

# Chemopreventive Efficacy and Pharmacokinetics of Curcumin in the Min/+ Mouse, a Model of Familial Adenomatous Polyposis<sup>1</sup>

Sarah Perkins, Richard D. Verschoyle, Kirsti Hill, Ifat Parveen, Michael D. Threadgill, Ricky A. Sharma, Marion L. Williams, William P. Steward, and Andreas J. Gescher<sup>2</sup>

Cancer Biomarkers and Prevention Group, Department of Oncology, University of Leicester, Leicester, LE1 9HN [S. P., R. D. V., K. H., R. A. S., M. L. W., W. P. S., A. J. G.], and Department of Pharmaceutical Sciences, University of Bath, Bath BA2 7AY [I. P., M. D. T.], United Kingdom

## Abstract

Curcumin, the major yellow pigment in turmeric, prevents the development of adenomas in the intestinal tract of the C57Bl/6J Min/+ mouse, a model of human familial APC. To aid the rational development of curcumin as a colorectal cancer-preventive agent, we explored the link between its chemopreventive potency in the Min/+ mouse and levels of drug and metabolites in target tissue and plasma. Mice received dietary curcumin for 15 weeks, after which adenomas were enumerated. Levels of curcumin and metabolites were determined by high-performance liquid chromatography in plasma, tissues, and feces of mice after either long-term ingestion of dietary curcumin or a single dose of [<sup>14</sup>C]curcumin (100 mg/kg) via the i.p. route. Whereas curcumin at 0.1% in the diet was without effect, at 0.2 and 0.5%, it reduced adenoma multiplicity by 39 and 40%, respectively, compared with untreated mice. Hematocrit values in untreated Min/+ mice were drastically reduced compared with those in wild-type C57Bl/6J mice. Dietary curcumin partially restored the suppressed hematocrit. Traces of curcumin were detected in the plasma. Its concentration in the small intestinal mucosa, between 39 and 240 nmol/g of tissue, reflects differences in dietary concentration. [<sup>14</sup>C]Curcumin disappeared rapidly from tissues and plasma within 2–8 h after dosing. Curcumin may be useful in the chemoprevention of human intestinal malignancies related to *Apc* mutations. The comparison of dose, resulting curcumin levels in the intestinal tract, and chemopreventive potency suggests tentatively that a daily dose of 1.6 g of curcumin is required for efficacy in humans. A clear advantage of curcumin over nonsteroidal anti-inflammatory drugs is its ability to

decrease intestinal bleeding linked to adenoma maturation.

## Introduction

Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] is the major yellow pigment extracted from turmeric, a commonly used spice, derived from the rhizome of the plant *Curcuma longa*. In India and Southeast Asia, turmeric has long been used as a treatment for inflammation, skin wounds, and tumors. Curcumin has broad spectrum cancer chemopreventive activity in preclinical animal models (reviewed in Ref. 1). Especially intriguing is its ability to prevent carcinogen-induced intestinal premalignancies and malignancies in rats (2, 3) and in the Min/+ mouse, a model of hereditary FAP<sup>3</sup> (4). The Min/+ mouse has an autosomal dominant heterozygous nonsense mutation of the mouse *Apc* gene (5); homologous to human germ-line and somatic *APC* mutations. The C57Bl/6J Min/+ inbred mouse model is particularly advantageous for investigating chemopreventive agents targeted toward early-stage lesions because, in this mutant strain, scores of adenomas grow to a detectable size within a few months on a defined genetic background (6). Because Min/+ mice develop adenomas as a result of inactivation of the same tumor suppressor gene known to underlie the pathogenesis of most colon cancers in humans, experiments using this model are likely to be germane to the design of human chemoprevention trials (7). This notion has recently been illustrated impressively by the selective COX-2 inhibitor celecoxib, the development of which was advanced substantially by evaluation of its efficacy in Min/+ mice (8). This mouse model predicted the significant reduction, or retardation, of adenoma development that was subsequently seen in FAP patients (9), a result that, in turn, led to the approval by the United States Food and Drug Administration of celecoxib for the treatment of FAP (10).

The cancer chemopreventive activity of curcumin in humans has yet to be confirmed. A recent clinical pilot study in colorectal cancer patients (11) and results of experiments in rodents (12–14) suggest that the systemic availability of curcumin is poor. This conclusion mitigates against its use for the chemoprevention of malignancies remote from the site of absorption, but it would not preclude its development for the prevention of gastrointestinal neoplasias. We wished to evaluate the link between the pharmacological activity of curcumin in the Min/+ mouse and levels of curcumin and its metabolites in target tissue and plasma. To that end, curcumin was administered in the diet at three dose levels, efficacy was assessed in terms of adenoma load, and agent and metabolites were quantitated. A major complication associated with adenoma load in

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<sup>2</sup> To whom requests for reprints should be addressed, at Medical Research Council Toxicology Unit, University of Leicester, P. O. Box 138, Leicester, LE1 9HN, United Kingdom. Phone: 44-116-252-5618; Fax: 44-116-252-5616; E-mail: ag15@le.ac.uk.

<sup>3</sup> The abbreviations used are: FAP, familial adenomatous polyposis; APC, adenomatous polyposis coli; COX, cyclooxygenase; HPLC, high-performance liquid chromatography; NSAID, nonsteroidal anti-inflammatory drug; pd, per diem.

Min/+ mice is gastrointestinal bleeding, which is thought to contribute substantially to morbidity and mortality (6). To find out to what extent curcumin affects adenoma-related gastrointestinal bleeding, we measured the hematocrit in treated and control animals and compared values with adenoma numbers. Finally, to define the disposition of curcumin accurately, we synthesized  $^{14}\text{C}$ -labeled curcumin, administered it via the i.p. route, and determined the disappearance of radioactivity from tissues and plasma. Overall, the study was designed to aid the program of clinical evaluation of curcumin in the treatment of FAP and the prevention of colorectal cancer in humans.

## Materials and Methods

**Chemicals.** Curcumin was purchased from Apin Chemicals (Abingdon, United Kingdom) and its purity verified by HPLC analysis (see HPLC analysis below). This material contained 3% desmethoxycurcumin. Curcumin was blended into RM3 high-protein breeders diet, bought from Specialist Dietary Services (SDS, Witham, United Kingdom), using a mechanical mixer to ensure uniform distribution, confirmed by HPLC analysis. The synthesis of [ $^{14}\text{C}$ ]curcumin has been described previously (15). The labeled material had a specific activity of 13.5 MBq/mmol. For the distribution study after i.p. injection, [ $^{14}\text{C}$ ]curcumin was diluted with four parts unlabelled curcumin. Ethyl acetate, acetonitrile (both HPLC grade), and DMSO were obtained from Fisher Laboratory Supply Ltd. (Loughborough, United Kingdom); tissue solubilizer (Optisolve) and scintillation fluid (Optisafe High Phase) came from Wallac Scintillation Products (Milton Keynes, United Kingdom).

**Animals and Treatments.** To establish a breeding colony, male C57Bl/6J Min/+ mice (referred to in the following as "Min/+ mice") were purchased from the Jackson Laboratory (Bar Harbor, ME) and mated with female C57Bl/6J mice (wild-type) obtained from Charles River (Margate, United Kingdom). Male Min/+ mice offspring of this cross breeding and C57Bl/6J female wild-type mice maintained the Min/+ breeding colony. Tissue samples were obtained from the weaners by ear punch and genotyped for Min/+ status by PCR and *HindIII* digest of the product, essentially as described previously (16). Male C57Bl/6J mice (Charles River) were used in accompanying pharmacokinetic studies. All of the mice were kept in positive pressure isolators, and routine bacteriological and serological tests established that they were free of pathogens. Breeding pairs and all of the offspring were maintained throughout on a RM3 diet. This diet consists of cereal products (wheat, barley) 64%, vegetable protein (extracts of soya beans and dried yeast) 16.5%, soya oil 2%, animal protein (fish meal, whey powder) 15%, and supplements (vitamins, minerals, amino acids) 2.5%. Offspring were weaned at 3 weeks. At 4 weeks, littermates were divided following a randomized block design into control (RM3 diet only) or treatment groups, which constituted curcumin at 0.1, 0.2, or 0.5% mixed in with the RM3 diet. The choice of these curcumin concentrations was determined by the intention to span a dose range from subefficacious to markedly efficacious.

**Adenoma Enumeration.** Experiments in mice were conducted as stipulated by the Animals (Scientific Procedures) Act 1986 Project License 80/1250 granted to Leicester University by the United Kingdom Home Office, and the experimental design was vetted and approved by the Leicester University Ethical Committee for Animal Experimentation. Each experimental group comprised between 10 and 15 mice. At the end of the experiment when Min/+ mice reached age 18 weeks, they were killed by cardiac exsanguination under terminal anesthe-

sia (halothane). The entire gastrointestinal tract was removed for dissection and flushed with PBS (~10 ml) to remove intestinal content. Tissue was opened longitudinally and washed extensively with PBS. Stomach and cecum were omitted from the analysis. Small intestine and colon were fixed flat in methacarn (methanol:chloroform:acetic acid, 6:3:1) for 2–4 h, after which the tissue was examined under 3-fold magnification. The small intestine was divided visually into three segments of approximately equal length (referred to in the following as proximal, middle, and distal segments). Multiplicity, location, and size of adenomas were recorded within these segments and the colon. Adenomas were differentiated by size (diameter) into <1 mm, 1–3 mm, and >3 mm.

**Measurement of Hematocrit.** Blood samples were collected and drawn by capillary force into heparinized microhematocrit tubes (75 mm; Richardsons, Leicester, United Kingdom). The hematocrit, which constitutes the proportion of the volume of the blood sample occupied by the erythrocytes, was determined as described previously (17).

**Study of Curcumin and Metabolites in Tissues, Blood, and Excreta.** Male C57Bl/6J mice (8 weeks of age, ~25g) received the RM3 diet containing 0.1, 0.2, or 0.5% curcumin for 8 days. Animals were placed into metabolism cages for 24 h, and urine and feces were collected. After killing (exsanguination under terminal anesthesia), liver, small intestine and colon tissue were isolated, and gut epithelium was scraped off by brushing gently with a metal spatula. Plasma was separated from blood by centrifugation ( $10,000 \times g$  for 5 min). Tissue samples were homogenized in acetate buffer [1 M (pH 4.5), 2 ml] and extracted with 10 volumes of ethyl acetate or acetonitrile. Aliquots of plasma or blood were extracted after the addition of an equal volume of acetate buffer with twice the volume of ethyl acetate. The mixtures were centrifuged ( $2,800 \times g$  at  $4^\circ\text{C}$  for 15 min), the organic layer was removed, and the mixtures were evaporated under nitrogen. Fecal samples (~200 mg) were homogenized with acetate buffer (2 ml) and extracted into 10 volumes of ethyl acetate. The extraction efficiency from plasma for curcumin at 0.1  $\mu\text{g}/\text{ml}$ , determined by HPLC, was  $92 \pm 7\%$  (mean  $\pm$  SD;  $n = 6$ ); its extraction efficiency from feces was  $75 \pm 10\%$  and from liver and mucosal scrapings,  $50 \pm 12\%$ . Curcumin glucuronide and curcumin sulfate were extracted at 50% efficiency.

**HPLC Analysis and Mass Spectrometry.** Samples of blood, tissues, and excreta were analyzed for the presence of curcumin and its metabolites curcumin sulfate, curcumin glucuronide, hexahydrocurcumin, and hexahydrocurcuminol, using a reversed-phase HPLC method as described previously (18). The limits of detection for curcumin, curcumin glucuronide, and curcumin sulfate under the conditions of this assay were 5 pmol/ml of plasma or 10 pmol/g of tissue. The identity of curcumin and metabolites was established by cochromatography with authentic standard compounds and confirmed by electrospray mass spectrometry in the ion-selected mode as described previously (12).

**Study of [ $^{14}\text{C}$ ]Curcumin Distribution.** Male C57Bl/6J mice (8 weeks of age, ~25g) received [ $^{14}\text{C}$ ]curcumin (100 mg/kg) dissolved in DMSO (injection volume, ~50  $\mu\text{l}$ ) via the i.p. route. Mice were killed after 0.25, 0.5, 1, 2, 4, 8, or 24 h (four mice per time point). Samples (0.2–0.3 g) of brain, heart, lung, liver, spleen, kidney, small intestine, and blood were dissolved in Optisolve solubilizer at  $37^\circ\text{C}$  (1.5 ml), after which, scintillation fluid was added. In the case of plasma samples, the scintillation fluid was added directly. Samples were analyzed in a Wallac 1410 liquid scintillation counter using a 10-min  $^{14}\text{C}$

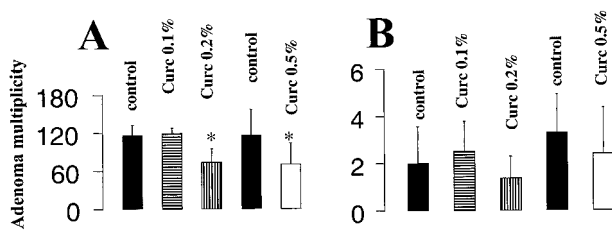


Fig. 1. Effect of dietary curcumin (*Curc*) on adenoma multiplicity in the small intestine (A) and colon (B) of Min/+ mice. Numbers of mice per group were between 10 and 12; \*, number of adenomas is significantly different from that in control animals ( $P < 0.05$ ). There were two control groups (untreated Min/+ mice), one for the study of curcumin 0.1 and 0.2% and the other for the mice on curcumin 0.5%. For details of animal husbandry and adenoma enumeration see "Materials and Methods."

counting program. The measured radioactivity was converted into nmol curcumin equivalents per milliliter of plasma or gram of tissue. The plasma and tissue peak values quoted in the "Results" are the mean  $\pm$  SD obtained in three animals.

**Statistical Evaluation.** Adenoma numbers and hematocrit values were subjected to ANOVA using Excel and Minitab software packages (Microsoft Windows 1997). Statistical significance ( $P < 0.05$ ) was established by *post hoc* Tukey's pairwise comparison.

## Results

**Effect of Curcumin on Multiplicity of Intestinal and Colonic Adenomas.** Min/+ mice received curcumin mixed in with their diet at three concentrations, 0.1, 0.2, and 0.5% commencing one week *post*-weaning. Animals were killed at 18 weeks of age, and tumor multiplicity and size were inspected *postmortem*. Fig. 1 shows that dietary curcumin at 0.1% was without effect on overall gastrointestinal tumor burden. However, at dietary concentrations of 0.2 and 0.5%, curcumin reduced intestinal tumor load significantly by 39 and 40%, respectively. Inspection of adenomas revealed a flattened morphology in the case of mice that had received curcumin at 0.5%, compared with untreated mice or mice on the lower doses of curcumin (result not shown). There were only a few adenomas in the colon of untreated Min/+ mice ( $3.5 \pm 3.8$ ,  $n = 22$ ). Although dietary curcumin (0.2 and 0.5%) reduced their number by 30 and 27%, respectively, this decrease was not significant (Fig. 1). In a separate experiment, animals received curcumin (0.2%) for either the first (weeks 3–10.5 of age) or second (10.5–18 weeks) half of their postweaning life span. Such early or late treatment did not affect gastrointestinal adenoma multiplicity significantly (results not shown), in contrast to the effect of treatment when curcumin was applied continuously for the whole 15-week postweaning period.

Although total tumor multiplicity decreased significantly as a result of treatment with curcumin (0.2 or 0.5%), its efficacy varied depending on gastrointestinal tumor location and stage of tumor development (Fig. 2). Curcumin at 0.2% affected predominantly small adenomas (<1 mm diameter) and, to a lesser extent, middle-size tumors (1–3 mm) in both the distal and middle segments of the small intestine. Curcumin at 0.5% diminished mainly middle-size adenomas in the distal portion and reduced small and middle-size adenomas in the middle part of the small intestine. Both of the efficacious curcumin dose levels reduced numbers of large adenomas (>3 mm), rather than those of small and middle-sized ones, in the proximal

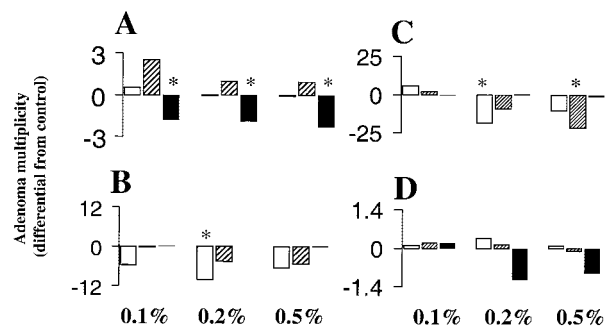


Fig. 2. Effect of dietary curcumin on multiplicity of small (<1-mm diameter, □), medium size (1–3 mm, ▨) or large (>3 mm, ■) adenomas in the proximal (A), middle (B), distal (C), or colonic (D) sections of the intestine of Min/+ mice. Results are expressed as mean number of adenomas over or below mean adenoma numbers in untreated (control) Min/+ mice. Adenoma numbers (mean  $\pm$  SD) in untreated (control) Min/+ mice were as follows (in the order small, medium size, and large adenomas, respectively; because there were two control groups in separate sets of experiments, the numbers without brackets denote values for the control mice for the 0.1 and 0.2% curcumin treatment groups, and numbers in brackets refer to the control mice for the 0.5% curcumin treatment group): proximal intestine,  $0.5 \pm 1.0$  ( $0.6 \pm 0.8$ ),  $2.4 \pm 1.4$  ( $1.9 \pm 1.7$ ), and  $2.0 \pm 1.3$  ( $3.7 \pm 1.6$ ); middle intestine,  $25.6 \pm 11.6$  ( $19.5 \pm 11.3$ ),  $15.2 \pm 6.4$  ( $16.7 \pm 9.6$ ), and  $0$  ( $0.4 \pm 1.3$ ); distal intestine,  $43.9 \pm 10.7$  ( $34.2 \pm 12.7$ ),  $26.5 \pm 11.7$  ( $37.4 \pm 20.0$ ), and  $0.3 \pm 0.7$  ( $2.2 \pm 3.2$ ); colon  $0$  ( $0$ ),  $0.6 \pm 0.8$  ( $1.4 \pm 1.1$ ), and  $1.4 \pm 1.2$  ( $1.9 \pm 1.1$ ). Numbers of mice per group were between 10 and 12. \*, number of adenomas was significantly different from that in control animals ( $P < 0.05$ ). The effect of 0.2 and 0.5% dietary curcumin on the numbers of small and middle-size adenomas in the middle portion of the small intestine, and of large adenomas in the colon, approached significance ( $P < 0.1$  but  $> 0.05$ ). For details of animal husbandry and tumor enumeration see "Materials and Methods."

small intestine and colon. Curcumin at 0.1%, a concentration that lacked effect on overall tumor number (see Fig. 1), seemed to decrease the numbers of small adenomas in the middle region and large adenomas in the proximal region of the small intestine (Fig. 2). This decrease was outweighed by increased numbers of medium-size adenomas in the proximal and small adenomas in the distal regions.

**Effect of Curcumin on Hematocrit.** To investigate whether dietary curcumin affects gastrointestinal bleeding associated with adenoma load in Min/+ mice, the hematocrit was studied in individual animals at the termination of the experiment. The mean hematocrit value in untreated Min/+ mice was reduced to 25% of that seen in healthy C57Bl/6J mice (Fig. 3). Dietary curcumin increased the hematocrit in Min/+ mice in a dose-dependent fashion. The highest dietary concentration (0.5%) of curcumin partially restored hematocrit values in Min/+ mice to 60% of values observed in healthy C57Bl/6J mice (Fig. 3). When individual hematocrit values were plotted against adenoma number for control C57Bl/6J mice, Min/+ mice on control diet, and those on the curcumin-containing diet, the resulting line of best fit was characterized by a correlation coefficient of  $-0.84$  (result not shown).

**Steady-State Levels of Curcumin in Plasma, Target Tissue, and Excreta.** We wished to relate the chemopreventive efficacy of curcumin in Min/+ mice with the concentration of the agent or its metabolites. To that end, steady-state levels were determined in the plasma, excreta, and gastrointestinal mucosa, the target tissue, and for comparative purposes also in liver tissue of C57Bl/6J mice, which had received dietary curcumin at either 0.1, 0.2, or 0.5% for 1 week. Curcumin was not detectable in the urine. Irrespective of the dose, curcumin was present in the plasma at levels near the limit of detection (5 pmol/ml). Large amounts of curcumin were found in the feces



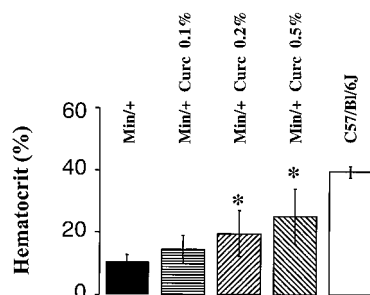


Fig. 3. Hematocrit values in C57Bl/6J mice, untreated Min/+ mice, and Min/+ mice that received curcumin (*Curc*) at 0.1, 0.2, or 0.5% in their diet. Values represent percentage proportion of the volume of the blood sample occupied by the erythrocytes and are the mean  $\pm$  SD of 10–12 mice. For details of animal husbandry and determination of hematocrit see “Materials and Methods.” \*, values are significantly different from those in untreated Min/+ mice ( $P < 0.05$ ).

(3.2–3.8  $\mu\text{mol/g}$ ; Table 1). In the mucosa of the small intestine, its concentration varied between 39 and 240 nmol/g of tissue and, in the colonic mucosa, between 15 and 715 nmol/g of tissue. Curcumin levels in the small intestine reflected satisfactorily differences in dose, whereas levels in the colon and feces did not mirror dose levels (Table 1). The concentration of curcumin in liver tissue of mice that were fed 0.2% curcumin in the diet was  $119 \pm 31$  pmol/g of tissue ( $n = 3$ ), a value which constitutes  $\sim 0.001$  of that observed in the intestinal mucosa. Products of metabolic conjugation or reduction of curcumin were not detected, except in the colonic mucosa and feces, in which HPLC analysis revealed the presence of traces of a species coeluting with authentic curcumin sulfate. Mass spectral investigation of the HPLC peak in the colon mucosa by selected ion monitoring afforded the molecular ion of  $m/z = 447$ , corroborating the identity of the peak as curcumin sulfate. In a confirmatory experiment, plasma levels of curcumin were measured in Min/+ mice at the end of the life-time feeding study, and levels were found to be very similar to those seen in wild-type C57Bl/6J mice kept on a curcumin-containing diet for 1 week.

**Disposition of Curcumin.** We wished to explore how rapidly curcumin levels decline on termination of treatment. This question was addressed in two ways: firstly, C57Bl/6J mice, which had received curcumin (0.2%) in their diet for 1 week, were changed onto a curcumin-free diet, and levels of curcumin in plasma and in gastrointestinal and hepatic tissues were analyzed for curcumin for up to 16 days after the cessation of curcumin feeding. Secondly, for a more general analysis, mice received [ $^{14}\text{C}$ ]curcumin (100 mg/kg) via the i.p. route, and the disappearance of radioactivity associated with the curcumin molecule was studied, not only in plasma and gastrointestinal and liver tissues, but also in heart, lung, kidney, brain, and muscle tissues. After termination of dietary curcumin intake, tissue levels of curcumin declined rapidly to unquantifiable amounts within 3 to 6 h after the termination of curcumin feeding, whereas fecal curcumin declined more slowly with a half-life of  $\sim 23$  h (results not shown). Radioactivity measured in the plasma and tissues after i.p. injection of [ $^{14}\text{C}$ ]curcumin achieved the following peak levels, expressed as nmol curcumin equivalents per milliliter of plasma or per gram of tissue: plasma,  $25 \pm 2$ ; liver,  $73 \pm 20$ ; intestinal mucosa,  $200 \pm 23$  (see Fig. 4); brain,  $2.9 \pm 0.4$ , heart,  $9.1 \pm 1.1$ ; lungs,  $16 \pm 3$ ; muscle  $8.4 \pm 6.0$ ; and kidney,  $78 \pm 3$ . Beyond the peak, radioactivity declined swiftly to reach levels of between 20 and

Table 1 Concentration of curcumin in small intestinal and colonic mucosa and feces of mice that received curcumin at 0.1, 0.2, or 0.5% in their diet for 1 week

Curcumin content of diet (%)	Curcumin levels <sup>a</sup> (nmol/g)		
	Small intestinal mucosa	Colonic mucosa	Feces
0.1	$39 \pm 9$	$15 \pm 9$	$3770 \pm 1246$
0.2	$111 \pm 40$	$508 \pm 149$	$3590 \pm 231$
0.5	$240 \pm 69$	$715 \pm 448$	$3186 \pm 2411$

<sup>a</sup> Values are the mean  $\pm$  SD of four animals.

33% of peak values at 4 h, or in the case of the small intestine, 8 h, after dosing (Fig. 4). From this time point onwards, radioactivity levels decreased little, or hardly at all, up to 24 h. A similar pattern of disposition was observed in heart, lung, kidneys, brain, and muscle tissues, in that levels decreased within 2–4 h after dosing to 10–20% of peak levels, and radioactivity levels remained at this residual level for up to 24 h (results not shown). It needs to be stressed that the curcumin pharmacokinetics observed in plasma and tissues after i.p. administration cannot be compared directly with those observed after dietary intake, not least because the agent was formulated for i.p. administration in DMSO, an amphiphilic solvent that seemed to enhance curcumin absorption considerably.

## Discussion

This study defines for the first time the relationship between chemopreventive efficacy of curcumin and its concentration in the Min/+ mouse, a colon neoplasia model of relevance to human cancer (7). The results of this investigation allow five novel conclusions that may aid with the design, and eventual interpretation, of future clinical intervention studies with this dietary constituent. Firstly, the chemopreventive activity of curcumin in the Min/+ mouse model seems to be governed by a rather narrow therapeutic window. Intake of curcumin at a dietary level of 0.1%, approximately equal to 150 mg/kg pd, lacked overall efficacy, whereas at 0.2%, which equates to  $\sim 300$  mg/kg pd, curcumin prevented, or retarded, adenoma formation. A further increase in dietary level by a factor of 2.5 to  $\sim 750$  mg/kg pd failed to yield any additional gain in efficacy, irrespective of the fact that curcumin concentrations in the small intestine adequately mimicked the stepwise increase in dose consumed with the diet. The effect of curcumin on the multiplicity of tumors varied depending on their location along the intestinal tract. It was the population of small and medium-size adenomas that was most susceptible to the preventive efficacy of curcumin, and reduction in adenoma number was most prominent in the middle and distal regions of the intestinal tract, the areas in which the majority of tumors occurred. This finding is consistent with previous experience in the Min/+ mouse model using piroxicam (19) or the selective COX-2 inhibitor celecoxib (8).

The second conclusion from the work described here is that for curcumin to be effective in the Min/+ mouse model, the gastrointestinal mucosa, the target tissue, needs to be exposed to curcumin at concentrations near or above the 100-nmol/g of tissue mark. Tissue concentrations below this value are apparently without measurable chemopreventive efficacy. Thirdly, to achieve chemopreventive activity, exposure to curcumin needs to persist for the whole postweaning lifetime of Min/+ mice.

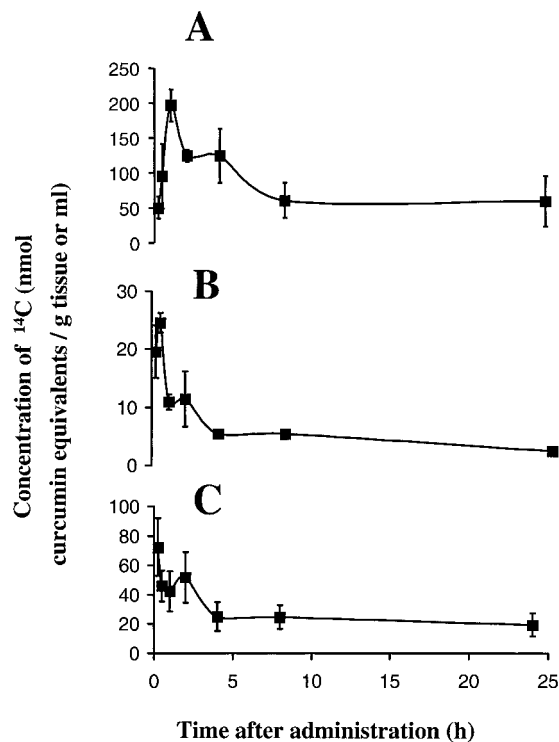


Fig. 4. Elimination of radioactivity derived from [ $^{14}\text{C}$ ]curcumin from the intestinal tract mucosa (A), plasma (B), and liver (C) of C57Bl/6J mice, which had received a single dose of [ $^{14}\text{C}$ ]curcumin (100 mg/kg) via the i.p. route. Values are expressed as nmol curcumin equivalents per gram (g tissue; A, C) or milliliters (ml) of plasma (B), and are the mean  $\pm$  SD of four mice. For details of the analysis see "Materials and Methods."

Restriction of exposure to either the first half of this time period, the preneoplastic phase, during which adenomas are usually not yet observed, or the second half, when adenomas are established, was insufficient to decrease adenoma formation.

Fourthly, the beneficial effect of curcumin on adenoma load, as adjudged by tumor number, was accompanied by an elevation in hematocrit values, which reflect intraluminal hemorrhage consequent to adenoma maturation. Dose-dependent activity of curcumin was illustrated by the change in hematocrit. This finding is especially intriguing, when advantages and disadvantages of treatment with curcumin are juxtaposed with those reported for cancer chemopreventive NSAIDs such as aspirin, sulindac, or piroxicam. NSAIDs have the notorious drawback that they can elicit severe adverse effects in the gastrointestinal tract (20). Curcumin not only failed to exacerbate adenoma-induced intestinal bleeding, it actually ameliorated it significantly. Furthermore, there was no hint of agent-induced histopathological lesions in the gastrointestinal tract of Min/+ mice in the study described here, nor in a pilot study performed by us in which animals were fed curcumin at a dietary level as high as 2%, corresponding to  $\sim 3$  g/kg pd.<sup>4</sup>

The fifth conclusion from the work described above is the fact that curcumin disappears relatively rapidly from rodent tissues, including the target tissue, once treatment is discontinued. This conclusion is consistent with the previous observation

of rapid disappearance of curcumin and its conjugates from the plasma of rats, which received curcumin by the i.v. route (12). The conclusion is important given the observation (discussed above) that curcumin needs to be present consistently in the gastrointestinal mucosa of Min/+ mice for several months to achieve chemopreventive activity. Potentially rapid dispositional removal of curcumin from the target tissue needs to be taken into account, if sustained levels are to be achieved in humans. Some curcumin metabolites, such as tetrahydrocurcumin, may contribute to the biological potency of curcumin (21, 22), whereas, in contrast, curcumin conjugates are probably devoid of biological activity (12). Therefore, it is important to note that no significant amounts of products of the metabolic reduction or glucuronidation of curcumin were found in the plasma, gastrointestinal mucosa, or feces of mice that received a curcumin diet in the study described here. Only a trace of curcumin sulfate was detected in the intestinal mucosa of treated animals.

As to the mechanisms by which curcumin interferes with the process of carcinogenesis, there are a host of biochemical candidate processes that might be compromised by curcumin at 0.1–0.5  $\mu\text{mol/g}$  of mucosal tissue, the range of levels defined here as necessary to prevent, or retard, adenoma formation in the gastrointestinal tract of Min/+ mice. This concentration range is roughly equivalent to 0.1–0.5 mM in experiments *in vitro* using cells or cellular fractions. In such systems curcumin has been shown to act as a scavenger of oxygen species, such as hydroxyl radical, superoxide anion, and singlet oxygen (23–27), and to interfere with lipid peroxidation (28–30). Curcumin also suppresses a number of key elements in cellular signal transduction pathways pertinent to growth: survival, promotion, angiogenesis, differentiation, and malignant transformation. Prominent among the signaling events inhibited by curcumin are phosphorylations catalyzed by protein kinases (31), c-Jun/AP-1 activation (32), prostaglandin biosynthesis (33), and activity and expression of the enzyme COX-2 (34, 35). All of these inhibitory actions of curcumin require concentrations of the agent in the 10–100- $\mu\text{M}$  range, which is well within the levels achieved in the target tissue of Min/+ mice at efficacious dietary doses.

In conclusion, the study presented here corroborates the notion that curcumin possesses chemopreventive activity in a model germane to human colorectal carcinogenesis involving *Apc* mutations. This finding encourages, in principle, the potential evaluation of curcumin for adenoma-retarding efficacy in FAP patients. The dose of curcumin required for efficacy in humans equivalent to the 0.2% dietary concentration or 300 mg/kg pd dose, which was active in mice, when calculated on the basis of equivalent body surface area (900 mg/m<sup>2</sup> in the mouse), would be 1.6 g per person pd, assuming a body surface area of 1.8 m<sup>2</sup> accompanying a body weight of 70 kg (36). This putative efficacious clinical dose of curcumin is well within the dose range, 0.5, 1.2, 2.1, and 8 g pd for up to 6 weeks, which according to the literature has been administered to humans apparently without adverse effect (37–40). The ability of curcumin to decrease intestinal blood loss linked to adenoma maturation, as adjudged by its significant effect on Min/+ mouse hematocrit, renders the clinical exploration of combinations of curcumin with NSAIDs potentially attractive.

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# BLOOD CANCER DISCOVERY

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Sarah Perkins, Richard D. Verschoyle, Kirsti Hill, et al.

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