

Phase IIa Clinical Trial of Curcumin for the Prevention of Colorectal Neoplasia

Robert E. Carroll¹, Richard V. Benya¹, Danielle Kim Turgeon², Shaiju Vareed², Mallore Neuman³, Luz Rodriguez⁴, Madhuri Kakarala^{2,5}, Philip M. Carpenter², Christine McLaren^{6,7}, Frank L. Meyskens, Jr⁶, and Dean E. Brenner^{2,3,5}

Abstract

Curcumin is derived from the spice turmeric and has antiinflammatory and antineoplastic effects *in vitro* and in animal models, including preventing aberrant crypt foci (ACF) and adenomas in murine models of colorectal carcinogenesis. Inhibiting the production of the procarcinogenic eicosanoids prostaglandin E₂ (PGE₂) and 5-hydroxyeicosatetraenoic acid (5-HETE) can suppress carcinogenesis in rodents. Curcumin reduces mucosal concentrations of PGE₂ (via inhibition of cyclooxygenases 1 and 2) and 5-HETE (via inhibition of 5-lipoxygenase) in rats. Although preclinical data support curcumin activity in many sites, the poor bioavailability reported for this agent supports its use in the colorectum. We assessed the effects of oral curcumin (2 g or 4 g per day for 30 days) on PGE₂ within ACF (primary endpoint), 5-HETE, ACF number, and proliferation in a nonrandomized, open-label clinical trial in 44 eligible smokers with eight or more ACF on screening colonoscopy. We assessed pre- and posttreatment concentrations of PGE₂ and 5-HETE by liquid chromatography tandem mass spectroscopy in ACF and normal-tissue biopsies; ACF number via rectal endoscopy; proliferation by Ki-67 immunohistochemistry; and curcumin concentrations by high-performance liquid chromatography in serum and rectal mucosal samples. Forty-one subjects completed the study. Neither dose of curcumin reduced PGE₂ or 5-HETE within ACF or normal mucosa or reduced Ki-67 in normal mucosa. A significant 40% reduction in ACF number occurred with the 4-g dose ($P < 0.005$), whereas ACF were not reduced in the 2-g group. The ACF reduction in the 4-g group was associated with a significant, five-fold increase in posttreatment plasma curcumin/conjugate levels (versus pretreatment; $P = 0.009$). Curcumin was well tolerated at both 2 g and 4 g. Our data suggest that curcumin can decrease ACF number, and this is potentially mediated by curcumin conjugates delivered systemically. *Cancer Prev Res*; 4(3); 354–64. ©2011 AACR.

Introduction

The phytochemical curcumin is derived from the spice turmeric and has substantial, complex antiinflammatory, antioxidant, and antineoplastic effects *in vitro* and in animal carcinogenesis models, including preventing colorectal aberrant crypt foci (ACF) and adenomas in mice (1). Long

used for treating inflammation, skin wounds, and tumors in India and Southeast Asia (2, 3), curcumin inhibits the production of at least 2 eicosanoids, prostaglandin E₂ (PGE₂) and 5-hydroxyeicosatetraenoic acid (5-HETE), via inhibiting cyclooxygenase 1 (Cox-1) and Cox-2 (to reduce PGE₂), and 5-lipoxygenase (5-Lox; to reduce 5-HETE; ref. 4). PGE₂ and 5-HETE are important procarcinogenic factors, the inhibition of which is associated with reduced carcinogenesis in rodent models of colorectal carcinogenesis (4). Clinical effects of curcumin on potential colorectal cancer biomarkers in humans have not been reported previously.

We and others found ACF in the rectum of current and former smokers. This association was independent of age and increased with years of tobacco use (5, 6). These findings led us to speculate that the ability of curcumin to reduce concentrations of PGE₂ and 5-HETE in the flat mucosa of the colorectum would reduce colorectal epithelial crypt proliferation and ACF formation in current smokers. ACF reduction has been used in both human (7, 8) and rodent models (9) to assess the potency of cancer-preventive drugs and dietary supplements.

Authors' Affiliations: ¹Department of Medicine, University of Illinois at Chicago, and Chicago Veterans Administration Medical Center (West Side Division), Chicago, Illinois; Departments of ²Internal Medicine and ³Pharmacology, University of Michigan, Ann Arbor, Michigan; ⁴Division of Cancer Prevention, National Cancer Institute, Bethesda, Maryland; ⁵Veterans Administration Medical Center, Ann Arbor, Michigan; ⁶Chao Family Comprehensive Cancer Center (Orange) and ⁷Department of Epidemiology, University of California, Irvine, Irvine, California

Note: Supplementary data for this article are available at Cancer Prevention Research Online (<http://cancerprevres.aacrjournals.org/>).

Corresponding Author: Robert E. Carroll, Department of Medicine, University of Illinois at Chicago, 840 South Wood Street (M/C 787), Chicago, IL 60612. Phone: 312-996-6651; Fax: 312-996-3186. E-mail: rcarroll@uic.edu

doi: 10.1158/1940-6207.CAPR-10-0098

©2011 American Association for Cancer Research.

Several phase I studies have shown that curcumin is well tolerated at doses up to 12 g (no maximum tolerated dose has been defined; refs. 10–12) but has very poor bioavailability (12, 13). Current curcumin or curcuminoid formulations may be more beneficial in the gastrointestinal tract, where they come into contact with gastrointestinal mucosa without need of systemic absorption. This suggestion is supported by a pilot study of 5 patients showing that curcumin plus quercetin reduced adenoma number and recurrence in subjects with familial adenomatous polyposis (14). Single-agent curcumin may also prevent relapse of ulcerative colitis (15), which is a chronic inflammatory process and precursor of colon cancer. All of these preliminary data led us to conduct the phase IIa clinical prevention trial of single-agent curcumin in colorectal neoplasia reported here.

Materials and Methods

Materials

Pure (Good Manufacturing Practice) curcumin powder [98.0% by high performance liquid chromatography (HPLC); Sabinsa Corp.] was micronized and provided by the National Cancer Institute's Division of Cancer Prevention for human use. Sodium acetate, sodium phosphate, β -17-estradiol acetate, and the enzymes β -glucuronidase (type IX-A from *Escherichia coli*) and sulfatase (type H-1 from *Helix pomatia*) were purchased from Sigma Chemical Co.. HPLC-grade ethyl acetate, hexane, methanol, and water were purchased from Burdick and Jackson (Honeywell International Inc.).

Participants

Participants were recruited from patients referred to the University of Illinois at Chicago (UIC) for screening colonoscopy or to the Colorectal Screening Clinic at the University of Michigan (UM) for screening flexible sigmoidoscopy. To be eligible, men and women had to be 40 years or older and a current smoker with a smoking history of more than 3 pack-years and had to have 8 or more rectal ACF by magnification chromoendoscopy. Written consent was obtained from each subject recruited to the study, which was reviewed and approved by the Institutional Review boards of UIC and UM.

Subjects were excluded for use of nonsteroidal antiinflammatory drugs including acetylsalicylic acid (ASA, or aspirin) more than 10 days per month unless they completed a 30-day washout period, and for a history of chronic inflammatory bowel disease, prior pelvic irradiation, or a history of endoscopically confirmed peptic-ulcer disease less than 5 years from the enrollment date.

Study plan

We conducted a phase IIa cancer prevention trial of oral curcumin given daily for 30 days to reduce the concentrations of PGE₂ and 5-HETE within ACF (the primary endpoint) and in associated normal mucosa. Secondary endpoints included total ACF number and an estimate

of proliferation in normal mucosa using the proliferation marker Ki-67. The trial had 2 stages – curcumin at 2 g (8 capsules) once daily in the first stage and at 4 g (16 capsules) once daily in the second, succeeding stage, with a planned 20 evaluable participants in each stage (total = 40). The protocol mandated a formal toxicity review and finding of acceptable toxicity after completion of stage 1 (2 g) prior to initiating stage 2 (4 g). The criteria for acceptable toxicity in stage 1 were less than 20% grade-1 or -2 toxicity requiring drug discontinuation and less than 10% grade-3 toxicity attributable to drug; these criteria were established in consultation with the Food and Drug Administration and Division of Cancer Prevention (National Cancer Institute) prior to opening this study. ACF were counted via magnifying endoscopy before and after treatment. Biopsies of ACF and normal rectal mucosa were taken both at baseline and posttreatment. All participants had venous blood drawn in a fasting state for a complete blood count, liver function tests, blood urea nitrogen, and creatinine levels after the baseline and posttreatment endoscopic exam. The posttreatment endoscopic (flexible sigmoidoscopy) exam was performed between day 30 and day 35 and curcumin was continued until day of exam to ensure biopsies were obtained <4h post dose. Adherence to treatment was tracked by a follow-up telephone call at day 14. At the posttreatment endoscopy procedure, adherence was assessed by pill counts and a diary in which participants recorded the date and time of each dose taken. Adherence to intervention was defined as taking 80% or more of the required doses. After completing the intervention with curcumin at 2 g, the study protocol was amended to evaluate plasma for curcumin and its conjugates pre- and postintervention with curcumin at 4 g. Curcumin was continued until the day of posttreatment flexible sigmoidoscopy. We provided extra capsules if day 30 fell on a weekend. The subjects took their morning dose prior to the final sigmoidoscopy exam, which was typically between 8 am and 10 am. We defined this dosing in the protocol to ensure biopsies and plasma would be obtained < 4 h after the last study dose. All laboratory endpoints were blinded as to subject and timing (pre- vs. posttreatment) and dose of curcumin. The clinical endpoint ACF reduction could not be blinded, as the same investigator performed pre- and postintervention examinations.

Magnification endoscopy (ACF quantification)

The Fujinon XL-401 videoscope (Fujinon Inc.) at UIC and Olympus GIF 200Z (Olympic Optical) at UM were used to perform the initial standard colonoscopy or sigmoidoscopy. These videoscopes can magnify the mucosa by a factor of 35 and have an autofocusing device. The initial exam was followed by a magnified survey of the rectum for ACF using a modified dye infusion technique that is significantly faster and better tolerated by subjects (16). ACF were counted in a sequential fashion during a single withdrawal of the scope to prevent double counting. The rectum was divided into thirds using the 15-cm mark on the scope and counting proximal (15–10 cm), middle

(10–5 cm), and distal (5 cm–out) thirds. The ACF structures were identified using the criteria of McLellan and Bird (17).

Biopsy and sample management procedure

During endoscopy, subjects underwent 9 cold forceps biopsies – 3 from ACF and 6 from normal mucosa. Normal mucosa was defined as a control area of normal crypts more than 5 cm from an ACF. Two ACF biopsies and 3 normal mucosal biopsies were placed in an indomethacin solution to prevent prostaglandin degradation (18) and then snap frozen in liquid nitrogen and stored at -70°C until assayed. The remaining biopsies were fixed in 10% buffered formalin solution.

Laboratory methods

Eicosanoid assays Frozen mucosa was pulverized to fine powder under dry ice to extract PGE_2 and 5-HETE. The samples were homogenized in ice-cold PBS buffer containing 0.1% BHT and 1 mmol/L EDTA using an ultrasonic Processor. Eicosanoids were extracted with hexane:ethyl acetate (1:1, v/v, 2 mL) after adding citric acid (1 N, 20 μL) and deuterated internal standards. The organic layers from 3 extractions were dried and reconstituted in HPLC mobile phase [methanol: ammonium acetate buffer (10 mmol/L, pH 8.5, 70:30)]. Eicosanoids were separated using a ThermoElectron Finnegan triple quadrupole mass spectrometer equipped with a Waters HPLC inlet and a Luna-3 μm , phenyl hexyl, 2- \times 150-mm analytical column (Phenomenex) with ammonium acetate:methanol gradient. PGE_2 and 5-HETE were detected using electrospray negative ionization with multiple-reaction monitoring similar to the methods of Yang and colleagues (19).

Crypt cell proliferation (Ki-67 immunohistochemistry) The Ki-67 antibody is a rabbit polyclonal antibody to a nuclear protein expressed in proliferating cells and is a validated marker of proliferation and cancer risk in both malignant (20) and normal colonic mucosa (21). Mucosal proliferation was determined from cold forceps biopsy specimens, oriented flat at the time of biopsy, and processed separately in a Sakura VIP-3000 bench-top automated tissue processor within 6 to 8 hours of fixation in formalin, and paraffin was embedded with the crypt axis parallel to block surface prior to shipment. Immunohistochemistry was performed using a standard but automated 2-stage modification of an immunoperoxidase technique (22). Briefly, for each biopsy, 4- μm -thick sections were placed on capillary gap slides and deparaffinized with Histoclear (National Diagnostics). Sections were then rehydrated through decreasing concentrations of isopropyl alcohol. Sections for immunoperoxidase staining were steam-pretreated for 20 minutes in Antigen Retrieval Citra Buffer (Biogenex). Avidin–biotin complex immunoperoxidase reactions were done using an Immunotech 500 automated immunostainer (Ventana Systems, Inc.) according to the manufacturer's instructions. Briefly, the automated steps included blockage of endogenous peroxidase with 3% hydrogen peroxide and reaction with monoclonal antibodies against human Ki-67 (clone MIB-1; Dako)

diluted 1:100. The reaction was followed by a biotinylated goat anti-mouse IgG secondary antibody and then an avidin–biotin peroxidase complex. The chromogen was diaminobenzadine for all reactions. Cells were counterstained by 0.4% methyl green in 0.1 mol/L sodium acetate buffer (pH 4.0), followed by 3 washes each of water, 1-butanol, and Histoclear. Negative controls were done in the same fashion, except that the primary antibody was substituted with mouse immunoglobulin. A section of squamous cell carcinoma provided the positive controls for Ki-67.

Image analysis of sections stained by immunohistochemistry was performed using the Quantitative Proliferation Index Program of the CAS 200, according to the manufacturer's instructions. The operator chose thresholds at a wavelength of 620 nm for nuclei exhibiting methyl green counterstaining for machine identification as a cell nucleus and at a wavelength of 500 nm for the program to identify a nucleus as having a positive immunohistochemical reaction. This was done because the green counterstain is transparent at 500 nm; thus, the instrument will detect only nuclei with a positive immunoperoxidase reaction. The analysis was displayed as the percentage of positively staining cells/total cells.

Detection of curcumin and its conjugates in tissue and in plasma Curcumin was extracted from biopsy tissue similar to the extraction procedure in a previously published study (23). Frozen normal colon tissue (8–10 mg wet weight) was ground to a fine powder in a precooled Multi-sample Biopulverizer (Research Products International Co.) under dry ice. The sample was then transferred into a precooled sealed microcentrifuge tube and treated with ice-cold sodium acetate buffer (0.20 mL, pH 5.0). The powdered tissue was then homogenized by an ultrasonic Processor (Misonix) at 0°C for 3 minutes, with a 20-second homogenization cycle and a 20-second brake cycle to cool the homogenization probe to reduce the degradation of curcumin and its metabolites. An aliquot (0.1 mL) of the colon tissue suspension was then mixed with β -17-estradiol acetate (internal standard, 10 μL), sodium acetate buffer (0.5 mol/L, 0.1 mL, pH 5.0) and vortexed for 20 seconds. The solution was extracted 3 times with extracting reagent and vortexed for 3 minutes. The sample was centrifuged at 3,500 rpm for 15 minutes at 4°C . The upper organic layer was collected. The organic phases from the 3 extractions were pooled and evaporated under a stream of argon at room temperature.

The extraction of plasma was performed by adding 200 μL of plasma to 2 mL microcentrifuge tubes (USA Scientific). To each tube, 80 μL deionized water was added. The tubes were capped and mixed for 20 seconds at medium speed by vortex (Fisher Scientific). A total of 40 μL internal standard (250 $\mu\text{g}/\text{mL}$ β -17-estradiol acetate) was added to each tube. The tubes were capped and mixed by vortexing for 30 seconds. Then, 500 μL of the extraction reagent (95% ethyl acetate/5% methanol) were added to each tube. The tubes were capped and vortexed at high speed for 30 seconds and then centrifuged at 13,500 rpm

for 5 minutes in an Eppendorf microcentrifuge (Brinkmann Instruments). Following centrifugation, the supernatant organic layer, approximately 420 μL , was carefully removed into a clean microcentrifuge tube and dried under a stream of room air, using a low heat setting. For assay of conjugates, a second 200 μL plasma sample was mixed with β -glucuronidase (50 μL , 446 units) in 0.1 mol/L phosphate buffer (pH 6.8), and sulfatase (45 μL , 52 units) in 0.1 mol/L sodium acetate buffer (pH 5.0), incubated at 37°C for 3.5 hours, and then extracted. All the extraction procedures were performed under dim light to prevent the degradation of curcumin. The extract was then reconstituted in methanol (100 μL) before ultra-flow liquid chromatograph (UFLC) analysis.

Curcumin in colon biopsies was quantified by using Prominence UFLC (Shimadzu Corp.) equipped with EZStart software, Prominence UV-Vis detector. The separation was performed on a Symmetry C_{18} column (2.1 \times 100-mm i.d.; 3.5 μm ; Waters) with a Waters absorbosphere 30- \times 4.6-mm C_{18} guard column (Waters) at room temperature. The mobile phase used under gradient conditions was 25% acetonitrile with 74.9% deionized water containing 0.1% acetic acid (25:74.9:0.1; v/v/v; A) and 100% acetonitrile containing 0.1% acetic acid (99.9:0.1; v/v; B). The gradient conditions start from 20% B to 37% B in 8 minutes and then to 100% B in 4 minutes and maintained for another 2 minutes at 100% B before returning to opening conditions over 4 minutes. The flow rate was 0.3 mL/minute and injection volume for all samples was 10 μL . The detection of curcumin and β -17-estradiol acetate were performed at 420 and 280 nm, respectively.

The assays were validated by within and between day calibration curves and quality control low, medium, and high accuracy standards. The lower limit of detection for curcumin from human plasma was 5 ng/mL and from human colonic mucosa was 10 $\mu\text{g/g}$ protein.

Statistical considerations

We calculated that a sample size of 20 patients at each dose level would be sufficient for the 2-sided distribution-free Sign test, which is a nonparametric Sign test for detecting any significant reduction or change of value (either positive or negative), such that the change is not due to the null hypothesis of random symmetric variability, in the primary endpoint (level of PGE_2 in ACF tissue) with a significance level of 0.05 and power of 80%. The Sign test was chosen because 3 previous studies, even though demonstrating a significant reduction in prostaglandin concentrations, showed that subjects had a wide variation in prostaglandin concentrations at baseline (24–26). A 30-day intervention period was chosen because two of these studies demonstrated a significant reduction in prostaglandin concentrations in fewer than 30-days administration of Cox inhibitors (25, 26). We estimated a dropout rate of 15% and therefore set the accrual goal at 24 to end up with 20 evaluable participants for each dose level. Changes from baseline of tissue PGE_2 and 5-HETE concentrations were evaluated by the distribution-free Sign test. To assess pro-

liferation and ACF change, mean change in labeling per crypt compartment and ACF number were examined by paired *t* test. Pearson's correlation coefficient was calculated to assess the correlation between pre- and postintervention concentrations of curcumin.

Results

Participants

We consented 93 eligible patients (current smokers) referred for colorectal screening from October 2006 through October 2008 into the 2 stages of the trial (Fig. 1). Of these eligible patients, 62 (67%) were enrolled into the trial and 31 failed to meet study entry criteria for the following reasons: 19 for endoscopy failure (<8 ACF); 6 unable to complete the 30-day aspirin washout period; 6 for disqualifying illnesses on chart review. Forty-four enrolled patients began treatment after 7 withdrew consent and 11 were lost to follow-up prior to starting the curcumin intervention. Three of these 44 patients were not fully evaluable (1 was lost to follow-up; 1 withdrew; and 1 was noncompliant with treatment) and were not further evaluated because the risk of a second endoscopy was thought to be unjustified. Forty-one patients completed the trial and were included in our final toxicity and biomarker analyses, 22 on the 2-g and 19 on the 4-g dose (Table 1); 32 of these patients were enrolled at UIC and 9 at UM. We evaluated 17 males and 24 females; overall, 66% of patients were African American, 27% Caucasian, and 4% Latin American. The 2 participant groups (2 g and 4 g) were similar with regard to mean age ($P = 0.91$) and pack-years of smoking ($P = 0.74$).

Effects on PGE_2 and 5-HETE tissue concentrations

PGE_2 concentrations did not change significantly in the 2-g or 4-g cohort between baseline and postintervention (Table 2); all results [picogram $\mu\text{g/g}$ protein] are shown as mean \pm SD. In the 2-g cohort, PGE_2 concentrations in ACF tissue were (1.1 \pm 1.9 $\mu\text{g/g}$ protein (baseline) vs. 1.5 \pm 1.9 $\mu\text{g/g}$ protein (postintervention; $P = 0.344$) and in normal mucosa were 2.2 \pm 3.2 $\mu\text{g/g}$ protein (baseline) vs. 2.9 \pm 3.8 $\mu\text{g/g}$ protein (postintervention; $P = 0.119$). Although higher at baseline (with greater variability/standard deviations) than in the 2-g cohort (Supplementary Fig. 1 and 2), PGE_2 concentrations also did not change significantly in the 4-g cohort between baseline and postintervention either in ACF tissue [3.4 \pm 4.8 $\mu\text{g/g}$ protein (baseline) vs. 3.5 \pm 3.2 $\mu\text{g/g}$ protein (post-intervention); $P = 0.388$] or in normal mucosa [2.8 \pm 1.9 $\mu\text{g/g}$ protein (baseline) vs. 2.5 \pm 3.0 $\mu\text{g/g}$ protein (post-intervention); $P = 0.092$].

Curcumin also did not reduce 5-HETE concentrations in ACF or normal mucosa of the 2-g or 4-g cohort (Table 2). 5-HETE concentrations in the 2-g cohort were 1.4 \pm 1.1 $\mu\text{g/g}$ protein (postintervention) vs. 1.4 \pm 0.9 $\mu\text{g/g}$ protein (baseline) in ACF ($P = 0.453$) and 2.3 \pm 1.3 $\mu\text{g/g}$ protein (postintervention) vs. 2.4 \pm 1.1 $\mu\text{g/g}$ protein (baseline) in normal mucosa ($P = 0.688$). Concentrations in the 4-g

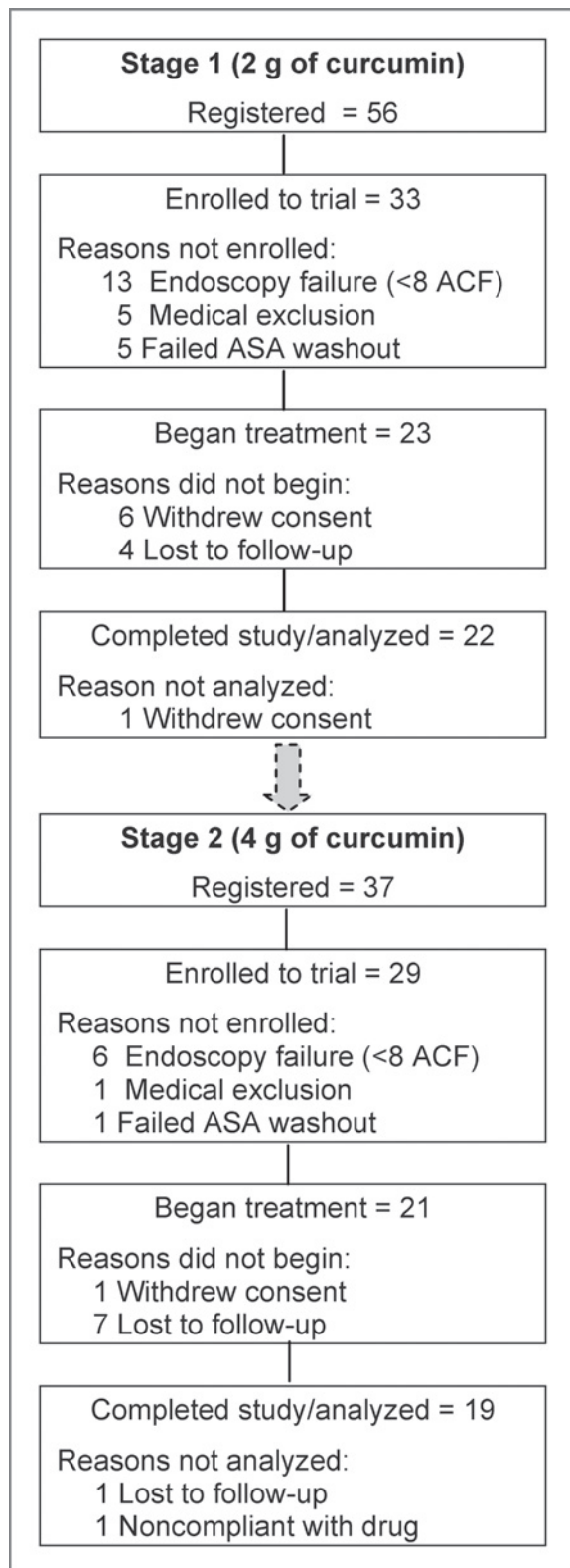


Figure 1. Study flow diagram. The overall 93 patients registered to this study included 56 registered in stage 1 and 37 in stage 2. Stage 2 (4 g) did not begin until a formal toxicity review of stage 1 (2 g) approved continuation of the study. ASA, acetylsalicylic acid (aspirin)

cohort were 2.4 ± 1.6 $\mu\text{g/g}$ protein (postintervention) vs. 1.8 ± 1.0 $\mu\text{g/g}$ protein (baseline) in ACF ($P = 0.727$) and 2.5 ± 1.5 $\mu\text{g/g}$ protein (postintervention) versus 1.6 ± 1.6 $\mu\text{g/g}$ protein (baseline) in normal mucosa ($P = 0.344$).

Effects on ACF number and mucosal proliferation

All ACF number results are shown as mean \pm SEM. The number of rectal ACF remained similar between baseline (15.0 ± 4.8) and postintervention (16.3 ± 6.6 ; $P = 0.51$) after curcumin at 2 g (Fig. 2). Curcumin at 4 g significantly decreased the number of rectal ACF (17.8 ± 2.0 , baseline, vs. 11.1 ± 2.8 , postintervention; $P < 0.005$; Fig. 2).

No significant differences in Ki-67-detected mucosal proliferation corresponded to the reduced ACF number in the 4-g group; Ki67 was assessed, however, in normal mucosa (Table 3), not in ACF tissue. Proliferation in the proximal, middle, and distal thirds of the crypt remained unchanged in response to 2 g and 4 g of curcumin, showing that the expected increase in proliferative index from the apex to the base of the crypt was maintained (at either dosing level; Table 3).

Curcumin and curcumin-conjugate concentrations

Concentrations of curcumin (parent compound) and curcumin conjugates were assayed in rectal mucosal biopsies and venous plasma and are shown as mean \pm SD in Table 4. Rectal mucosal biopsy samples for these assays were obtained from 39 of the 41 participants who completed curcumin treatment (21 at 2 g and 18 at 4 g). Curcumin was detected in baseline biopsy samples from 1 patient in the 2-g cohort and in postintervention samples from 5 patients in the 2-g and 3 in the 4 g cohort (Table 4). Curcumin conjugates were detected in baseline biopsy samples from 1 patient in the 4-g cohort and in postintervention samples from 13 patients in the 2-g and 12 in the 4-g cohort (a total of 25, or 64%, of the 39 patients with assayed biopsies). The mean \pm SD postintervention level of curcumin was 8.2 ± 2.9 $\mu\text{g/g}$ protein (range, 4.7–11.6 $\mu\text{g/g}$ protein) in the 2-g cohort (5 samples) and 3.8 ± 0.6 $\mu\text{g/g}$ protein (range, 3.1–4.3 $\mu\text{g/g}$ protein) in the 4-g cohort (3 samples). The detection of curcumin or its conjugates in rectal mucosal biopsies did not correlate with significant changes in concentrations of either tissue PGE₂ or 5-HETE or reductions in ACF formation (data not shown).

Venous plasma samples for curcumin/curcumin-conjugate assays were obtained from all 19 participants who completed study in the 4-g cohort (Table 4); no venous plasma samples were assayed in the 2-g cohort; all concentrations are shown in mean \pm SD. Concentrations at baseline were 7.3 ± 8.1 ng/mL (range, 1.4–19.1) for curcumin (detected in 4 of 19 samples) and 15.8 ± 14.8 ng/mL (range, 4.6–56.5 ng/mL) for curcumin conjugates (detected in all 19 samples). Two post-intervention plasma samples had detectable curcumin (3.8 ± 1.3 ng/mL; range, 2.9–4.7 ng/mL), whereas all 19 samples had detectable curcumin conjugates (78.5 ± 84.3 ng/mL; range 9.2–382.7). Postintervention curcumin conjugate concentrations rose significantly during treatment in the 4-g group ($P = 0.009$).

Table 1. Summary of patient demographics

	Curcumin dose group	
	2 g (n = 22)	4 g (n = 19)
Age, y (mean ± SD)	57 ± 5	54 ± 7
Range, y	51–70	42–69
Personal/family history		
Adenoma	1/1 (9%)	1/2(15.8%)
Colon Cancer	0/2 (9%)	0/0 (0%)
Gender		
Female	13 (59%)	11 (58%)
Male	9 (41%)	8 (42%)
Race/ethnicity, %		
Caucasian	3 (13.6%)	8 (42.1%)
African American	18 (81.8%)	9(47.4%) ^a
Latino American	1 (4.6%)	1(5.3%)*
Amerindian	0 (0%)	1(5.3%)
Asian	0 (0%)	1(5.3%)
Tobacco pack-years (mean)	21.75	22.8
1–15 pack-years	10 (45%)	5(26%)
16–29 pack-years	5 (23%)	8(42%)
≥ 30 pack-years	7 (32%)	6(32%)
ACF number pretreatment (mean ± SEM)	15.0 ± 4.8	17.8 ± 2.0

^aOne participant self-identified as both Latino- and African American.

Toxicity

Toxicity was reviewed by site principal investigators and Data Monitoring boards at the University of California Irvine and UM. Curcumin was well tolerated at either 2 g or 4 g. Stage 2 (curcumin at 4 g) was initiated after a formal review of stage 1 (2 g) found that toxicity at 2 g was acceptable (according to criteria described in Methods). In the 22 stage-1 patients, 13 (59%) had grade-1 and -2 toxicity (none requiring drug discontinuation) and none had grade-3 toxicity. In the 19 stage-2 participants, 12 (63%) had grade-1 and -2 toxicity and 1 (2%) had

grade-3 toxicity. Overall, 25 of 41 participants (61%) had grade-1 and -2 toxicity, primarily gastrointestinal disturbances [diarrhea (most prevalently) and distension and gastroesophageal reflux disease, which responded to loperamide and metoclopramide, respectively], with none requiring drug discontinuation. The single grade-3 toxicity was atypical chest pain and was unrelated to curcumin. Liver function tests were monitored for subclinical toxicity, and no clinically significant elevations were detected for curcumin at 2 g or 4 g.

Discussion

Curcumin at either 4 g or 2 g per day for 30 days did not reduce concentrations of PGE₂ (the primary endpoint) or 5-HETE in ACF or normal flat mucosa, in contrast to previous data in preclinical rodent colorectal cancer models (27). Curcumin at 4 g/day for 30 days, however, significantly reduced ACF formation.

Unlike previously reported elevated concentrations of the eicosanoids PGE₂ and 5-HETE measured in the bronchial epithelium of smokers compared with nonsmokers (28), PGE₂ and 5-HETE were not significantly altered in flat rectal mucosa of smokers compared with that described in the same mucosa of nonsmokers (25). This similarity in concentrations suggests a lack of an inflammatory response to tobacco components in the epithelium of the distal gastrointestinal tract, in marked contrast to inflammatory changes that occur in bronchial mucosa of smokers. Neither dose of curcumin reduced or changed the concentrations of these 2 eicosanoids in the present study. A trial of celecoxib in familial adenomatous polyposis patients similarly found that the intervention failed to reduce PGE₂ concentrations in adenomas or normal rectal mucosa despite significantly reducing the number of adenomas (29). Furthermore, a recent study showed no significant change in leukotriene B₄ (which is downstream of 5-HETE) during colorectal carcinogenesis (30). Unlike data in rodent models (31), our present human data suggest that Cox-1 or -2 or 5-Lox activity does not correlate with a reduction in ACF. Curcumin may exert its anticarcinogenic effects via alternative signaling pathways or via inhibition

Table 2. Effects of curcumin on PGE₂ and 5 HETE

	PGE ₂ and 5-HETE concentrations			
	2 g pre	2 g post	4 g pre	4 g post
PGE-2 ACF	1.1 ± 1.8*	1.6 ± 1.8	3.4 ± 4.8*	3.7 ± 3.2
PGE-2 NL mucosa	2.1 ± 3.2*	2.7 ± 3.7	2.7 ± 1.9*	2.6 ± 2.9
5-HETE ACF	1.4 ± 0.9*	1.4 ± 1.0	2.3 ± 1.6*	1.9 ± 0.9
5-HETE NL mucosa	2.3 ± 1.3*	2.4 ± 1.2	2.5 ± 1.5*	2.2 ± 1.6

*NOTE: Data are expressed as mean ± SD, µg/g protein.

No significant difference between pre- and posttreatment values by the Sign test at a significance level of 0.05.

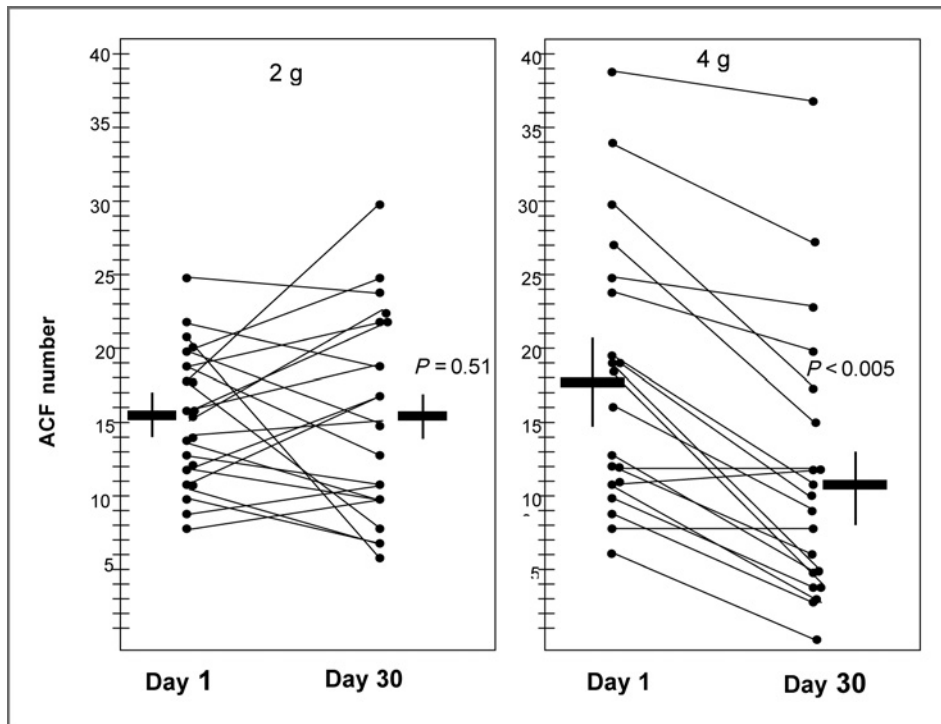


Figure 2. Human rectum with ACF number at initial exam and 30 days posttreatment with 2 g or 4 g of curcumin. For each dose group, horizontal lines indicate the mean value for pre- and posttreatment levels in each dose group, with the standard error of the mean indicated by the endpoints of the corresponding vertical lines. Pre- and posttreatment levels of ACF number are plotted for each individual patient. The paired *t* test was applied to examine change from pretreatment levels in ACF number at a significance level 0.05.

of upstream events, for example, its known inhibition of nuclear factor kappa B (NF-κB) release (32) upstream of the eicosanoid system.

Our and others' previous data (10, 11) suggest that the systemic bioavailability of curcumin is very poor in humans. Is the present study's lack of curcumin effect on eicosanoids in flat rectal mucosa due to poor bioavailability of both directly delivered/topical and systemic curcumin? Our present data demonstrate very low detection rates for curcumin in rectal flat mucosa or ACF before or after 30 days of daily administration. On the other hand, we found a far higher rate of detection for glucuronide and sulfate conjugates of curcumin following a month of daily curcumin. Although curcumin conjugates were detected pretherapy in plasma, their levels were much higher following the month-long daily dosing (4 g). Our previous data did not find circulating conjugate concentrations prior to curcumin administration (10,12). This discrepancy with

curcumin conjugate detection at baseline (pretreatment) in the present study may be explained by improved analytical sensitivity. Our prior analytical sensitivity ranged from 50 ng/mL to 75 ng/mL (12, 33). Using UFLC and improved UV detection technology, we have reduced our low limit of quantitation of curcumin extracted from plasma to 5 ng/mL, similar to other recently reported UFLC analytical detection limits (34). The concentrations we now report are below our previously reported analytical detection limits (10, 12) because of the more-sensitive analytical assay for curcumin.

These data might be interpreted as demonstrating that curcumin is not absorbed in rectal mucosa and that conjugate concentrations detected in the rectal mucosa are delivered systemically from absorption and conjugation higher in the gastrointestinal tract. Our matched plasma concentrations support this hypothesis. This result is surprising because the stools in our participants turned yellow,

Table 3. Proliferation index (% labeled cells) before and after treatment

Crypt Segment	2 g pre	2 g post	4 g pre	4 g post
Proximal third	0.5 ± 0.9 ^a	0.6 ± 1.4	0.3 ± 0.6 ^a	0.3 ± 0.8
Middle third	11.9 ± 4.2 ^a	11.8 ± 7.2	12.8 ± 6.1 ^a	16.4 ± 10.3
Distal third	25.2 ± 5.3 ^a	23.5 ± 8.0	28.7 ± 9.1 ^a	30.9 ± 7.7

NOTE: Data are expressed as mean ± SD. Proliferation index is calculated as number of labeled cells/number of total cells in that portion of the crypt which is arbitrarily divided into 3 segments.

^aNo significant difference between pre- and posttreatment values by paired *t* test, at a significance level of 0.05.

Table 4. Curcumin concentrations in tissue and plasma by dose

	2 g		4 g	
	Pre	Post	Pre	Post
Rectal mucosa ^a		<i>n</i> = 21		<i>n</i> = 18
Curcumin	4.03 (1)	8.2 ± 2.9 (5)	ND	3.8 ± 0.6 (3)
Conjugates	ND	5.9 ± 2.6 (13)	4.21 (1)	4.5 ± 1.7 (12)
Plasma ^b		<i>n</i> = 0		<i>n</i> = 19
Curcumin	NA	NA	7.3 ± 8.1 (4)	3.8 ± 1.3 (2)
Conjugates	NA	NA	15.8 ± 14.8 (19)	78.5 ± 84.3 ^c (19)

^aMeasures are mean ± SD in µg/g protein (number of samples with detectable levels).

^bMeasures are mean ± SD in ng/mL (number of samples with detectable levels).

^c*P* = 0.009 for increased post-intervention level (versus pre-intervention level).

Abbreviations: Pre, pre-intervention; Post, post-intervention; ND, not detected; NA, not assayed.

the color of curcumin. We did not assay stool samples to confirm that the yellow color indeed reflected the presence of curcumin. Alternatively, our data might suggest that curcumin is absorbed and rapidly and fully conjugated in the rectal mucosa. The recently published rodent data of Marczyklo and colleagues suggest much higher curcumin concentrations in intestinal mucosa compared with plasma in rats given 340 mg/kg of Meriva, a phosphatidylcholine formulation of curcumin (35). This formulation may protect curcumin from rapid conjugation in intestinal mucosa and the liver.

Our present data suggest that healthy people eating a Western diet may ingest regular, small quantities of curcumin perhaps in the form of mustard or spice mixtures. The absorbed, dietary curcumin is conjugated, circulates systemically and partitions to tissues as shown by measured curcumin conjugate concentrations in rectal biopsies pre- and postcurcumin treatment in this trial, thus leading to a large volume of distribution and long conjugate half-lives. We previously demonstrated the long half-lives (6–24 hours) of curcumin glucuronide and sulfate conjugates in human plasma (12).

Curcumin has anticarcinogenesis effects when delivered systemically in immunodeficient mouse models (36) and in humans with pancreatic adenocarcinoma (23). Yet in humans, we and Dhillon and colleagues infrequently detected curcumin concentrations systemically (plasma) and locally (rectal mucosa). These findings raise the question of whether curcumin conjugates or other breakdown products of curcumin, such as vanillin, ferulic acid, and feruloylmethane, may be active metabolites. A mixture of these curcumin breakdown products did not alter the numbers or the mitotic cycle of Ishikawa cells (37), but no *in vitro* or *in vivo* data assessing the conjugate cellular pharmacology or potential anticarcinogenesis effects are available to date.

Despite the evidence of poor local tissue concentrations and a lack of effect on key rectal mucosal eicosanoids, our data demonstrate an important anticarcinogenic effect of

curcumin – significant reduction of ACF at the 4-g dosing level. This finding raises the question of the *in vivo* mechanism(s) by which curcumin may have cancer-preventive effects in the setting of early preinvasive neoplastic lesions. Potential mechanisms may involve downregulation of different signaling cascades such as epidermal growth factor receptor (EGFR) or insulin-like growth factor receptor (IGFR) signaling, either of which has been linked to ACF formation (38, 39) and is known to be inhibited by curcumin in cell-culture models (40). Alternatively, curcumin may act via influencing Notch signaling, as shown in pancreatic cell lines (41), and Wnt signaling, as shown in breast-cancer stem cells (42). These Wnt and Notch signaling pathways have recently been shown to cooperatively influence proliferation and tumor formation in colon crypts (43).

Another potential curcumin mechanism involves tobacco-smoke biology. Tobacco smoke is known to contain the carcinogen benzo-[a]-pyrene, which is activated by colonic cytochrome P450 1B1 (CYP 1B1) to more-mutagenic and -carcinogenic epoxide intermediates that induce DNA adduct formation (44). Curcumin is capable of inhibiting CYP 1B1 activity *in vitro* and may inhibit the phase-1 activation of tobacco carcinogens, subsequently reducing DNA damage directly (45).

The reduction of ACF by agents in preclinical animal models of chemically induced carcinogenesis typically is associated with reduced proliferation (46), reduced inflammation (47), and/or increased apoptosis (48). Although we did not see changes in either proliferation or inflammatory markers in the present clinical study, we did not assay apoptotic markers in ACF or normal mucosa. Curcumin enhances apoptosis effects in colon adenocarcinoma cell lines through sphingomyelinase inhibition (49) and p21-independent mechanisms (50). Furthermore, apoptotic markers have recently been shown to be reduced in cancer subjects with ACF (51), suggesting that cancer-reductive agents that upregulate apoptotic pathways could reduce the incidence of ACF.

ACF as a biomarker of colon carcinogenesis in human studies remains controversial. Two clinical studies in Japan showed a significant association between increased ACF number in the distal rectum with colorectal adenomas and/or cancer, compared with normal controls (52, 53). Recent studies from the United States, however, failed to show a significant correlation between ACF number and true adenomas (54, 6). Such differences may be related to racial disparities. In such a context, the positive effect of curcumin at 4 g on ACF in the predominately African American population of our trial may be important. African Americans have increased rates of and mortality from colon cancer (21), in addition to a disproportionate burden of smoking-attributable cancer mortality (55), compared with Caucasian Americans.

Tobacco smoke contains active carcinogens that concentrate in colonic mucosa (56). For example, benzo-[a]-pyrene is known to produce ACF within the colons of rats and mice (57) and is associated with increased DNA adduct formation. Similar adducts that have been identified in the lung tissue of human smokers, increase linearly with tobacco consumption (58) and are strongly linked to colorectal cancer when present in the distal colon (59). In a study of curcumin given for 7 days preoperatively to colorectal cancer patients, 3.6 g of curcumin was found to reduce DNA adducts in malignant but not adjacent normal mucosa, an effect not observed at lower doses (0.45 g and 1.8 g; ref. 60), consistent with our findings of an ACF reduction at 4 g but not at 2 g. These data support the concept that ACF are a neoplastic lesion and might be a useful biomarker of both DNA damage and smoking-associated carcinogen exposure in humans. These earlier and our present data suggest that ACF reduction is a potentially important biomarker for monitoring chemopreventive efficacy in African Americans.

A major direction of future curcumin studies should be the optimization of its bioavailability through improved gastrointestinal absorption and systemic distribution to tissues (61). Pharmaceutical approaches to overcome these

limitations include synthesis of curcumin analogues, the use of agents to increase curcumin absorption (e.g., piperine), and the development of modified drug-delivery systems, including liposomal, nanoparticulated, and phospholipid complex formulations of curcumin (62, 63).

In conclusion, we have demonstrated that a short duration of curcumin treatment reduces ACF number. Our finding that oral, 98%-pure curcumin significantly reduced ACF number in humans confirms preclinical observations regarding changes in ACF in response to curcuminoid mixtures (consisting of curcumin, bisdemethoxy, and demethoxycurcumin), but the mechanism(s) by which this occurs remain unanswered. The treatment-related increase in conjugate concentrations in plasma suggests that the ACF reduction resulted from systemic rather than local delivery of conjugates. These data verify the data of others: despite a lack of systemically bioavailable concentrations of curcumin, anticarcinogenic activity occurs in tissue targets. If these results can be confirmed in further trials in high-risk adenoma populations and if the mechanisms by which curcumin reduces ACF can be identified, they would further support the use of curcumin as a cancer prevention agent and would strengthen the utility of ACF as a biomarker for clinical colon-cancer studies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

This work was supported by NIH, National Cancer Institute contract NCI-N01-CN-35160 (to R. E. Carroll), NCI-K07-CA12884 (to M. Kakarala), and grants M01-RR000042 and UL1RR024986 to the University of Michigan, and the Kutche Family Professorship (to D.E. Brenner). P30-CA62203 and contract NO-1 CN-25000(39; to F. L. Meyskens, Jr)

Received June 15, 2010; revised November 30, 2010; accepted January 17, 2011; published online March 3, 2011.

References

- Rao CV, Rivenson A, Simi B, Reddy BS. Chemoprevention of colon carcinogenesis by dietary curcumin, a naturally occurring plant phenolic compound. *Cancer Res* 1995;55:259-66.
- Mukhopadhyay A, Basu N, Ghatak N, Gujral PK. Anti-inflammatory and irritant activities of curcumin analogues in rats. *Agents Actions* 1982;12:508-15.
- Srimal RC, Dhawan BN. Pharmacology of diferuloyl methane (curcumin), a non-steroidal anti-inflammatory agent. *J Pharm Pharmacol* 1973;25:447-52.
- Huang M, Lysz T, Ferraro T, Abidi T, Laskin J, Conney A. Inhibitory effects of curcumin on in vitro lipoxygenase and cyclooxygenase activity. *Cancer Res* 1991;51:813-9.
- Moxon D, Raza M, Kenney R, Ewing R, Arozullah A, Mason JB, et al. Relationship of aging and tobacco use with the development of aberrant crypt foci in a predominantly African-American population. *Clin Gastroenterol Hepatol* 2005;3:271-8.
- Mutch MG, Schoen RE, Fleshman JW, Rall CJ, Dry S, Seligson D, et al. A multicenter study of prevalence and risk factors for aberrant crypt foci. *Clin Gastroenterol Hepatol* 2009;7:568-74.
- Bruce WR, Archer MC, Corpet DE, Medline A, Minkin S, Stamp D, et al. Diet, aberrant crypt foci and colorectal cancer. *Mutat Res* 1993; 290:111-8.
- Hosono K, Endo H, Takahashi H, Sugiyama M, Sakai E, Uchiyama T, et al. Metformin suppresses colorectal aberrant crypt foci in a short-term clinical trial. *Cancer Prev Res* 2010;3:1077-83.
- Corpet DE, Tache S. Most effective colon cancer chemopreventive agents in rats: a systematic review of aberrant crypt foci and tumor data, ranked by potency. *Nutr Cancr* 2002;43:1-21.
- Lao CD, Ruffin MT, Normolle D, Heath DD, Murray SI, Bailey JM, et al. Dose escalation of a curcuminoid formulation. *BMC Complement Altern Med* 2006;6:6-10.

11. Sharma RA, Euden SA, Platton SL, Cooke DN, Shafayat A, Hewitt HR, et al. Phase I clinical trial of oral curcumin: biomarkers of systemic activity and compliance. *Clin Cancer Res* 2004;10:6847–54.
12. Vareed SK, Kakarala M, Ruffin MT, Crowell JA, Normolle DP, Djuric Z, et al. Pharmacokinetics of curcumin conjugate metabolites in healthy human subjects. *Cancer Epidemiol Biomarkers Prev* 2008;17:1411–7.
13. Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB. Bioavailability of curcumin: problems and promises. *Mol Pharm* 2007;4:807–18.
14. Cruz-Correa M, Shoskes DA, Sanchez P, et al. Combination treatment with curcumin and quercetin of adenomas in familial adenomatous polyposis. *Clin Gastroenterol Hepatol* 2006;4:1035–8.
15. Hanai H, Iida T, Takeuchi K, Watanabe F, Maruyama Y, Andoh A, et al. Curcumin maintenance therapy for ulcerative colitis: randomized, multicenter, double-blind, placebo-controlled trial. *Clin Gastroenterol Hepatol* 2006;4:1502–6.
16. Carroll RE. Colon preparation for magnification endoscopy: a rapid novel approach. *Endoscopy* 2004;36:1–4.
17. McLellan EA, Bird RP. Aberrant crypts: Potential preneoplastic lesions in the murine colon. *Cancer Res* 1988;48:6187–92.
18. Finley PR, Bogert CL, Alberts DS, Einspahr J, Earnest DL, Blackwell G, et al. Measurement of prostaglandin E2 in rectal mucosa in human subjects: a method study. *Cancer Epidemiol Biomarkers Prev* 1995;4:239–44.
19. Yang P, Chan D, Felix E, Madden T, Klein RD, Shureiqi I, et al. Determination of endogenous tissue inflammation profiles by LC/MS/MS: COX- and LOX-derived bioactive lipids. *Prostaglandins Leukot Essent Fatty Acids* 2006;75:385–95.
20. Rego RL, Foster NR, Smyrk TC, Le M, O'Connell MJ, Sargent DJ, et al. Prognostic effect of activated EGFR expression in human colon carcinomas: comparison with EGFR status. *Br J Cancer* 2010;102:165–72.
21. O'Keefe SJ, Chung D, Mahmoud N, Sepulveda AR, Manafe M, Arch J, et al. Why do African Americans get more colon cancer than Native Africans? *J Nutr* 2007;137:175S–82S.
22. Carpenter PM, Linden KG, McLaren CE, Li KT, Arain S, Barr RJ, et al. Nuclear morphometry and molecular biomarkers of actinic keratosis, sun-damaged, and nonexposed skin. *Cancer Epidemiol Biomarkers Prev* 2004;13:1996–2002.
23. Dhillon N, Aggarwal BB, Newman RA, Wolff RA, Kunnumakkara AB, Abbruzzese JL, et al. Phase II trial of curcumin in patients with advanced pancreatic cancer. *Clin Cancer Res* 2008;14:4491–9.
24. Giardello FM, Casero RJ, Hamilton S, Hyland L, Trimbath J, Geiman D, et al. Prostanoids, ornithine decarboxylase, and polyamines in primary chemoprevention of familial adenomatous polyposis. *Gastroenterology* 2004;126:425–31.
25. Krishnan K, Ruffin MT, Normolle D, Shureiqi I, Burney K, Bailey J, et al. Colonic mucosal prostaglandin-E2 and cyclooxygenase expression before and after low aspirin doses in subjects at high risk or at normal risk for colorectal cancer. *Cancer Epidemiol Biomarkers Prev* 2001;10:447–53.
26. Ruffin MT, Krishnan K, Rock CL, Normolle D, Vaerten MA, Peters-Golden M, et al. Suppression of human colorectal mucosal prostaglandins: determining the lowest effective aspirin dose. *J Natl Cancer Inst* 1997;89:1152–60.
27. Huang M, Lou Y, Ma W, Newmark H, Reuhl K, Conney A. Inhibitory effects of dietary curcumin on forestomach, duodenal, and colon carcinogenesis in mice. *Cancer Res* 1994;54:5841–7.
28. Profita M, Sala A, Bonanno A, Riccobono L, Ferrara M, La Grutta S, et al. Chronic obstructive pulmonary disease and neutrophil infiltration: role of cigarette smoke and cyclooxygenase products. *Am J Physiol Lung Cell Mol Physiol* 2009;298:L261–9.
29. Sinicrope FA, Half E, Morris JS, Lynch PM, Morrow JD, Levin B, et al. Cell proliferation and apoptotic indices predict adenoma regression in a placebo-controlled trial of celecoxib in familial adenomatous polyposis patients. *Cancer Epidemiol Biomarkers Prev* 2004;13:920–7.
30. Shureiqi I, Chen D, Day RS, Zuo X, Hochman FL, Ross WA, et al. Profiling lipoxygenase metabolism in specific steps of colorectal tumorigenesis. *Cancer Prev Res* 2010;3:829–38.
31. Watanabe K, Kawamori T, Nakatsugi S, Ohta T, Ohuchida S, Yamamoto H, et al. Role of the prostaglandin E receptor subtype EP-1 in colon carcinogenesis. *Cancer Res* 1999;59:5093–6.
32. Sandur SK, Deorukhkar A, Pandey MK, Pabon AM, Shentu S, Guha S, et al. Curcumin modulates the radiosensitivity of colorectal cancer cells by suppressing constitutive and inducible NF-kappaB activity. *Int J Radiat Oncol Biol Phys* 2009;75:534–42.
33. Heath DD, Pruitt MA, Brenner DE, CL R. Curcumin in plasma and urine: quantitation by high-performance liquid chromatography. *J Chromatogr B Analyt technol biomed Life Sci* 2003;783:287–95.
34. Marczylo TH, Steward WP, Gescher AJ. Rapid analysis of curcumin and curcumin metabolites in rat biomatrices using a novel ultraperformance liquid chromatography (UPLC) method. *J Agric Food Chem* 2009;57:797–803.
35. Marczylo TH, Verschoyle RD, Cooke DN, Morazzoni P, Steward WP, Gescher AJ. Comparison of systemic availability of curcumin with that of curcumin formulated with phosphatidylcholine. *Cancer Chemother Pharmacol* 2007;60:171–7.
36. Bachmeier B, Nerlich AG, Iancu CM, Cilli M, Schleicher E, Vene R, et al. The chemopreventive polyphenol Curcumin prevents hematogenous breast cancer metastases in immunodeficient mice. *Cell Physiol Biochem* 2007;19:137–52.
37. Dempe JS, Pfeiffer E, Grimm AS, Metzler M. Metabolism of curcumin and induction of mitotic catastrophe in human cancer cells. *Mol Nutr Food Res* 2008;52:1074–81.
38. Carroll RE, Goodlad RA, Poole AJ, Tyner AL, Robey RB, Swanson SM, et al. Reduced susceptibility to azoxymethane-induced aberrant crypt foci formation and colon cancer in growth hormone deficient rats. *Growth Horm IGF Res* 2009;19:447–56.
39. Dougherty U, Sehdev A, Cerda S, Mustafi R, Little N, Yuan W, et al. Epidermal growth factor receptor controls flat dysplastic aberrant crypt foci development and colon cancer progression in the rat azoxymethane model. *Clin Cancer Res* 2008;14:2253–62.
40. Patel BB, Sengupta R, Qazi S, Vachhani H, Yu Y, Rishi AK, et al. Curcumin enhances the effects of 5-fluorouracil and oxaliplatin in mediating growth inhibition of colon cancer cells by modulating EGFR and IGF-1R. *Int J Cancer* 2008;122:267–73.
41. Wang Z, Zhang Y, Banerjee S, Li Y, Sarkar FH. Notch-1 down-regulation by curcumin is associated with the inhibition of cell growth and the induction of apoptosis in pancreatic cancer cells. *Cancer* 2006;106:2503–13.
42. Kakarala M, Brenner DE, Korkaya H, Cheng C, Tazi K, Ginestier C, et al. Targeting breast stem cells with the cancer preventive compounds curcumin and piperine. *Breast Cancer Res Treat* 2010;122:777–85.
43. Fre S, Pallavi SK, Huyghe M, Lae M, Janssen KP, Robine S, et al. Notch and Wnt signals cooperatively control cell proliferation and tumorigenesis in the intestine. *Proc Natl Acad Sci U S A* 2009;106:6309–14.
44. Port JL, Yamaguchi K, Du B, De Lorenzo M, Chang M, Heerdt PM, et al. Tobacco smoke induces CYP1B1 in the aerodigestive tract. *Carcinogenesis* 2004;25:2275–81.
45. Walle T, Walle UK. Novel methoxylated flavone inhibitors of cytochrome P450 1B1 in SCC-9 human oral cancer cells. *J Pharm Pharmacol* 2007;59:857–62.
46. Carroll RE, Goodlad RA, Poole AJ, Tyner AL, Robey RB, Swanson SM, et al. Reduced susceptibility to azoxymethane-induced aberrant crypt foci formation and colon cancer in growth hormone deficient rats. *Growth Horm IGF Res* 2009;19:447–56.
47. Hagos GK, Carroll RE, Kouznetsova T, Li Q, Toader V, Fernandez PA, et al. Colon cancer chemoprevention by a novel NO chimera that shows anti-inflammatory and antiproliferative activity in vitro and in vivo. *Mol Cancer Ther* 2007;6:2230–9.
48. Poole A, Heap D, Carroll RE, Tyner A. Tumor suppressor functions for the Cdk inhibitor p21 in the mouse colon. *Oncogene* 2004;23:8128–34.
49. Cheng Y, Kozubek A, Ohlsson L, Sternby B, Duan RD. Curcumin decreases acid sphingomyelinase activity in colon cancer Caco-2 cells. *Planta Med* 2007;73:725–30.
50. Watson JL, Hill R, Lee PW, Giacomantonio CA, Hoskin DW. Curcumin induces apoptosis in HCT-116 human colon cancer cells in a p21-independent manner. *Exp Mol Pathol* 2008;8:230–3.
51. Figueiredo P, Donato M, Urbano M, Goulao H, Gouveia H, Sofia C, et al. Aberrant crypt foci: endoscopic assessment and cell kinetics characterization. *Int J Colorectal Dis* 2009;24:441–50.

52. Takayama T, Katsuki S, Takahashi Y, Ohi M, Nojiri S, Sakamaki S, et al. Aberrant crypt foci of the colon as precursors of adenoma and cancer. *N Engl J Med* 1998;339:1277–84.
53. Seike K, Koda K, Oda K, Kosugi C, Shimizu K, Nishimura M, et al. Assessment of rectal aberrant crypt foci by standard chromoscopy and its predictive value for colonic advanced neoplasms. *Am J Gastroenterol* 2006;101:1362–9.
54. Adler D, Gostout C, Sorbi D, Burgart L, Wang L, Harmsen W. Endoscopic identification and quantification of aberrant crypt foci in the human colon. *Gastrointest Endosc* 2002;56:657–62.
55. Rivo ML, Kofie V, Schwartz E, Levy ME, R.V. T. Comparisons of black and white smoking attributable mortality, morbidity, and economic costs in the District of Columbia. *J Natl Med Assoc* 1989;81:1125–30.
56. Boysen G, Hecht SS. Analysis of DNA and protein adducts of benzo[a]pyrene in human tissues using structure-specific methods. *Mutat Res* 2003;543:17–30.
57. Tudek B, Bird RP, Bruce WR. Foci of aberrant crypts in the colons of mice and rats exposed to carcinogens associated with foods. *Cancer Res* 1989;49:1236–40.
58. Phillips DH, Hewer A, Martin CN, Garner RC, King MM. Correlation of DNA adduct levels in human lung with cigarette smoking. *Nature* 1988;336:790–2.
59. Pfohl-Leschkowicz A, Grosse Y, Carriere V, Cugnenc PH, Berger A, Carnot F, et al. High levels of DNA adducts in human colon are associated with colorectal cancer. *Cancer Res* 1995;55:5611–16.
60. Garcea G, Berry DP, Jones DJ, Singh R, Dennison AR, Farmer PB, et al. Consumption of the putative chemopreventive agent curcumin by cancer patients: assessment of curcumin levels in the colorectum and their pharmacodynamic consequences. *Cancer Epidemiol Biomarkers Prev* 2005;14:120–5.
61. Cui J, Yu B, Zhao Y, Zhu W, Li H, Lou H, et al. Enhancement of oral absorption of curcumin by self-microemulsifying drug delivery systems. *Int J Pharm* 2009;371:148–55.
62. Bisht S, Maitra A. Systemic delivery of curcumin: 21st century solutions for an ancient conundrum. *Curr Drug Discov Technol* 2009;6:192–9.
63. Shahani K, Swaminathan SK, Freeman D, Blum A, Ma L, Panyam J. Injectable sustained release microparticles of curcumin: a new concept for cancer chemoprevention. *Cancer Res* 2010;70:4443–52.