *et al.* (19) suggest that curcumin inhibits colon cancer cell proliferation, independent of its ability to inhibit PG synthesis. Furthermore, transformation of colorectal epithelium into adenomas and adenocarcinomas has been shown to be associated with progressive inhibition of apoptosis, suggesting that inhibition of apoptosis in colon carcinogenesis may contribute to tumor growth and promote neoplastic progression (30).

This study was designed to specifically investigate the chemopreventive efficacy and dose-response effect of curcumin when it is administered late in the premalignant stage, representing the promotion/progression phase of colon carcinogenesis in F344 rats. In addition, the effect of dietary curcumin on apoptosis in colon tumors was determined.

MATERIALS AND METHODS

Animals, Diets, and Carcinogen. Weanling male F344 rats were received from Charles River Breeding Laboratories (Kingston, NY). AOM was purchased from Ash Stevens (Detroit, MI). Synthetically derived curcumin (purity >99.9% diferuloylmethane) was kindly provided by Gene Print, Inc. Bala Cynwyd, PA as part of the National Cancer Institute's project for investigational studies of this compound. All ingredients for the semipurified diet were purchased from Dyets, Inc (Bethlehem, PA). The experimental diets were prepared weekly in our laboratory by adding curcumin at 0.2 and 0.6% levels instead of cornstarch (Table 1). The experimental and control diets were stored in a cold room.

Efficacy Study. The experimental protocols followed those detailed in our previous publications (27). Briefly, weanling male F344 rats were quarantined for 7 days and had access to modified AIN-76A control diet (Table 1). Following quarantine, at 5 weeks of age, all animals were randomly distributed by weight into the various experimental groups. As shown in Fig. 2, the points at which the animals received the test diets from 2 weeks before, during, and after carcinogen treatment to termination of the study were designated initiation and postinitiation stages, whereas promotion/progression stages represent the point at which the animals received test diets from 14 weeks after carcinogen treatment until the end of the study. Beginning at 5 weeks of age, groups of animals in the initiation and postinitiation study had access to either control diet or experimental diet containing 0.2% curcumin, whereas the rats for the assays testing efficacy during the promotion/progression stage received the control diet. At 7 weeks of age, all rats except those intended for vehicle treatment received s.c. injections of AOM at a dose rate of 15 mg/kg body weight, once weekly for 2 weeks. Rats in vehicle-treated control groups were injected with an equal volume of normal saline. The rats designated for the intervention during the promotion/progression stage and maintained on the control diet were then transferred to experimental diets containing 0.2 or 0.6% curcumin beginning 14 weeks after the second dose of AOM (Fig. 2). Our past experience on AOM-induced colon carcinogenesis suggests that the premalignant lesions in the colon would have developed by week 14 following carcinogen administration (26). This dietary regimen was continued until termination of the experiment 52 weeks after the last carcinogen treatment. Body weights were recorded every 2 weeks for the first 10 weeks and then every 4 weeks. At the scheduled termination, all animals were killed by CO_2 euthanasia. After laparotomy, the entire gastrointestinal tract was resected and opened longitudinally, and the contents were flushed with normal saline. Colon tumors were recorded by gross observation using a dissection microscope. All other organs, including kidney, liver, and lungs were grossly examined under the dissection microscope for any abnormalities. For histopathological evaluation, colon tumors were fixed in 10% neutral buffered formalin, embedded in paraffin blocks, cut into multiple sections, and processed. The slides were stained with H&E and examined. The histological criteria used for classification of intestinal tumors were as described previously (24, 26). Upon termination of this study, more than 90% of the colon tumors had developed into adenocarcinomas that were classified as invasive or noninvasive. The invasive adenocarcinomas were mostly signet-ring mucinous types, invading the muscularis mucosa deep into the intestinal wall and beyond. The noninvasive adenocarcinomas were those growing outward toward the intestinal lumen without invasion of the muscularis mucosa. They were usually well-differentiated adenocarcinomas.

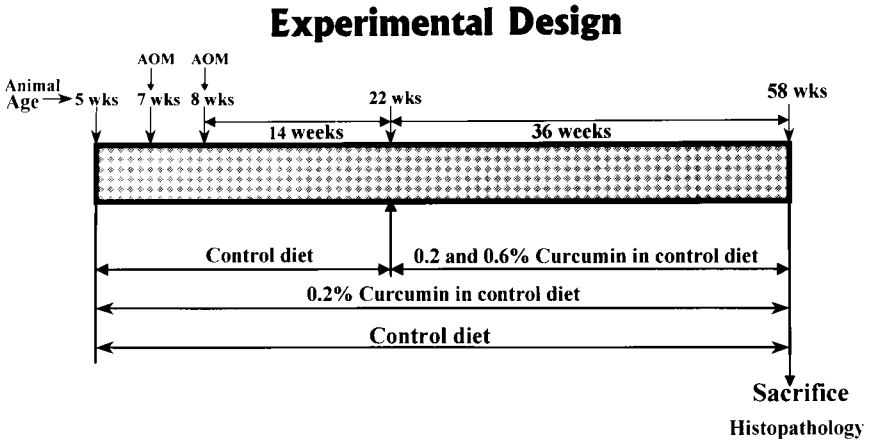

Fig. 2. Experimental design for evaluation of the chemopreventive activity of curcumin against colon carcinogenesis. Groups of male F344 rats were fed the experimental diets containing 0 or 0.2% curcumin beginning 2 weeks prior to exposure to AOM, during treatment, and until termination (initiation and postinitiation stages). Additional groups of animals who were on control diet (0% curcumin) 2 weeks prior to exposure of AOM, during treatment, and until 14 weeks after AOM treatment were transferred to experimental diets containing 0.2 and 0.6% curcumin and were on this regimen until termination (promotion/progression stage). AOM was given to the animals s.c. at the beginning of 7 and 8 weeks of age at 15 mg/kg body weight.

Table 2 *Effect of dietary curcumin on body weights of male F344 rats*

Experimental group	No. of animals/group	Body weights (g) of animals on experimental diets							
		Week 0	Week 1	Week 4	Week 12	Week 24	Week 36	Week 44	Week 52
AOM-treated									
Control diet	36	115 ± 10[a]	150 ± 12	224 ± 14	323 ± 22	395 ± 25	422 ± 27	437 ± 38	430 ± 40
0.2% curcumin[b]	36	115 ± 8	139 ± 11	219 ± 17	318 ± 20	384 ± 24	417 ± 40	428 ± 55	433 ± 54
0.2% curcumin[c]	36	115 ± 8	148 ± 10	221 ± 13	322 ± 19	392 ± 27	433 ± 32	444 ± 42	437 ± 49
0.6% curcumin[c]	36	112 ± 9	145 ± 12	213 ± 16	314 ± 22	379 ± 32	414 ± 30	423 ± 40	419 ± 40
Vehicle-treated									
Control diet	6	113 ± 9	147 ± 11	231 ± 8	340 ± 13	419 ± 15	463 ± 12	481 ± 12	484 ± 14
0.6% curcumin[c]	6	111 ± 10	152 ± 12	235 ± 13	337 ± 20	405 ± 30	436 ± 29	456 ± 37	461 ± 37

[a] Mean ± SD.
[b] Curcumin was administered 2 weeks before, during, and after carcinogen treatment.
[c] Curcumin was administered starting 14 weeks after the second dose of carcinogen treatment.

Detection of Apoptosis. Although apoptosis is characterized by DNA fragmentation, and the appearance of a "ladder" of nucleosomal-sized fragments on agarose gel electrophoresis has been used as a hallmark of apoptosis, DNA cleavage is not universally found in apoptosis (31). A ladder of DNA fragments has also been associated with necrosis in certain types of cells (32, 33). The gold standard for determination of apoptosis has been set through observation of characteristic morphological changes by electron microscopy (32, 34) or alternatively, by light microscopy (29, 35, 36). In this study, we examined the modulation of apoptosis by curcumin by quantifying the number of apoptotic cells in H&E-stained histological sections of colon tumors using light microscopy (29, 36). Apoptotic cells were identified by cell shrinkage, nuclear condensation, and formation of apoptotic bodies (29, 36). The light microscopic appearance of apoptotic bodies are quite diverse; most are round or roughly oval in shape. Apoptotic bodies vary in size, but they are a little smaller than the parent cells. Some apoptotic cells contain pyknotic chromatin, and some are devoid of a nuclear component (29, 36). The apoptotic index, which represents the percentage of cells exhibiting apoptosis, was determined by counting at least 300 cells in randomly chosen fields. All slides were scored by one person who was blinded to the experimental listing by means of code numbers.

Statistical Analysis. Data on body weights were compared among the levels of test agent using Student's t test. The comparative colon tumor incidence (total number of colon tumor-bearing rats with respect to the total number of rats at risk) in the animals fed the control diet and those given experimental diets was analyzed using Armitage's χ^2 method. Tumor multiplicities (total number of colon tumors per animal) were calculated for each dietary group; the significance of the differences between results in groups on the control diet and experimental diets containing curcumin was analyzed using the unpaired Student's t test, accounting for unequal variance. The apoptotic index, which is expressed as the percentage of cells exhibiting apoptosis was analyzed by unpaired Student's t test. Differences were considered statistically significant at $P < 0.05$.

RESULTS

General Observations. The body weights of rats who received the experimental diets containing 0.2% of curcumin starting from 2 weeks before, during, and after carcinogen treatment to termination of the study (initiation and postinitiation stages) and containing 0.2 or 0.6% curcumin beginning from 14 weeks after carcinogen treatment until the end of the study (promotion/progression stage) were comparable to weights of those fed the control diet only (Table 2). As expected, vehicle-treated animals in all groups weighed slightly more than those treated with AOM during the course of the study. In vehicle-treated rats, experimental diets containing curcumin did not produce any gross changes in any organs and, thus, showed no toxicity.

Tumor Data. There were no tumors among rats given vehicle only and maintained on control or experimental diets containing curcumin. The results, summarized in Table 3, indicate that administration of AOM induced adenomas and adenocarcinomas of the colon in ~9% and 82% of rats, respectively, who were fed the control diet. Because of long-term nature of this study (52 weeks), most of the colon tumors had become adenocarcinomas. Administration of 0.2% curcumin during the initiation and postinitiation stages (before, during and after carcinogen treatment) significantly inhibited the incidence of noninvasive adenocarcinomas (59% inhibition; $P < 0.05$), multiplicities of noninvasive adenocarcinomas (71% inhibition; $P < 0.01$), and total (noninvasive plus invasive) adenocarcinomas of the colon (34% inhibition; $P < 0.05$). The incidences of adenomas could not be compared among different groups because of low yield of this lesion. Administration of 0.2% curcumin during the promotion/progression stages (14 weeks after carcinogen treatment) also significantly inhibited the incidence of invasive adenocarcinomas of the colon (54% inhibition; $P < 0.05$). Although the inhibition of the incidences and multiplicities of noninvasive adenocarcinomas had reached 54 and 44%, respectively, in the rats given 0.2% curcumin during the promotion/progression stage, the differences were not statistically significantly ($P > 0.05$). It is noteworthy that administration of 0.2% curcumin during the promotion/progression stage significantly suppressed total colon tumor incidence and multiplicity (adenomas plus adenocarcinomas) as compared to results with the control diet

Table 3 *Effect of dietary curcumin on AOM-induced colon carcinogenesis in male F344 rats*

Experimental groups	Tumor incidence (% animals with tumors)					Tumor multiplicity (tumors/animal)				
		Adenocarcinomas					Adenocarcinomas			
	Adenomas	Noninvasive	Invasive	Total	Total	Adenomas	Noninvasive	Invasive	Total	Total
Control diet	9	41	76	82	85	0.09 ± 0.28[a]	0.59 ± 0.73	1.35 ± 1.08	1.94 ± 1.37	2.03 ± 1.42
0.2% curcumin[b]	3 (67)[c]	17 (59)[d]	57 (25)	71 (13)	71 (16)	0.03 ± 0.17	0.17 ± 0.38 (71)[e]	1.11 ± 1.14 (17)	1.29 ± 1.08 (34)[f]	1.31 ± 1.12 (35)[f]
0.2% curcumin[g]	3 (67)	19 (54)	50 (54)[d]	64 (22)	64 (25)[d]	0.03 ± 0.16	0.33 ± 0.78 (44)	1.00 ± 1.18 (30)	1.33 ± 1.25 (31)	1.36 ± 1.27 (33)[f]
0.6% curcumin[g]	6 (33)	9 (78)[h]	54 (29)	64 (22)	64 (25)[d]	0.06 ± 0.23	0.09 ± 0.28 (85)[i]	0.74 ± 0.87 (45)[f]	0.83 ± 0.84 (57)[i]	0.89 ± 0.85 (56)[i]

[a] Mean ± SD.
[b] Animals were administered curcumin beginning 2 weeks before, during, and after carcinogen treatment until termination of the study (initiation and postinitiation period).
[c] % inhibition from control diet groups is shown in parenthesis.
[d] Significantly different from control diet group by χ^2-test, $P < 0.05$.
[e] Significantly different from control diet group by Student's t test, $P < 0.01$.
[f] Significantly different from control diet group by Student's t test, $P < 0.05$.
[g] Animals were administered curcumin beginning 14 weeks after carcinogen treatment until termination of the study (promotion/progression period).
[h] Significantly different from control diet group by χ^2 test, $P < 0.01$.
[i] Significantly different from control diet group by Student's t test, $P < 0.001$.

Table 4 *Modulating effects of dietary curcumin on apoptosis in colon adenocarcinomas*

Experimental group	Apoptotic index[a] (%)
Control diet	5.33 ± 0.61[b]
0.2% curcumin[c]	9.17 ± 1.04[d]
0.2% curcumin[e]	7.56 ± 0.82[f]
0.6% curcumin[e]	8.40 ± 0.61[d]

[a] Apoptotic index represents percentage of cells exhibiting apoptosis.
[b] Mean ± SE; number of adenocarcinomas examined in each group: 10.
[c] Animals were administered curcumin beginning 2 weeks before, during, and after carcinogen treatment until termination of the study (initiation and postinitiation period).
[d] Significantly different from the control diet group, $P < 0.01$.
[e] Animals were administered curcumin beginning 14 weeks after carcinogen treatment until termination of the study (promotion/progression period).
[f] Significantly different from the control diet group, $P < 0.05$.

($P < 0.05$). As expected, administration of 0.6% curcumin during the promotion/progression stage also significantly inhibited the incidence of noninvasive adenocarcinomas (78% inhibition; $P < 0.01$) and multiplicities of noninvasive (85% inhibition; $P < 0.001$) and invasive (45% inhibition; $P < 0.05$) adenocarcinomas of the colon. In addition, the incidences and multiplicities of total colon tumors (adenomas plus adenocarcinomas) were reduced when rats were given 0.6% curcumin (25 and 56% inhibition; $P < 0.05$ and $P < 0.01$). These results were analyzed using the linear correlation method for a dose-response effect. This analysis yielded the correlation coefficients (r) for multiplicity of adenocarcinomas with increasing levels of curcumin in the diet from 0 to 0.6%, suggesting a dose-dependent inhibition of colon tumors ($P < 0.05$): noninvasive adenocarcinomas, −0.97; invasive adenocarcinomas, −0.95; total adenocarcinomas, −0.97; and total tumors, −0.96.

Apoptosis. Having established the inhibition of colon carcinogenesis by dietary administration of 0.2% curcumin during the initiation and postinitiation stages and the effects by 0.2 and 0.6% curcumin given during the promotion/progression period, we investigated whether the inhibition of colon tumorigenesis by curcumin is associated with the modulation of apoptosis in the colon tumors. Results summarized in Table 4 indicate that continual administration of 0.2% curcumin during the initiation and postinitiation stages and feeding 0.2 and 0.6% curcumin during the promotion/progression period significantly increased the apoptotic index in the colon tumors as compared to that in tumors of rats given control diet ($P < 0.05$–$P < 0.002$).

DISCUSSION

This study is part of a large-scale evaluation of phytochemicals that have anti-inflammatory and antioxidant properties for their potential chemopreventive activities against colon carcinogenesis. The primary mission of these studies is to identify effective and safe chemopreventive agents that will facilitate the development of cancer-preventive strategies and their application in a clinical setting. Curcumin, a naturally occurring anti-inflammatory agent and antioxidant, has been shown to inhibit tumors in several organs, including 7,12-dimethylbenz[*a*]anthracene-induced and TPA-promoted skin tumors, benzo(*a*)pyrene-induced forestomach tumors, and AOM-induced intestinal tumors in mice (16, 17, 20), to cite a few. Recent studies from our laboratory and elsewhere that demonstrated an inhibitory effect of dietary curcumin when administered continuously during the initiation and postinitiation phases (20–24) provided a rationale for elucidating the efficacy of this agent against premalignant lesions during the promotion/progression stage of colon carcinogenesis.

The results of this study are in agreement with earlier investigations showing that dietary curcumin inhibits colon carcinogenesis when administered during the initiation and postinitiation periods (20, 23, 24). Our results also demonstrate for the first time that curcumin, a naturally occurring anti-inflammatory agent and antioxidant, given as a dietary supplement during promotion/progression period still inhibits its tumorigenesis in the colon, suggesting that administration of curcumin may retard growth and/or development of existing neoplastic lesions in the colon. This also suggests the potential usefulness of this agent as a chemopreventive agent for individuals at high risk for colon cancer development, such as patients with polyps. This study further extends our earlier observations that synthetic NSAIDs, such as piroxicam and sulindac, given during the promotion/progression period protect against colon tumorigenesis in F344 rats (26, 37). Importantly, unlike synthetic NSAIDs curcumin does not produce any gastrointestinal toxicity, even at very high doses, which may provide advantage over synthetic agents.

With regard to the mode of chemopreventive action, curcumin exhibits a diverse array of metabolic, cellular, and molecular activities including inhibition of arachidonic acid formation and its further metabolism to eicosanoids. Studies from our laboratory have demonstrated that dietary curcumin significantly inhibits phospholipase A_2 in colonic mucosa and tumors leading to the release of arachidonic acid from phospholipids, alters COX and LOX activities, and modifies PGE_2 levels (24). Several lines of evidence also indicate that the mechanism of action of curcumin is not limited to PG inhibition. We had observed earlier that dietary curcumin inhibits LOX activity, and the production of the LOX metabolites, 5(*S*)-, 8(*S*)-, 12(*S*)-, and 15(*S*)-HETEs, in the colonic mucosa and in tumors (24). Importantly, LOX metabolites such as 12(*S*)-HETE have been shown to promote tumor cell adhesion, stimulate the spreading of tumor cells, and augment metastatic potential (38–40). Also, a positive correlation was observed between the levels of 8(*S*)-HETE and hyperproliferation and tumor development induced by TPA (41). Moreover, curcumin inhibits several mediators and enzymes involved in cell mitogenic signal transduction pathways (42) and activator protein-1 and nuclear factor κB activation (43). Hanif *et al.* (19) provided evidence that curcumin inhibits cell proliferation and induces cell cycle changes in the colonic adenocarcinoma cell lines, HT-29 and HCT-15, and that this effect is independent of its ability to inhibit PG synthesis. Here, the inhibitory effects of curcumin administered during the promotion/progression stage of chemically induced carcinogenesis is associated with increased apoptosis, suggesting that increased cell death through apoptosis may be one of the mechanisms by which dietary curcumin affects this inhibition. The results of this and other studies support the concept that the capacity to induce apoptosis may be common to many chemopreventive agents (28, 44, 45). This had certainly been documented for NSAIDs and other agents that inhibit colon carcinogenesis, suggesting that cellular responses to these agents may contribute to chemopreventive effects (29, 35). The effects of curcumin demonstrated here resemble those of NSAIDs and thus seem to act strongly *via* inhibition of arachidonate metabolism and through reducing cell proliferation and inducing apoptosis.

In conclusion, the study described here demonstrates for the first time that dietary administration of curcumin during the promotion/progression stage of AOM induced colon carcinogenesis significantly inhibits tumor development in a dose-dependent manner and increases apoptosis in the colonic tumors. Similar levels of inhibition of colon tumorigenesis were achieved when 0.2% curcumin was administered either during initiation and postinitiation periods or promotion/progression stage, suggesting indirectly that most of chemopreventive efficacy of this agent is achieved during the promotion/progression phase in this model. Although the exact mechanisms of its chemopreventive action of curcumin remain to be elucidated, it would appear that modulation of tumorigenesis by this agent is associated

not only with the alteration of arachidonic acid metabolism through LOX and COX pathways (24) but also through mechanisms that are independent of eicosanoid metabolism, such as cell proliferation and apoptosis in the colon tumors.

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REFERENCES

1. American Cancer Society. Cancer Statistics 1998. CA Cancer J. Clin., *48:* 11–42, 1998.
2. Wynder, E. L., Kajitani, T., Ishidawa, S., Dodo, H., and Takano, A. Environmental factors in cancer of colon and rectum. Cancer (Phila.), *23:* 1210–1220, 1969.
3. Willett, W. C., Stampfer, M. J., Colditz, G. A., Rosner, B. A., and Speizer, F. E. Relation of meat, fat and fiber intake to the risk of colon cancer in a prospective study among women. N. Engl. J. Med., *323:* 1664–1672, 1990.
4. Reddy, B. S. Nutritional factors and colon cancer. Crit. Rev. Food Sci. Nutr., *35:* 175–190, 1995.
5. Potter, J. D., and Steinmatz, K. Vegetables, fruits and phytoestrogens as preventive agents. IARC Sci. Publ., *139*: 61–90, 1996.
6. Wattenberg, L. W. Chemoprevention of cancer by naturally occurring and synthetic compounds. *In:* M. Wattenberg, C. W. Lipkin, C. W. Boone, and G. J. Kelloff (eds.), Cancer Chemoprevention, pp. 19–39. Boca Raton, FL: CRC Press, 1992.
7. Kelloff, G. J., Boone, C. W., Malone, W. E., and Steele, V. E. Recent results in preclinical and clinical drug development of chemopreventive agents at the National Cancer Institute. *In:* L. W. Wattenberg, M. Lipkin, C. W. Boone, and G. J. Kelloff (eds.), Cancer Chemoprevention, pp. 41–56. Boca Raton, FL: CRC Press, 1992.
8. Greenwald, P., Kelloff, G. J., Boone, C. W., and McDonald, S. N. Genetic and cellular changes in colorectal cancer: proposed targets of chemopreventive agents. Cancer Epidemiol. Biomark. Prev., *4:* 691–702, 1995.
9. Ammon, H. P. T., and Wahl, M. A. Pharmacology of *Curcuma longa*. Planta Med., *57:* 1–7, 1991.
10. Nadkarani, K. M. *Curcuma longa*. *In:* K. M. Nadkarani (ed.), India Materia Medica, pp. 414–416. Bombay: Popular Prakashan Publishing Co., 1976.
11. Tonnesen, H. H. Chemistry of curcumin and curcuminoids. *In:* C-T. Ho, C. Y. Lee, and M-T. Haung (eds.), Phenolic Compounds in Food and their Effect of Health, Vol. 1: Analysis, Occurrence and Chemistry, ACS Symposium Series No. 506, pp. 143–153. Washington, DC: American Chemical Society, 1992.
12. Srimal, R. C., and Dhawan, B. N. Pharmacology of diferuloylmethane (curcumin), a non-steroidal anti-inflammatory agent. J. Pharm. Pharmacol., *25:* 447–452, 1973.
13. Satoskar, R. R., Shah, S. J., and Shenoy, S. G. Evaluation of antiinflammatory property of curcumin (diferuloylmethane) in patients with postoperative inflammation. Int. J. Clin. Pharmacol. Ther. Toxicol., *24:* 651–654, 1986.
14. Sharma, O. P. Antioxidant activity of curcumin and related compounds. Biochem. Pharmacol., *25:* 1811–1812, 1976.
15. Toda, S., Miyase, T., Arichi, H., Tanizawa, H., and Takino, Y. Natural antioxidant III. Antioxidative components isolated from rhizome of *Curcuma longa* L. Chem. Pharm. Bull. (Tokyo), *33:* 1725–1728, 1985.
16. Huang, M-T., Smart, R. C., Wong, G-Q., and Conney, A. H. Inhibitory effect of curcumin, chlorogenic acid, caffeic acid, and ferulic acid on tumor promotion in mouse skin by 12-*O*-tetradecanoylphorbol-13-acetate. Cancer Res., *48:* 5941–5946, 1988.
17. Huang, M-T., Wang, Z. Y., Georgiadis, C. A., Laskin, J. D., and Conney, A. H. Inhibitory effect of curcumin on tumor initiation by benzo[*a*]pyrene and 7,12-dimethylbenz[*a*]anthracene. Carcinogenesis (Lond.), *13:* 2183–2186, 1992.
18. Huang, M. T., Ma, W., Yen, P., Xie, J. G., Han, J., Frenkel, K., Grunberger, D., and Conney, A. H. Inhibitory effects of topical application of low doses of curcumin on 12-*O*-tetradecanoylphorbol-13-acetate-induced tumor promotion and oxidized DNA bases in mouse epidermis. Carcinogenesis (Lond.), *18:* 83–88, 1997.
19. Hanif, R., Qiao, L., Shiff, S. J., and Rigas, B. Curcumin, a natural plant phenolic food additive, inhibits cell proliferation and induces cell cycle changes in colon adenocarcinoma cell lines by a prostaglandin-independent pathways. J. Lab. Clin. Med., *130:* 576–584, 1997.
20. Huang, M-T., Lou, Y-R., Ma, W., Newmark, H., Reuhl, K., and Conney, A. H. Inhibitory effect of dietary curcumin on forestomach, duodenal and colon carcinogenesis in mice. Cancer Res., *54:* 5841–5847, 1994.
21. Huang, M. T., Deschner, E. E., Newmark, H. L., Wang, Z-Y., Ferraro, T. A., and Conney, A. H. Effect of dietary curcumin and ascorbyl palmitate on azoxymethane-induced colonic epithelial cell proliferation and focal areas of dysplasia. Cancer Lett., *64:* 117–121, 1992.
22. Rao, C. V., Simi, B., and Reddy, B. S. Inhibition by dietary curcumin of azoxymethane-induced ornithine decarboxylase, tyrosine protein kinase, arachidonic acid metabolism and aberrant crypt foci formation in the rat colon. Carcinogenesis (Lond.), *14:* 2219–2225, 1993.
23. Pereira, M. A. Grubbs, D. J., Barnes, L. H., Li, H., Olson, G. R., Eto, I., Juliana, M., Whitaker, L. M., Kelloff, G. J., Steele, V. E., and Lubet, R. A. Effects of the phytochemicals, curcumin and quercetin, upon azoxymethane-induced colon cancer and 7,12-dimethylbenz[a]anthracene-induced mammary cancer in rats. Carcinogenesis (Lond.), *17:* 1305–1311, 1996.
24. Rao, C. V., Rivenson, A., Simi, B., and Reddy, B. S. Chemoprevention of colon carcinogenesis by dietary curcumin, a naturally occurring plant phenolic compound. Cancer Res., *55:* 259–266, 1995.
25. Reddy, B. S., Rao, C. V., Rivenson, A., and Kelloff, G. Inhibitory effect of aspirin on azoxymethane-induced colon carcinogenesis in F344 rats. Carcinogenesis (Lond.), *14:* 1493–1497, 1993.
26. Rao, C. V., Rivenson, A., Simi, B., Zang, E., Kelloff, G., Steele, V., and Reddy, B. S. Chemoprevention of colon carcinogenesis by sulindac, a nonsteroidal anti-inflammatory agent. Cancer Res., *55:* 1464–1472, 1995.
27. Rao, C. V., Tokumo, K., Rigotty, J., Zang, E., Kelloff, G., and Reddy, B. S. Chemoprevention of colon carcinogenesis by dietary administration of piroxicam, α-difluoromethylornithine, 16α-fluoro-5-androsten-17-one, and ellagic acid individually and in combination. Cancer Res., *51:* 4528–4534, 1991.
28. Boolbol, S. K., Dannenberg, A. J., Chadburn, A., Martucci, C., Guo, X., Ramonettti, J. T., Abreu-Goris, M., Newmark, H. L., Lipkin, M. L., DeCosse, J. J., and Bertagnolli, M. M. Cyclooxygenase-2 overexpression and tumor formation are blocked by sulindac in a murine model of familial adenomatous polyposis. Cancer Res., *56:* 2556–2560, 1996.
29. Samaha, H. S., Hamid, R., El-Bayoumy, K., Rao, C. V., and Reddy, B. S. The role of apoptosis in the modulation of colon carcinogenesis by dietary fat and by the organoselenium compound 1,4-phenylenebis(methylene)selenocyanate. Cancer Epidemiol. Biomark. Prev., *6:* 699–704, 1997.
30. Bedi, A., Pasricha, P. J., Akhtar, A. J., Barber, J. P., Bedi, G. C, Giardiello, G. M., Zehnbauer, B. A., Hamilton, S. R., and Jones, R. J. Inhibition of apoptosis during development of colorectal cancer. Cancer Res., *55:* 1811–1816, 1995.
31. Schultz-Osthoff, K., Wakzcak, H., Droge, W., and Krammer, P. H. Cell nucleus and DNA fragmentation are not required for apoptosis. J. Cell Biol., *127:* 15–20, 1994.
32. Collins, R. J., Harmon, B. V., Gobi, G. C., and Kerr, J. F. R. Internucleosomal DNA cleavage should not be the sole criterion for identifying apoptosis. Int. J. Radiat. Biol., *61:* 451–453, 1992.
33. Schulte-Herman, R., Buisch, W., and Grasl-Krupp, B. Active cell death (apoptosis) in liver biology and disease. *In:* J. L. Boyer and R. D. Ockner (eds.), Progress in Liver Diseases, Vol. 13, pp. 1–35. Philadelphia: W. B. Saunders, 1995.
34. Kerr, J. F. R., Wyllie, A. H., and Currie, A. R. Apoptosis: basic biological phenomenon with wide-ranging implication in tissue kinetics. Br. J. Cancer, *26:* 239–257, 1972.
35. Hall, P., Coates, P. J., Ansari, B, and Hopwood, D. Regulation of cell number in the mammalian gastrointestinal tract: the importance of apoptosis. J. Cell Sci., *107:* 3569–3577, 1994.
36. Samaha, H. S., Kelloff, G. J., Steele, V., Rao, C. V., and Reddy, B. S. Modulation of apoptosis by sulindac, curcumin, phenylethyl-3-methylcaffeate, and 6-phenylhexyl isothiocyanate: apoptotic index as a biomarker in colon cancer chemoprevention and promotion. Cancer Res., *57:* 1301–1305, 1997.
37. Reddy, B. S., Maruyama, H., and Kelloff, G. Dose-related inhibition of colon carcinogenesis by dietary piroxicam, a nonsteroidal anti-inflammatory drug, during different stages of rat colon tumor development. Cancer Res., *47:* 5340–5346, 1987.
38. Honn, K. V., Grossi, I. M., Steinert, B. W., Chopra, H., Onoda, J., Nelson, K. K., and Taylor, J. D. Lipoxygenase regulation of membrane expression of tumor cell glycoproteins and subsequent metastasis. Adv. Prostaglandin Thromboxane Leukotriene Res., *19:* 439–443, 1989.
39. Timar, J., Chen, Y. Q., Liu, B., Basaz, R., Taylor, J. D., and Honn, K. V. The lipoxygenase metabolite 12(*S*)-HETE promotes αIIb β3 integrin-mediated tumor-cell spreading on fibronectin. Int. J. Cancer, *52:* 594–603, 1992.
40. Honn, K. V., and Tang, D. G. Adhesion molecules and cancer cell interaction with endothelium and subendothelial matrix. Cancer Metastasis Rev., *11:* 353–375, 1992.
41. Furstenberger, G., Schurich, B., Kaina, B., Petrusevska, R. T., Fusenig, N. E., and Marks, F. Tumor induction in initiated mouse skin by phorbol esters and methylmethanesulfonate: correlation between chromosomal damage and conversion ("stage I of tumor promotion") *in vivo*. Carcinogenesis (Lond.). *10:* 749–752, 1989.
42. Jiang, M. C., Yang-Yen, H. F. J., Yen, J. J., and Lin, J. K. Curcumin induces apoptosis in immortalized NIH 3T3 and malignant cancer cell lines. Nutr. Cancer, *26:* 111–120, 1996.
43. Xu, Y. X., Pindolia, K. R., Janakiraman, N., Chapman, N., and Gautam S. C. Curcumin inhibits IL-1α and TNF-α induction of AP-1 and NF-κB DNA-binding activity in bone marrow stromal cells. Hematopathol. Mol. Hematol., *11:* 49–62, 1997–1998.
44. Piantadosi, S., Hamilton, S. R., and Giardiello, F. M. The effects of sulindac on colorectal proliferation and apoptosis in familial adenomatous polyposis. Gastroenterology, *109:* 994–998, 1995.
45. Elder, D. J. E., Hague, A., Hicks, D. J., and Paraskeva, C. Differential growth inhibition by the aspirin metabolite salicylate in human colorectal tumor cell lines: enhanced apoptosis in carcinoma and *in vitro*-transformed adenoma relative to adenoma cell lines. Cancer Res., *56:* 2273–2276, 1996.

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