

Pharmacodynamic and Pharmacokinetic Study of Oral *Curcuma* Extract in Patients with Colorectal Cancer¹

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ABSTRACT

Curcuma spp. extracts, particularly the dietary polyphenol curcumin, prevent colon cancer in rodents. In view of the sparse information on the pharmacodynamics and pharmacokinetics of curcumin in humans, a dose-escalation pilot study of a novel standardized *Curcuma* extract in proprietary capsule form was performed at doses between 440 and 2200 mg/day, containing 36–180 mg of curcumin. Fifteen patients with advanced colorectal cancer refractory to standard chemotherapies received *Curcuma* extract daily for up to 4 months. Activity of glutathione *S*-transferase and levels of a DNA adduct (M₁G) formed by malondialdehyde, a product of lipid peroxidation and prostaglandin biosynthesis, were measured in patients' blood cells. Oral *Curcuma* extract was well tolerated, and dose-limiting toxicity was not observed. Neither curcumin nor its metabolites were detected in blood or urine, but curcumin was recovered from feces. Curcumin sulfate was identified in the feces of one patient. Ingestion of 440 mg of *Curcuma* extract for 29 days was accompanied by a 59% decrease in lymphocytic glutathione *S*-transferase activity. At higher dose levels, this effect was not observed. Leukocytic M₁G levels were constant within each patient and unaffected by treatment. Radiologically stable disease was demonstrated in five patients for 2–4 months of treatment. The results suggest that (a) *Cur-*

cuma extract can be administered safely to patients at doses of up to 2.2 g daily, equivalent to 180 mg of curcumin; (b) curcumin has low oral bioavailability in humans and may undergo intestinal metabolism; and (c) larger clinical trials of *Curcuma* extract are merited.

INTRODUCTION

Curcumin (diferuloylmethane), a low molecular weight polyphenol derived from the rhizomes of *Curcuma* spp., has been shown to prevent cancer in the skin, forestomach, duodenum, and colon of mice and in the tongue, colon, mammary glands, and sebaceous glands of rats (1). Of particular interest is the ability of dietary curcumin to interfere with colon carcinogenesis in chemical and genetic rodent models (2–4). Curcumin has also been associated with regression of established malignancy in humans (5). Curcumin is the major constituent of the spice turmeric, which is abundantly used in the diet on the Indian subcontinent, an area that has a low incidence of colorectal cancer (6). Mechanisms by which curcumin prevents cancer are thought to involve up-regulation of carcinogen-detoxifying enzymes, such as GSTs³ (7–9), antioxidation (10–16), and suppression of expression of the enzyme cyclooxygenase-2 (17, 18). The pharmacokinetic properties of curcumin in humans remain unexplored. In rodents, curcumin undergoes avid metabolism by conjugation and reduction, and its disposition after oral dosing is characterized by poor systemic bioavailability (9, 17, 19, 20).

In view of the paucity of pharmacodynamic and pharmacokinetic information regarding curcumin in humans, we conducted a dose-escalation pilot study of a standardized *Curcuma* extract in patients with advanced colorectal cancer refractory to standard chemotherapy. The aims of the study were threefold. Firstly, we wished to evaluate the safety of curcumin administered p.o. as *Curcuma* extracts. Secondly, we wanted to investigate the suitability of two potential biomarkers of the pharmacological efficacy of curcumin in patients' blood leukocytes: GST activity and the levels of the adduct (M₁G) formed by the reaction of malondialdehyde with deoxyguanosine in DNA. GST enzyme activity has been shown to be up- or down-regulated in rat tissues after oral curcumin treatment, depending on the dose and route of administration (7–9). To aid the interpretation of GST activity data, patients were genotyped for *GSTM1*, *GSTT1*, and *GSTP1*. These represent the three major GST subclasses found in human lymphocytes, at least one of which is relevant to colorectal cancer and resistance to chemo-

Received 2/1/01; revised 4/10/01; accepted 4/13/01.

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¹ Supported by program grants from the United Kingdom Medical Research Council (to A. J. G. and M. M. M.), funds from Phytopharm plc. (to W. P. S.), NIH Grant CA77839 (to L. J. M.), and a fellowship from the University Hospitals of Leicester (to R. A. S.).

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³ The abbreviations used are: GST, glutathione *S*-transferase; NSAID, nonsteroidal anti-inflammatory drug; HPLC, high-performance liquid chromatography; CEA, carcinoembryonic antigen; CT, computed tomography; CDNB, 1-chloro-2,4-dinitrobenzene; AUC, area under curve.

therapy (21–23). Malondialdehyde is a naturally occurring product of lipid peroxidation and prostaglandin biosynthesis via cyclooxygenase (24, 25). These two cellular processes implicated in the pathogenesis of colorectal cancer (26, 27) are inhibited by curcumin in preclinical models (17, 28). In a recent study in rats, dietary curcumin was shown to up-regulate GST activity in the liver and diminish M₁G levels in colon mucosa, and these effects were accompanied by measurable tissue levels of curcumin (9). The third aim of the study described here was therefore to test the hypothesis that curcumin or products of its metabolism can be detected in blood or excreta of humans. Overall, the study was designed to define parameters that might help to optimize the clinical evaluation of curcumin in Phase I/II trials.

PATIENTS AND METHODS

Patients. The trial was approved by the local ethics committee and the United Kingdom Medicines Control Agency. Fifteen patients enrolled between September 1999 and September 2000 at the Leicester Royal Infirmary met the following eligibility criteria: (a) histologically proven adenocarcinoma of the colon or rectum for which no further conventional therapies were available; (b) measurable or evaluable disease; (c) age > 18 years; (d) WHO performance status of 0–2 and life expectancy greater than 12 weeks; (e) absolute neutrophil count $\geq 1.5 \times 10^9$ /liter; (f) hemoglobin ≥ 10 g/dl; (g) platelets $\geq 100 \times 10^9$ /liter; (h) aspartate aminotransferase and alanine aminotransferase $< 2.5 \times$ the upper limit of normal; (i) serum bilirubin and creatinine $< 1.5 \times$ the upper limit of normal; and (j) no previous investigational or chemotherapeutic drugs within 28 days prior to enrollment. Exclusion criteria included: (a) active chronic inflammatory or autoimmune disease; (b) active infection, including viral infection; (c) significant impairment of gastrointestinal function or absorption; (d) active peptic ulcer disease; (e) known biliary obstruction or biliary insufficiency; and (f) use of NSAIDs within 14 days of enrollment. Patients were asked to abstain from NSAID use and the consumption of foods containing the spice turmeric during the study period, and their general practitioners were asked not to prescribe NSAIDs. Written informed consent was obtained from each patient before enrollment. Demographic and baseline characteristics of patients are shown in Table 1. All patients were Caucasian, and all had undergone previous surgery. Three patients stopped NSAID medication 3 weeks before enrollment.

Study Design and Treatment. P54FP was provided in soft gelatin capsules by Phytopharm plc. (Godmanchester, United Kingdom). Each capsule contained 20 mg of curcuminoids (18 mg of curcumin and 2 mg of desmethoxycurcumin) suspended in 200 mg of essential oils derived from *Curcuma* spp. Typical constituents of *Curcuma* essential oil mixtures are turmerone, atlantone, and zingiberene. Chemical analysis by HPLC/mass spectrometry confirmed the content of curcuminoids. This formulation, which in the following text will be referred to as “*Curcuma* extract,” was selected on account of the curcumin dose, which equates to dietary intake of turmeric (see below), the reproducibility of curcuminoid content, and the fact that the capsules contained extracts of *Curcuma* plants used in traditional Indian and Southeast Asian medicine. There were

Table 1 Patient characteristics at enrollment

	Daily dose of <i>Curcuma</i> extract (mg)				
	440	880	1320	1760	2200
Sex					
Male	3	3	2	1	3
Female	0	0	1	2	0
Age (yrs)					
Mean	67.3	61	60.3	53	63.7
Range	65–71	53–72	59–66	42–73	51–71
WHO performance					
0	2	2	2	3	2
1	1	1	1	0	1
Sites of measurable disease					
Colorectum	1	0	0	0	0
Liver	2	2	2	2	3
Lung	1	0	0	0	1
Lymph nodes	2	2	0	0	1
Other	0	0	0	1	0
Peritoneum	0	1	2	0	1
Previous chemotherapy					
5-Fluorouracil	3	3	3	3	3
Other	2	3	2	2	2
Previous radiotherapy	1	1	0	2	1

three patients per dose level. After at least a 2-h fast, patients consumed 2, 4, 6, 8, or 10 capsules once daily with water. This translates to doses of 440, 880, 1320, 1760, and 2200 mg of *Curcuma* extract per day containing 36, 72, 108, 144, and 180 mg of curcumin, respectively. Treatment was continued until disease progression was established or consent was withdrawn.

Clinical Measurements. Blood, urine, and feces were collected on days 1, 2, 8, and 29 and protected from light and stored at -80°C . Blood was collected before dose administration and at 0.5, 1, 2, 3, 6, and 8 h after dose administration. Samples were collected in tubes pretreated with lithium-heparin (Sarstedt, Loughborough, United Kingdom). Full blood cell count and urea, electrolytes, liver, and bone function were measured in venous samples, and physical examination was performed before treatment and on treatment days 1, 2, 8, and 29 and monthly thereafter. Venous blood levels of the tumor markers CEA and CA19.9 were measured before treatment and after every month of treatment. Radiological assessment of target lesions was performed every 2 months by CT or magnetic resonance imaging scan, in addition to monthly chest X-rays. Blood samples for analysis of GST activity and M₁G levels were collected 1 week before treatment and on days 1, 2, 8, and 29 of treatment, immediately before dosing for M₁G or immediately before and 1 h after each dose for GST. Lymphocytes were separated from fresh blood using Ficoll-Paque Plus (Amersham Pharmacia Biotech, Bucks, United Kingdom), resuspended in 1 ml of 10 mM Tris-HCl (pH 7.8), and stored at -80°C . Patients completed the European Organization for Research and Treatment of Cancer quality of life questionnaire GLQ-C30 (version 2.0) before treatment and monthly during treatment (29).

Chemical Analysis. Extraction of curcumin and its metabolites curcumin glucuronide, curcumin sulfate, hexahydrocurcumin, and hexahydrocurcuminol from plasma and urine was performed, and their recovery efficiency was established, as described previously (17). Curcumin and curcumin sulfate were extracted from feces with 2 parts (w/v) of acetonitrile:water (7:3). Curcuminoids were separated from other fecal constituents by C18 solid phase extraction (Varian, Walton-on-Thames, United Kingdom) and eluted from the column with acetonitrile (2 ml). The reverse-phase HPLC method with UV-visible detection used to analyze curcuminoids in extracts of plasma, urine, or feces has been reported previously (17). The limit of detection for curcumin in plasma and urine was 5 pmol/ml. The synthesis of curcumin sulfate and its identification by electrospray mass spectrometry were performed as described previously (17).

Measurement of GST Activity and M₁G Levels. Glutathione and CDNB were purchased from Sigma Chemical Co. (Poole, United Kingdom). Once thawed, lymphocyte samples were sonicated for 30 s (Fisher 550 sonicator; Fisher, Pittsburgh, PA) on ice and centrifuged at 3000 × *g* (15 min, 4°C). Total GST activity in the supernatant was measured spectrophotometrically using glutathione and CDNB as substrates, in triplicate for each sample (30). Results were corrected for protein levels using the Bio-Rad protein assay (Bio-Rad, Hemel Hempstead, United Kingdom). The GST activity values were quoted as nmol CDNB conjugated with glutathione/min/mg lymphocytic protein. The *GSTM1*, *GSTT1*, and *GSTP1* genotypes were determined by PCR methods described previously (31, 32). Murine M₁G monoclonal antibody D10A1 was prepared as described previously (33). Antirabbit and antimurine horseradish peroxidase antibodies were purchased from Dako (Ely, United Kingdom). M₁G standards were synthesized and characterized, genomic DNA was extracted from whole blood, and leukocytic M₁G adduct levels were analyzed by immunoslot blot in triplicate as described previously (34). Discrepancies in the amount of DNA/slot were corrected for by staining the nitrocellulose filter with propidium iodide and performing UV light densitometry (9). The detection limit for M₁G was 5 adducts/10⁸ nucleotides. The assay had been validated previously by collaborating laboratories at the University of Leicester and the Vanderbilt Cancer Center by exchange of samples.

Statistical Evaluation. GST and M₁G values were subjected to a balanced repeated measure ANOVA and linear regression analysis using Minitab (version 10.2) software package. Plots of residuals were used to ensure that variances were homogeneous and that the residuals had a normal distribution.

RESULTS

Tolerability of Oral *Curcuma* Extract. Patients with advanced colorectal cancer ingested P54FP capsules once daily for up to 4 months at doses between 440 and 2200 mg of *Curcuma* extract containing between 36 and 180 mg of curcumin. The treatment was well tolerated at all dose levels, and there was no dose-limiting toxicity. Two types of adverse events, both gastrointestinal, were possibly related to *Curcuma* consumption. One patient on 1320 mg of *Curcuma* extract daily

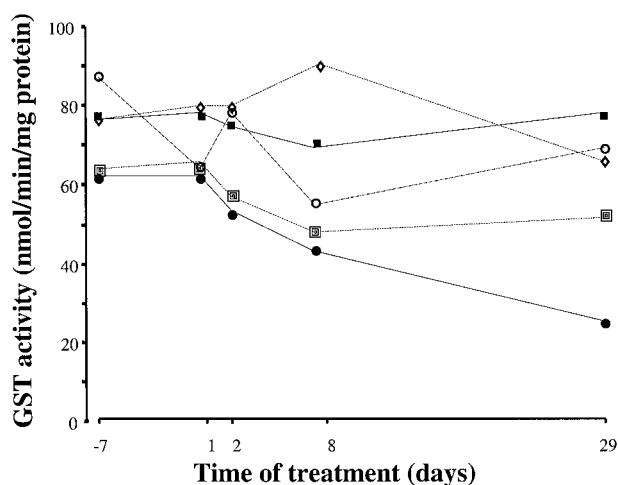


Fig. 1 Mean lymphocytic GST levels for patients on the following daily doses of *Curcuma* extract: 440 (●), 880 (concentric squares), 1320 (◇), 1760 (■), and 2200 mg (○), containing 36, 72, 108, 144, and 180 mg of curcumin, respectively. Each point represents means of values for three patients, calculated from triplicate readings for two samples per patient per day (immediately before dose administration and 1 h after dose administration). Time is relative to first dose on day 1. The pooled SD, including all data points, is 10.9 nmol/min/mg protein. For details of patients and measurement, see "Materials and Methods."

experienced nausea during the first month of treatment (National Cancer Institute toxicity grade 1), which resolved spontaneously despite continuation of treatment. Two patients (one each on 880 and 2200 mg of *Curcuma* extract daily) developed diarrhea (National Cancer Institute grades 2 and 1, respectively) 4 months and 1 month into treatment, respectively. Both patients withdrew from the study before the cause of the diarrhea could be investigated.

Pretreatment GST and M₁G Levels in Relation to GST Polymorphisms. Lymphocytic total GST activity and leukocytic M₁G levels differed substantially between patients (Figs. 1 and 2). Patients were genotyped for GST isoenzymes *GSTM1*, *GSTP1*, and *GSTT1*. Two-thirds of the patients lacked *GSTM1*, slightly more than the 40–60% proportion expected in healthy Caucasians (21), with an even distribution across the five dose levels. In patients who displayed the null genotype for *GSTM1*, pretreatment levels of leukocytic M₁G were 7.6 ± 4.3 adducts/10⁷ nucleotides, 74% higher than those in patients expressing *GSTM1*, in whom adduct levels were 4.3 ± 2.6 adducts/10⁷ nucleotides ($P < 0.001$ by ANOVA). Two patients were null for *GSTT1*. Their pretreatment levels of leukocytic M₁G (mean levels pooling triplicate readings from both time points, 5.8 ± 1.6 adducts/10⁷ nucleotides) were marginally lower than those in patients expressing *GSTT1* (6.6 ± 4.3 adducts/10⁷ nucleotides; $P = 0.02$ by ANOVA). Leukocytic levels of M₁G did not correlate with total GST activity, active smoking status ($n = 2$), vegetarianism ($n = 2$), or age.

Biological Effects of Oral *Curcuma* Extract. In patients taking 440 mg of *Curcuma* extract (36 mg of curcumin) daily, lymphocytic GST activity decreased gradually with time from a pretreatment GST value of 64 ± 19 nmol/min/mg protein

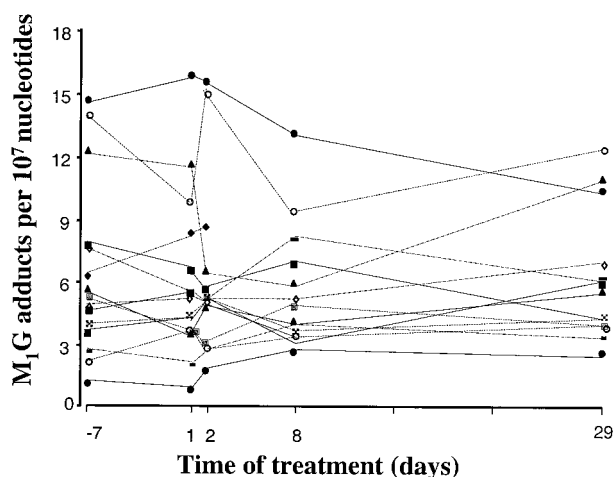


Fig. 2 Leukocytic M₁G adduct levels for patients on *Curcuma* extract over a 5-week period. Each line represents one patient; each point is the mean of three to five readings from one sample taken before dose administration on each day (shown relative to first dose on day 1). For experimental details, see "Materials and Methods." The interpatient variation is significant ($P < 0.001$ by ANOVA), but there is no significant inpatient change with time. The pooled SD, including all data points, is 5.4 adducts/ 10^7 nucleotides.

(measured in triplicate at two time points for each patient) to 26 ± 13 nmol/min/mg protein on day 29 of *Curcuma* consumption ($P < 0.001$ by ANOVA; Fig. 1). This decline was not observed at the higher dose levels. Retrospective subset analysis revealed that pretreatment lymphocytic GST levels (53 ± 10 nmol/min/mg protein) in the five patients who subsequently exhibited stable disease radiologically for more than 3 months of treatment were lower ($P = 0.001$ by ANOVA) than those in the other 10 patients (84 ± 25 nmol/min/mg protein). Leukocytic M₁G levels varied considerably between patients, with no significant difference between subsets of patients as defined by GST polymorphisms or radiological criteria. Consumption of *Curcuma* extract did not affect M₁G levels (Fig. 2).

Levels of Curcuminoids in Blood and Excreta. Neither curcumin nor its metabolites curcumin glucuronide, curcumin sulfate, hexahydrocurcumin, or hexahydrocurcuminol were found in the plasma or urine at up to 29 days of daily treatment. Incubation of plasma and urine samples with glucuronidase and sulfatase enzymes, which would hydrolyze curcumin conjugates, did not generate measurable amounts of curcumin. To test the hypothesis that curcumin, a very lipophilic molecule, is sequestered in blood cells and/or total blood lipoproteins, which may confound its detection in the plasma, these fractions were also analyzed. Curcuminoids were not detected. Feces obtained from patients on days 8 and 29 of consumption of *Curcuma* extract were investigated for the presence of curcuminoids. HPLC analysis furnished a prominent peak in fecal samples from all patients, which coeluted with curcumin. The mass spectrum of this peak was characterized by the presence of the molecular ion ($m/z = 367$) and major fragments of authentic curcumin including its base peak ($m/z = 149$), confirming the identity of this species as curcumin. Levels of curcumin in day 29 fecal samples from patients consuming 1760 and 2200 mg of

Curcuma extract daily (containing 144 and 180 mg of curcumin) were between 144 and 519 nmol/g dried feces at the lower dose and between 64 and 1054 nmol/g dried feces at the higher dose. The feces of one of the patients on the highest dose contained a species which on HPLC analysis coeluted with curcumin sulfate. Mass spectral investigation of the HPLC peak by selected ion monitoring afforded the molecular ion of $m/z = 447$, and the fragmentation pattern was compatible with that of the authentic reference compound, corroborating the identity of the peak as curcumin sulfate.

Chemotherapeutic Efficacy of Oral *Curcuma* Extract.

All patients enrolled exhibited radiological evidence of progressive malignant disease before recruitment. Levels of the tumor marker CEA in venous blood were above the normal range in all patients, and those of CA19.9 were abnormal in 80% of patients. In one patient, who received 440 mg of *Curcuma* extract (equivalent to 36 mg of curcumin) daily, venous blood CEA levels decreased from a pretreatment value of 310 ± 15 to 175 ± 9 $\mu\text{g/liter}$ after 2 months of treatment. This patient experienced stabilization of disease in the colon but progression in the liver, as demonstrated on CT scan. None of the other patients had measurable disease in the colon because the primary tumor had been resected previously. Levels of CA19.9 did not change with treatment. Five patients exhibited stable disease on CT scan [three (on 440, 880, and 1760 mg of *Curcuma* extract) for 3 months and two (on 880 and 1320 mg of *Curcuma* extract) for 4 months of treatment]. Significant changes in quality of life variables were not recorded.

DISCUSSION

The study presented here constitutes the first clinical evaluation of a standardized *Curcuma* extract in patients with cancer including pharmacodynamic and pharmacokinetic measurements. The results allow three conclusions, which will help to optimize the design of future clinical trials of curcumin or *Curcuma* extracts: (a) oral administration of *Curcuma* extract for several months at doses of up to 2.2 g daily (equivalent to 180 mg of curcumin) appears safe in the framework of this Phase I study; (b) the systemic bioavailability of p.o. administered curcumin is low in humans; and (c) *Curcuma* extract may cause clinical benefit in patients with advanced refractory colorectal cancer.

Our first conclusion regarding the apparent safety of *Curcuma* extracts is consistent with previous reports of clinical studies of curcumin and turmeric. Soni and Kuttan (35) treated 10 volunteers with 500 mg of curcumin daily for 7 days and failed to observe clinical toxicity. Two clinical trials designed to study the efficacy of curcumin as an anti-inflammatory agent in the treatment of arthritis or postoperative inflammation found that daily doses of 1.2–2.1 g of curcumin for 2–6 weeks did not cause adverse effects (36, 37). In a pilot study published in abstract form (38), tablets of turmeric extract containing 99.8% curcumin did not cause any treatment-related toxicity at doses as high as 8 g/day. Furthermore, a single dose of 50–200 mg of micronized curcumin formulated as capsules or sachets was administered to 18 volunteers without causing significant tox-

icity.⁴ Clinical trials of oral curcumin incorporating larger subject populations will be required to establish the safety of chronic administration. Although certain communities in the Indian subcontinent consume up to 1.5 g of dietary turmeric per person per day, curcumin constitutes only 2–8% of most turmeric preparations (39). The acceptable daily intake of curcumin as an additive has been defined by the WHO as 0–1 mg/kg body weight (40). Thus the largest dose administered in the study presented here (2.2 g of *Curcuma* extract, containing 180 mg of curcumin) exceeds that of dietary consumption.

Our finding that curcumin was detectable only in the feces of patients and not in plasma, blood cells, or urine is in keeping with the low systemic bioavailability of p.o. administered curcumin seen in rodents (9, 17, 19, 20) and suspected in humans (38).⁴ After a single oral dose of 2 g, curcumin levels were transiently detectable in the serum of healthy volunteers (41). In that study, coingestion of curcumin with the pepper constituent l-piperoylpiperidine, which is thought to inhibit xenobiotic glucuronidation, appeared to increase curcumin serum AUC by a factor of 20. The presence of curcumin sulfate in the feces of one patient at the highest dose level described here is consistent with the suspicion that curcumin can undergo metabolic conjugation in the gut (42). We are currently testing the hypothesis that sulfation is the major biotransformation route of the curcumin molecule catalyzed by intestinal tissue.

Two potential biomarkers of the systemic efficacy of curcumin were evaluated in the pilot study described here. Lymphocytic GST activity decreased with time in the three patients who received the lowest dose level of *Curcuma* extract. This decrease may have been associated with the treatment, but in light of the small number of patients studied and the fact that GST activity was not decreased in patients on higher dose levels, the interpretation of this observation has to be tentative. Rats fed dietary curcumin at approximately 250 mg/kg body weight and above were found to have decreased hepatic GST activity compared to controls, and competitive enzyme inhibition by the curcumin molecule was thought to be responsible (7). It is unlikely that this observation can be used to rationalize the decline observed in our patients because the dose used in the rats was more than 60-fold higher than that given to the patients. Lymphocytic GST activity, as measured by the CDNB assay, has been shown to be independent of age and gender (43) and constant within subjects, as borne out by measurements on at least three occasions over a 2–4-week period in normal individuals and those at increased risk of developing colorectal cancer (44). The observations made in the study reported here propose similar consistency for patients with advanced cancer. Whether or not lymphocytic GST activity correlates with colon mucosal GST levels in patients with colon cancer, as was demonstrated in individuals at risk of developing colon cancer (44), remains to be established.

This study provides the first description of leukocytic M₁G

levels in patients with colorectal cancer. The lower levels shown in Fig. 2 are comparable with those reported previously in healthy volunteers, whereas the highest levels resemble those seen in humans on pro-oxidant diets (45). M₁G adduct levels were unaffected by *Curcuma* consumption. It is conceivable that higher doses of curcumin, which furnish measurable plasma curcumin concentrations, are required to elicit an antilipid peroxidative effect in the blood. Nevertheless, the intraindividual reproducibility over time of M₁G adduct levels supports the potential suitability of this adduct as a biomarker of the systemic effects of curcumin or other chemopreventive antioxidants. The putative link between the *GSTM1* null genotype and elevated leukocytic M₁G adduct levels observed in the patients in this trial is congruous with associations reported previously between *GSTM1* genotype and levels of aflatoxin B₁-induced DNA adducts (46) but is the first suggestion of such an association for an adduct formed by an endogenous product of lipid peroxidation.

One-third of the patients in this study experienced stable disease for 3 months or longer, and in one additional patient, *Curcuma* extract may have been linked to a decrease in venous tumor marker level and abatement of progression of the primary colon tumor without a cytostatic effect on liver metastases. The possibility that patients with colorectal cancer may benefit from consumption of *Curcuma* extract merits evaluation at higher dose levels and ultimately within the framework of larger studies incorporating control groups.

In conclusion, despite the lack of reproducible effects of *Curcuma* extracts on the biomarkers studied, this pilot study of *Curcuma* extract in patients with colorectal cancer provides information that might help optimize the design of the future clinical evaluation of curcumin. Doses of up to 2.2 g of *Curcuma* extract (containing 180 mg of curcumin) per day can be administered to patients with cancer for up to 4 months, and in this pilot study, such treatment was safe. Clinical trials of *Curcuma* extracts as potential cancer chemopreventive agents should focus on the effects of such doses in target tissues, particularly colon epithelium. Moreover, because consumption of *Curcuma* extract was not detrimental to patients with advanced cancer, future trials of *Curcuma* extracts as potential cancer chemotherapeutic agents should study the systemic effects of higher dose levels. Leukocytic GST activity and M₁G levels merit further exploration as potentially suitable biomarkers of pharmacological efficacy in this regard.

ACKNOWLEDGMENTS

We thank the following: the patients who participated in this study; N. Rush, C. Sangane, A. Alfirevic, and S. Feehan (Leicester and Liverpool Universities) for help with the patients' samples; Drs. R. Singh, D. Jones, S. Plummer, M. Festing, M. Williams, and C. K. Lim and Profs. D. Shuker and P. B. Farmer (MRC Toxicology Unit) for help with experiments and useful discussions; Prof. D. Barnett and Dr. P. Woll for reviewing the case report forms; and Phytopharm plc., particularly Drs. R. Grover and A. Kelly, for advice and provision of P54FP capsules.

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Clin Cancer Res 2001;7:1894-1900.

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