European Journal of Cancer 41 (2005) 1955–1968

European Journal of Cancer

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Curcumin: The story so far

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Received 15 March 2005; accepted 13 May 2005
Available online 2 August 2005

Abstract

Curcumin is a polyphenol derived from the herbal remedy and dietary spice turmeric. It possesses diverse anti-inflammatory and anti-cancer properties following oral or topical administration. Apart from curcumin's potent antioxidant capacity at neutral and acidic pH, its mechanisms of action include inhibition of several cell signalling pathways at multiple levels, effects on cellular enzymes such as cyclooxygenase and glutathione *S*-transferases, immuno-modulation and effects on angiogenesis and cell–cell adhesion. Curcumin's ability to affect gene transcription and to induce apoptosis in preclinical models is likely to be of particular relevance to cancer chemoprevention and chemotherapy in patients. Although curcumin's low systemic bioavailability following oral dosing may limit access of sufficient concentrations for pharmacological effect in certain tissues, the attainment of biologically active levels in the gastrointestinal tract has been demonstrated in animals and humans. Sufficient data currently exist to advocate phase II clinical evaluation of oral curcumin in patients with invasive malignancy or pre-invasive lesions of the gastrointestinal tract, particularly the colon and rectum.

Keywords: Carcinogenesis; Colorectal cancer; Chemoprevention

1. Introduction

Phytochemicals are naturally occurring substances found in plants. There has been considerable public and scientific interest in the use of phytochemicals derived from dietary components to combat human diseases, especially the two commonest killers in the developed world, cardiovascular disease and cancer. The dried ground rhizome of the perennial herb *Curcuma longa* Linn., called turmeric in English, haldi in Hindi and ukon in Japanese, has been used in Asian medicine since the second millenium BC [1]. Its utility is referred to in the ancient Hindu scripture, the Ayurveda. In addition to its aromatic, stimulant and colouring properties in the diet, turmeric is mixed with other natural compounds such as slaked lime and has been used topically as a treatment for wounds, inflammation and tumours. In contrast to the maximum dietary consumption of 1.5 g per person per day in certain South East Asian communities, smaller quantities of turmeric tend to be used for medicinal purposes [2]. The appeal of turmeric as a colouring, food preservative and flavouring is global – according to the Food and Agriculture Organization of the United Nations, over 2400 metric tons of turmeric are imported annually into the USA for consumer use.

Curcuma spp. contain turmerin (a water-soluble peptide), essential oils (such as turmerones, atlantones and zingiberene) and curcuminoids including curcumin [1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione]. Curcuminoids can be defined as phenolic compounds derived from the roots of *Curcuma* spp. (Zingiberaceae). Curcumin (diferuloylmethane) is a low

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0959-8049/$ - see front matter
doi:10.1016/j.ejca.2005.05.009

molecular weight polyphenol, first chemically characterised in 1910, that is generally regarded as the most active constituent of and comprises 2–8% of most turmeric preparations [3,4]. Curcumin has been the subject of hundreds of published papers over the past three decades, studying its antioxidant, anti-inflammatory, cancer chemopreventive and potentially chemotherapeutic properties. The pharmacology and putative anti-cancer properties of curcumin have been the subject of several review articles published since 1991 [5–7], which predate a number of clinical studies of curcumin which have been completed and published within the last 2 years. The purpose of the current article is to present an appraisal of the current level of knowledge regarding the potential of curcumin as an agent for the chemoprevention of cancer, particularly of the gastrointestinal tract, *via* an understanding of its pharmacology at preclinical and clinical levels. It will be argued on the basis of the data presented that the current state of knowledge on this phytochemical is sufficient to advocate its advancement into phase II clinical studies.

2. Chemical properties

Curcumin is a bis-α,β-unsaturated β-diketone. As such, curcumin exists in equilibrium with its enol tautomer. The bis-keto form predominates in acidic and neutral aqueous solutions and in the cell membrane [8]. At pH 3–7, curcumin acts as an extraordinarily potent H-atom donor [9]. This is because, in the keto form of curcumin, the heptadienone linkage between the two methoxyphenol rings contains a highly activated carbon atom, and the C–H carbon bonds on this carbon are very weak due to delocalisation of the unpaired electron on the adjacent oxygens (Fig. 1). In contrast, above pH 8, the enolate form of the heptadienone chain predominates, and curcumin acts mainly as an electron donor, a mechanism more typical for the scavenging activity of phenolic antioxidants [9]. Curcumin is relatively insoluble in water, but dissolves in acetone, dimethylsulphoxide and ethanol.

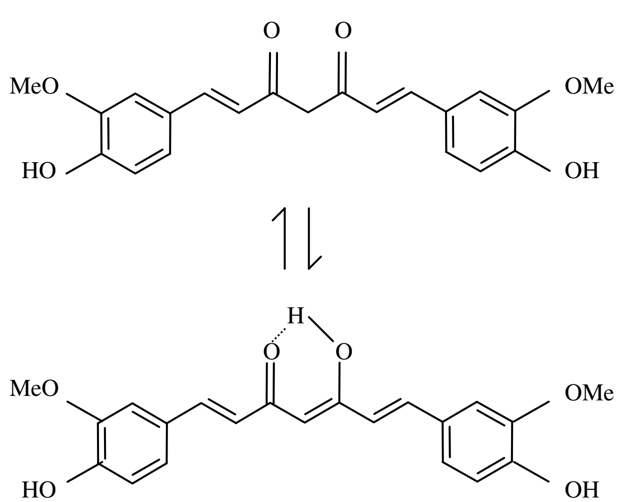

Fig. 1. Tautomerism of curcumin under physiological conditions. Under acidic and neutral conditions, the bis-keto form (top) predominates, whereas the enolate form is found above pH 8.

Curcumin is unstable at basic pH, and degrades within 30 min to *trans*-6-(4′-hydroxy-3′-methoxyphenyl)-2,4-dioxo-5-hexanal, ferulic acid, feruloylmethane and vanillin [10]. The presence of foetal calf serum or human blood, or addition of antioxidants such as ascorbic acid, *N*-acetylcysteine or glutathione, completely blocks this degradation in culture media or phosphate buffer above pH 7 [8]. Under acidic conditions, the degradation of curcumin is much slower, with less than 20% of total curcumin decomposed at 1 h [8]. Other investigators have also found that curcumin is more stable in cell culture medium containing 10% foetal calf serum or in human blood, with less that 20% decomposition within 1 h compared to 90% within 30 min in serum-free medium [8]. The complex kinetics of pH-dependent degradation of curcumin in aqueous solution were first reported by Tonnesen and Karlsen [11]. These investigators went on to study curcumin's photochemical stability, offering the first suggestions of its potential antimicrobial activity by photosensitisation [12]. As a result of light sensitivity, samples containing curcumin should be protected from light. Above pH 7, curcumin's hue is less yellow and more red.

Curcumin has a molecular weight of 368.37 and a melting point of 183 °C. Commercial grade curcumin contains the curcuminoids desmethoxycurcumin (MW 338; typically 10–20%) and bisdesmethoxycurcumin (MW 308; typically less than 5%, for structures see Fig. 2). On ultraviolet–visible spectrophotometric investigation, maximum light absorption of curcumin occurs at 420 nm. Studies in preclinical models of carcinogenesis have demonstrated that commercial grade curcumin has the same inhibitory effects as pure curcumin [13,14]. It is not known whether essential oils derived from *Curcuma* spp. have intrinsic activity akin to curcumin [15].

3. Pharmacokinetic properties

3.1. Preclinical pharmacokinetics

The absorption, metabolism and tissue distribution of curcumin has been studied in at least 10 studies performed in rodents over the past three decades. In an early study, a dose of 1 g/kg was administered to rats in the diet [16]. About 75% of the dose was excreted in the faeces and negligible amounts appeared in the urine. A few years later, a study of oral curcumin administered to rats demonstrated 60% absorption of curcumin and presented evidence for the presence of glucuronide and

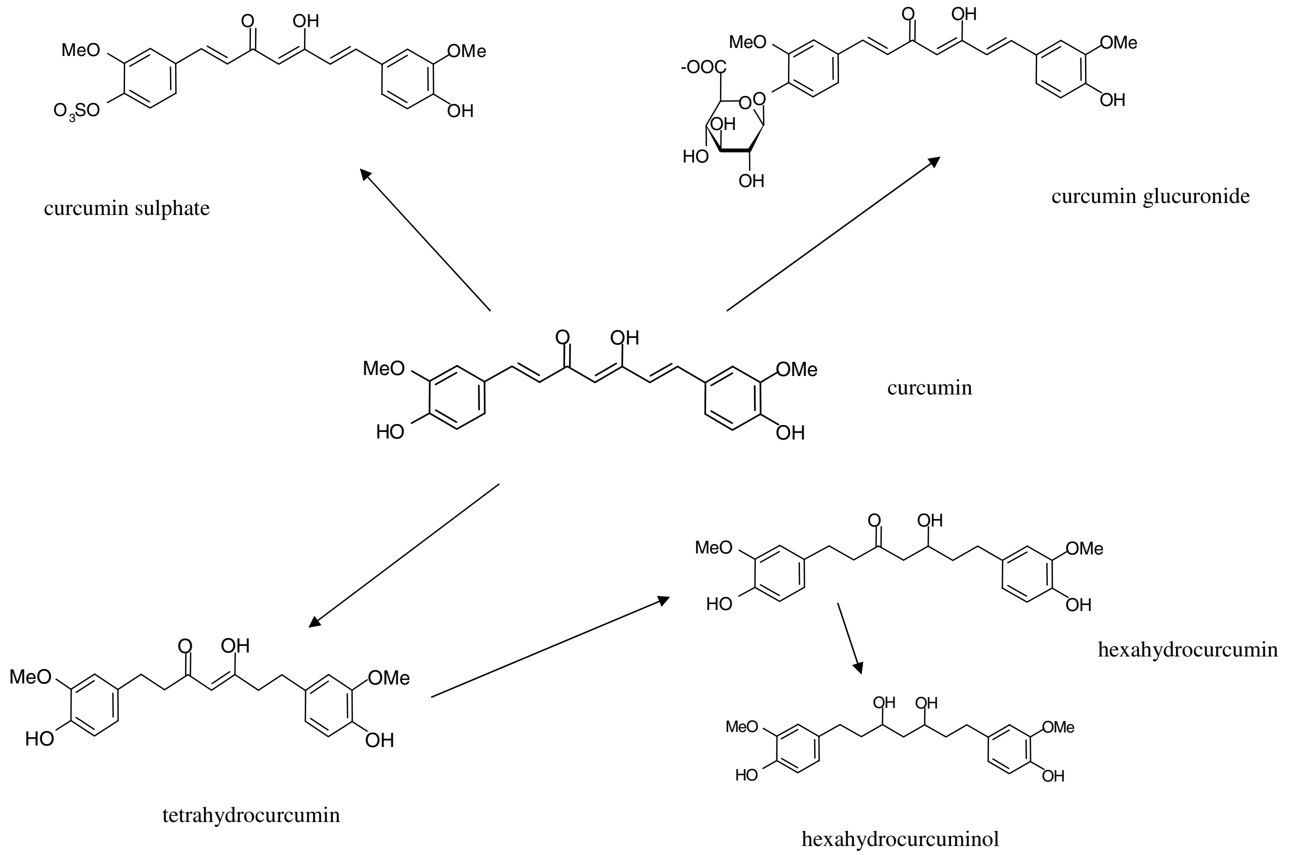

Fig. 2. Chemical structures of major metabolites of curcumin in rodents and humans.

sulphate conjugates in urine [17]. The same investigators proceeded to study the bioavailability of curcumin using ^{3}H-radiolabelling; oral administration resulted in the vast majority of the oral dose being excreted in faeces, and only one-third was excreted unchanged [18]. Intravenous and intraperitoneal administration of curcumin in rodents resulted in large quantities of curcumin and metabolites in bile, which were characterised as mainly tetrahydrocurcumin and hexahydrocurcumin glucuronides [19,20]. After intravenous dosing, more than 50% of the dose was excreted in the bile within 5 h; these data were presented as evidence that curcumin undergoes transformation during absorption *via* the intestine and is possibly subject to entero-hepatic recirculation [20]. Such an hypothesis was originally presented by Holder, Plummer and Ryan [19] based on their studies of the fate of curcumin in rats.

A more recent study of intraperitoneal curcumin (0.1 g/kg) in the mouse has suggested that curcumin was first biotransformed to dihydrocurcumin and tetrahydrocurcumin, and that these compounds were subsequently converted to monoglucuronide conjugates [21]. Preclinical studies of oral dosing of curcumin in rats using modern high pressure liquid chromatography (HPLC) techniques demonstrate small amounts of curcumin in plasma with higher levels of curcumin glucuronide and curcumin sulphate in plasma, and small quantities of hexahydrocurcumin, hexahydrocurcuminol and hexahydrocurcumin glucuronide [22], as summarised in Fig. 3. This preclinical work was extended using suspensions of isolated human hepatocytes or liver or gut microsomes [23]. The data suggested that metabolic reduction occurs very rapidly, in a matter of minutes. A study of high dose curcumin (2% in the diet, equating to approximately 1.2 g curcumin per kg body weight) for 14 days has shown that low nanomolar levels are detectable in plasma, with concentrations in liver and colon mucosal tissue ranging from 0.1 to 1.8 nmol/g tissue [24]. In a study of oral curcumin (2 g/kg) in rats performed in Bangalore, India, the investigators suggested that co-administration of piperine may increase systemic bioavailability following oral dosing by as much as 154%, potentially by inhibition of xenobiotic glucuronidation [25]. Piperine is primarily found in the fruit of the pepper vine, piper nigrum, and can also be found in other vegetables and spices such as hot jalapeno peppers. It is also said to give peppercorns their hot, biting and pungent taste.

In summary, curcumin exhibits low oral bioavailability in rodents and may undergo intestinal metabolism; absorbed curcumin undergoes rapid first-pass metabolism and excretion in the bile.

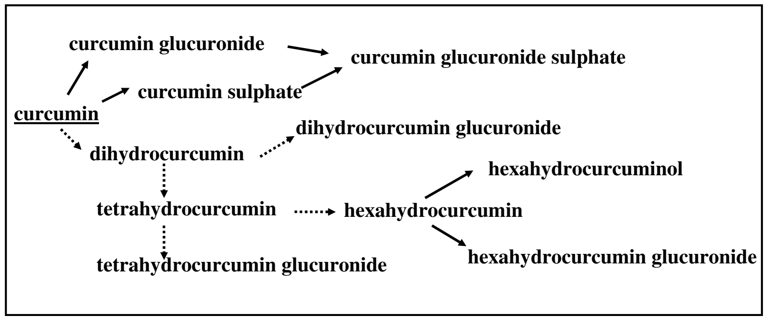

Fig. 3. Metabolic pathways of curcumin in rodents and *ex vivo* culture of rat and human hepatocytes. Solid arrows show metabolic pathways demonstrated in rat hepatocyte culture, human hepatocyte culture or in rat plasma *in vivo*. Broken arrows show metabolic pathways demonstrated in mice *in vivo*.

3.2. Clinical pharmacokinetics

In comparison to the preclinical work presented above, comprehensive pharmacokinetic data in humans is lacking. In a clinical study to parallel their work in rats, Shoba and colleagues administered 2 g of pure curcumin powder to fasting volunteers resulting in low curcumin concentrations detected in plasma (less than 10 ng/ml) 1 h post-dose [25]. In the same study, co-ingestion of curcumin with 20 mg of the pepper constituent l-piperoylpiperidine appeared to increase curcumin's bioavailability by 2000%. In a study of high dose oral curcumin performed in Taiwan, Cheng and colleagues administered 0.5–8 g daily of curcumin for 3 months to patients with pre-invasive malignant or high risk pre-malignant conditions of the bladder, skin, cervix, stomach or oral mucosa [26]. Serum curcumin concentrations were found to peak 1–2 h after oral intake and gradually decline within 12 h. The 8 g/day dose resulted in a peak serum concentration of 1.75 ± 0.80 μM. In a study performed in Michigan, USA, 50–200 mg of micronised curcumin was administered to 18 healthy volunteers as an oral dose with orange juice, resulting in no evidence for the presence of curcumin in the serum at the limit of quantitation, which was approximately 0.63 ng/ml [27].

Two clinical phase I dose escalation studies have been performed in Leicester, England, over the past 5 years. In the first study using a standardised oral *Curcuma* extract, doses up to 180 mg of curcumin per day were administered to patients with advanced colorectal cancer for up to 4 months without overt toxicity or detectable systemic bioavailability [28]. In a subsequent phase I study in 15 patients with advanced colorectal cancer refractory to standard chemotherapies, a curcuminoid formulation was consumed orally for up to 4 months, equating to curcumin doses between 0.45 and 3.6 g daily [29]. Levels of curcumin and its metabolites in plasma, urine and faeces were analysed by HPLC and mass spectrometry. Oral consumption of 3.6 g of curcumin daily resulted in levels of drug and conjugates in plasma near the limit of detection of the assays used. Surprisingly, analysis of urine suggested the presence of curcumin and its conjugates in all samples from patients consuming this dose. Such chromatographic peaks were not seen in any extracts of urine samples from patients on the lower doses. In the six patients consuming 3.6 g of curcumin daily, urinary levels varied between 0.1 and 1.3 μM (curcumin), 19 and 45 nM (curcumin sulphate) and 210 and 510 nM (curcumin glucuronide). Since the measurement of compliance is increasingly perceived to be an important component of intervention trials, the consistent presence of curcumin and its conjugates in urine observed in patients consuming 3.6 g of curcumin daily is of potential relevance to the clinical advancement of curcumin as a chemopreventive agent. Since urinary analysis of drug-derived species constitutes an easily accessible and a reproducible test for ensuring general compliance, larger studies of this dose level are merited to confirm the consistent presence of curcumin and its conjugates and to define inter- and intra-individual variability.

In parallel with dose escalation trials to determine the systemic bioavailability of curcumin in plasma and excreta, exploratory studies have also been performed in patients undergoing operations for colorectal cancer who have offered consent to tissue being obtained for designated research purposes [30,31]. Twelve patients with confirmed colorectal cancer received oral curcumin at 0.45, 1.8 or 3.6 g *per diem* for 7 days prior to surgery. Levels of agent-derived species were determined in the peripheral circulation and in colorectal tissue obtained at the time of surgical resection. The concentrations of curcumin in normal and malignant colorectal tissue of patients consuming 3.6 g daily of curcumin were 12.7 ± 5.7 and 7.7 ± 1.8 nmol/g tissue, respectively [30]. Curcumin sulphate and curcumin glucuronide were identified in the intestinal tissues of these patients. Trace levels of curcumin were found in the peripheral circulation. Compatible with the preclinical data presented above, these preliminary results in human subjects suggest that a daily dose of 3.6 g curcumin achieves measurable levels in colorectal tissue with negligible distribution of the parent drug outside the gut.

In order to study the levels of curcumin in hepatic tissue following oral dosing, 12 patients with liver metastases from colorectal cancer received 0.45–3.6 g of oral curcumin daily for 7 days prior to hepatic surgery [31]. Levels of curcumin and its metabolites were measured by HPLC in portal and peripheral blood, bile and liver tissue. Low nM levels of the parent compound and its glucuronide and sulphate conjugates were found in peripheral blood samples taken 1 h after the seventh dose of curcumin and in portal blood samples taken 6–7 h after the seventh dose of curcumin. Whilst curcumin was not found in liver tissue resected and preserved 6–7 h after the seventh dose of curcumin, trace levels of products of its metabolic reduction were detected. It was concluded from this pilot study that doses of oral curcumin required to produce hepatic levels sufficient to exert pharmacological activity are probably not feasible in humans using this pharmaceutical formulation.

Summarising the data from pilot and Phase I clinical studies performed with curcumin, it appears that low systemic bioavailability following oral dosing is consistent with the findings in preclinical models presented above. Efficient first-pass and some degree of intestinal metabolism of curcumin, particularly glucuronidation and sulphation, may explain its poor systemic availability when administered *via* the oral route, as suggested by the detection of metabolites in the plasma from patients consuming high doses of curcumin daily. A daily oral dose of 3.6 g of curcumin results in pharmacologically efficacious levels in colorectal tissue, with negligible distribution of the parent drug in hepatic tissue or other tissues outwith the gastrointestinal tract. Urinary analysis of drug-derived species may constitute an easily accessible and a reproducible test for ensuring general compliance with high doses of oral curcumin.

4. Safety

Recent concerns regarding the safety of selective enzyme inhibitors in large-scale chemoprevention trials emphasise the importance of carefully evaluating any potential toxicity of agents at the preclinical and early clinical trial levels. It cannot be assumed that diet-derived agents will be innocuous when administered as pharmaceutical formulations at doses likely to exceed those consumed in the dietary matrix. Anecdotal reports suggest that dietary consumption of turmeric up to 1.5 g per person per day, equating to a maximum of 150 mg/day of curcumin, are not associated with adverse effects in humans [2].

Studies of curcumin in animals have confirmed a lack of significant toxicity since an early report in which doses up to 5 g/kg were administered orally to Sprague–Dawley rats [16]. Systematic preclinical studies funded by the Prevention Division of the US National Cancer Institute did not discover adverse effects in rats, dogs or monkeys of doses up to 3.5 g/kg body weight (BW) administered for up to 3 months [32]. One report of dietary curcumin suggested ulcerogenic activity in the stomach of the albino rat [33], but this finding has not been confirmed in subsequent studies. In more recent preclinical studies of curcumin, no toxicity has been observed from 2% dietary curcumin (approximately 1.2 g/kg BW) administered to rats for 14 days [24] or from 0.2% dietary curcumin (approximately 300 mg/kg BW) administered to mice for 14 weeks [34].

Although very few clinical studies of oral curcuminoids have reported any discernible toxicity, it has not been clearly stated by the investigators of most of these studies which methods or which scales have been used to assess potential toxicity. Administration of 1.2–2.1 g of oral curcumin daily to patients with rheumatoid arthritis in India for 2–6 weeks did not result in any reported adverse effects [35]. In a study of high dose oral curcumin in Taiwan, Cheng and colleagues administered up to 8 g daily of curcumin for 3 months to patients with pre-invasive malignant or high risk pre-malignant conditions, stating that no toxicity was observed [26]. In patients with advanced colorectal cancer treated in the UK, curcumin was well tolerated at all dose levels up to 3.6 g daily for up to 4 months [29]. Two types of gastrointestinal adverse events were reported by patients, which were probably related to curcumin consumption: one patient consuming 0.45 g daily and one patient consuming 3.6 g daily developed diarrhoea (US National Cancer Institute (NCI) grades 1 or 2) one month and four months into treatment, respectively. One patient consuming 0.9 g curcumin daily experienced nausea (NCI toxicity grade 2), which resolved spontaneously despite continuation of treatment. Two abnormalities were detected in blood tests, both possibly related to treatment: a rise in serum alkaline phosphatase level was observed in four patients, consistent with NCI grade 1 toxicity in two patients and grade 2 toxicity in two patients; serum lactate dehydrogenase rose to more than 150% of pre-treatment values in three patients. These abnormal blood test results may have been related to disease progression rather than treatment toxicity.

5. Biological activity in preclinical models

Curcumin is notable for the diversity of its biological actions in preclinical models of carcinogenesis at a very wide range of physiologically attainable and supra-physiological doses (see Fig. 4). Indeed, some studies have suggested that curcumin elicits systemic effects relevant

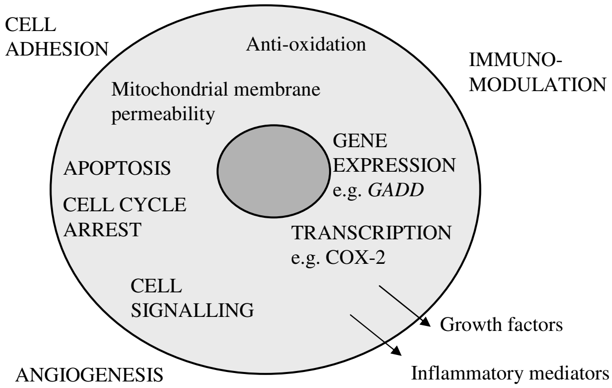

Fig. 4. Simplified representation of diverse cellular processes affected by curcumin. For supporting references, see main text.

to the chemoprevention of cancer in hepatic and mammary tissues of animals following attainment of levels of curcumin in these tissues that are only in the 10^{-9}–10^{-8} M range [36,37].

5.1. Cytotoxicity and induction of apoptosis

Studies in a variety of cell lines grown *in vitro* have demonstrated the cytotoxicity of curcumin in the micromolar to millimolar concentration range. In three different malignant colon cell lines, curcumin inhibited proliferation, induced apoptosis and caused accumulation of cells in the G2/M phase of the cell cycle [38–40]. Similar results have been observed in breast, kidney, hepatocellular, lymphoid, myeloid, melanoma, oral epithelial and prostate cell lines derived from malignant tumours [6,41–47]. In addition, curcumin has shown growth inhibitory effects *in vitro* in cancer cell lines derived from human prostate, large intestine, bone and leukaemia [48–51].

Curcumin's effects on the cell cycle are by no means consistent, and they may be tissue-specific. In mouse embryo fibroblast, mouse sarcoma, HT29 human colon carcinoma, human kidney carcinoma, and human hepatocellular carcinoma cell lines grown *in vitro*, Jiang and colleagues [41] observed cell shrinkage, chromatin condensation and DNA fragmentation secondary to 9 μM curcumin treatment. In other colon carcinoma cells cultured *in vitro*, curcumin induced apoptotic cell death by cell cycle arrest in the S and G2/M phases [39], and in the MCF-7 human breast tumour cell line the same was observed at G2 or M phases [42]. The presence of the diketone moiety may be a prerequisite for demonstration of antiproliferative activity [42]. Although one report has claimed that the inhibition of cell proliferation may be non-selective with regard to transformed/non-transformed cell lines *in vitro* [43], comparison in the SV40-virus transformed human colon epithelial cell line (HCEC) with the malignant colon adenocarcinoma cell line HT29 has shown some degree of tumour-specificity, with an IC_{50} for the malignant cells of about 5 μM compared to 14 μM for the non-malignant cells [44]. A subsequent study of curcumin's effects on colon cancer cells grown *in vitro* has demonstrated dose-dependent growth inhibition and stimulation of the trans-activating activity of peroxisome proliferator-activated receptor γ (PPAR-γ), which appears to mediate the suppression of gene expression of cyclin D1 and the epidermal growth factor receptor (EGFR) [49]. Noteworthy experiments in normal thymocytes and in colon carcinoma and myeloid leukaemia cells cultured *in vitro* have suggested that curcumin induces degradation of the tumour suppressor protein, p53, *via* inhibition of NAD(P)H:quinone oxidoreductase 1 (NQO1) activity [52].

G2/M arrest renders cells more susceptible to the cytotoxic effects of radiation, suggesting that curcumin may act as a radiosensitiser. Evidence in favour of this hypothesis has been presented from studies utilising prostate cancer and leukaemia cell lines grown *in vitro* [53,54], but contradictory results have also been published [55]. The antimicrobial and antiviral properties of curcumin have been suggested by numerous studies performed *in vitro* [56,57]. Curcumin also inhibits cell proliferation and induces apoptosis in fungi and viruses [58,59].

The mechanisms responsible for apoptosis induction by curcumin appear to be varied, including effects on the stability of p53, the release of cytochrome c and the generation of reactive oxygen species [6,52]. Inhibition of cell signalling pathways involving Akt, NF-κB, AP-1 or JNK have been implicated, as has up-regulation of growth arrest and DNA damage-inducible (*GADD)* genes and down regulation of the expression of survival genes *egr-1*, *c-myc*, *bcl-X(L)* and IAP or abnormal tumour suppressor genes such as *p53* [39,45,46,60–62]. Gene expression profiling by cDNA array has been used to compare total RNA extracted from curcumin-treated and untreated oral epithelial cancer cells [47]. The application of 12 625 probes identified 202 up-regulated mRNAs and 505 repressed transcripts (greater than or equal to twofold differential expression), including the pro-apoptotic activating transcription factor 3 (ATF3) that was induced more than four-fold [47].

Curcumin has also been shown to have immunomodulatory effects, involving activation of host macrophages and natural killer cells and modulation of lymphocyte-mediated function [46,63]. Since mitochondria play an important role in cell proliferation and apoptosis, it is interesting to note that curcumin increases mitochondrial membrane permeability in rat hepatocytes *via* opening of the permeability transition pore [64]. Like other proteasome inhibitors, curcumin targets proliferating cells more efficiently than differentiated ones [65].

5.2. Inhibition of carcinogenesis

Following oral administration, curcumin has been shown to prevent cancer in the colon, skin, stomach, liver, lung, duodenum, soft palate and breasts of rodents [32,66]. The effects of dietary curcumin (0.05–2.0%) on colon carcinogenesis in particular have been demonstrated in both chemical and genetic rodent models. This information is relevant to the study of carcinogenesis since inhibition of initiation has been demonstrated in chemical models, demonstrated *via* the adducts induced by benzo[a]pyrene or by aflatoxin B_1 [67,68], whereas genetic models such as the multiple intestinal neoplasia (*e.g.*, APC^{Min} mouse) model permit the study of inhibition of the promotion phase of carcinogenesis [34,69]. Chemical models of the promotion/progression of colon cancer have also permitted the study of the effects of oral curcumin. In intestinal cancer induced in mice by azoxymethane, 2000 parts per million of oral curcumin for 14 weeks produced a significant increase in the apoptotic histological index when compared to controls [70]. Curcumin interfered with adenoma formation in the Apc^{Min} mouse, which harbours an *adenomatous polyposis coli* (*APC*) gene mutation and is a model of the human disease familial adenomatous polyposis [71]. When administered in the diet for the animals' lifetimes at 0.1% and 0.2%, significant decreases in adenoma number were observed compared to control animals [34,69]. The latter dose, which equates to approximately 300 mg/kg BW per day, furnished only trace levels of curcumin and metabolites in the plasma, but concentrations of curcumin in the 100 nmol/g tissue range in the gastrointestinal mucosa [34]. It was argued by the investigators that this result provides a tentative "target concentration", although no reliable data currently exist on how extrapolation to the equivalent levels in human gastrointestinal mucosa can be calculated.

Topical application of curcumin has also been shown to inhibit chemical carcinogenesis of the skin [72]. In this series of studies, tumour initiation was induced by benzo[a]pyrene or 7,12-dimethylbenz[a]antracene (DMBA) and tumour promotion was induced by 12-*O*-tetradecanoylphorbol-13-acetate. Potential mechanisms of these effects were considered to involve inhibition of arachidonic acid-induced inflammation, inhibition of hydrogen peroxide formation, and inhibition of ornithine decarboxylase activity/transcription, which is a rate-limiting step in polyamine biosynthesis [72]. Application of curcumin thrice weekly to the buccal pouch of Syrian golden hamsters has also demonstrated inhibition of DMBA-induced oral carcinogenesis [73]. In this early example of combinatorial chemoprevention [73,74], the effect of topical curcumin appeared to be enhanced by the concomitant oral administration of green tea. Synergy of curcumin with the pro-differentiation agent, all-*trans* retinoic acid, has also been demonstrated in the induction of differentiation of promyelocytic leukaemia cells to granulocytes [75].

In summary, curcumin's cancer chemopreventive properties in rodents are impressively diverse, as reflected by the variety of chemical and genetic models of early and intermediate stages of carcinogenesis in which efficacy has been demonstrated. The diversity of its biological actions was recently highlighted by a noteworthy study demonstrating its beneficial effects in mice homozygous for a complete knockout of a gene linked with cystic fibrosis [76].

5.3. Inhibition of cyclooxygenase transcription

It has been known for over a decade that curcumin can inhibit cyclooxygenase (COX) activity in rat peritoneal neutrophils and human platelets [5]. COX is a key enzyme responsible for the conversion of arachidonic acid to prostaglandins and thromboxanes. It consists of two different isoforms, designated COX-1 and COX-2. COX-1 is a constitutive isoform present in most tissues and is generally regarded as an 'housekeeping' enzyme; its inhibition results in serious effects such as peptic ulceration or impairment of renal blood flow. In contrast, COX-2 is constitutively expressed only in brain and spinal cord tissue; it can be induced in a wide variety of normal tissues by the hormones of ovulation and pregnancy, cytokines, growth factors, oncogenes and tumour promoters [77]. COX-2 overexpression has been implicated in the carcinogenesis of tumours of colon, rectum, breast, head and neck, lung, pancreas, stomach and prostate [78].

Curcumin's ability to inhibit induction of COX-2 gene expression has been demonstrated in oral and colon epithelial cells *in vitro* [74,79]. At a concentration of 20 μM, curcumin's inhibition of chemically induced PGE_2 production in colon cells was significantly greater than that of its metabolites, tetrahydrocurcumin, hexahydrocurcumin, curcumin sulphate, and hexahydrocurcuminol [22]. One of the mechanisms for this effect is inhibition of the activity of the IKK signalling complex responsible for phosphorylation of IκB [80]. The IKK signalling complex is integral to activation of the transcription factor, NF-κB, and is also inhibited by aspirin, a drug whose regular use may decrease the incidence of colorectal cancer [81].

Unlike selective COX-2 inhibitors, which inhibit the catalytic activity of the COX enzyme, curcumin decreases COX-2 expression at the transcriptional level [80]. It may also inhibit COX-1 transcription (Dr. S. Plummer, unpublished data), an isozyme relevant to the local spread of malignancy and the cooperation between malignant cells and neighbouring stromal cells [82]. The ability of many natural agents derived from the diet to act at more than one level in a cellular pathway (reviewed in [83]) is likely be important in the prevention or treatment of diseases with multifactorial aetiologies such as cancer. Curcumin's ability to inhibit activation of pathways that interact with the NF-κB pathway, notably those involving activator protein-1 (AP-1) and c-Jun NH_2-terminal kinases (JNK) [60,61] provides an example of this concept. The AP-1 and JNK pathways constitute an important group of terminal kinases involved in cellular responses to environmental stress, pro-inflammatory cytokines, mitogen stimulation and apoptotic stimuli.

5.4. Effects on phase I and II carcinogen metabolising enzymes

The cytochromes P450 (CYP) enzyme system is important in the metabolic conversion and activation of many compounds, such as tetrachloromethane and aflatoxin B_1, to toxic reactive metabolites. Inhibition of CYP isoenzymes by curcumin has been demonstrated in cells cultured *in vitro* [84] and this may represent a mechanism by which dietary curcumin protects animals against the toxic effects of many chemicals. For example, in a mammary carcinoma cell line, curcumin's inhibition of CYP1A1-mediated activation of dimethylbenzanthracene resulted in diminished DNA adduct formation [85]. Understanding of curcumin's effects on metabolising enzymes such as CYP is also important in determining potential drug interactions in clinical usage.

In contrast to CYP enzymes, phase II metabolising enzymes such as glutathione *S*-transferases (GST) are regarded as detoxifiers; induction rather than inhibition is generally regarded as favourable in early carcinogenesis, potentially conferring a protective effect. Epoxide hydrolase and various hepatic GST isoenzymes were significantly increased upon curcumin feeding in mice [86] and total GST activity has been induced by dietary curcumin in both mice and rats in other studies [87–90]. A structure–activity study of the potency of curcumin analogues suggests that their ability to induce phase II enzymes may be linked to the presence of the hydroxyl groups at *ortho*-positions on the aromatic rings and the β-diketone functionality [90]. Although induction of GST activity may be desirable in the prevention of the early stages of carcinogenesis, in patients with advanced malignancy GST isozymes may be aberrantly overexpressed and linked with resistance to chemotherapy [91]. Curcumin also appears capable of inhibiting GST isoenzymes, for example *GSTP1* expression in leukaemia cells grown *in vitro* with an association between the level of inhibition and the induction of apoptosis [92].

5.5. Antioxidant actions

Reactive oxygen species, such as superoxide anions and hydroxyl radicals, play a pivotal role in atherosclerosis and are also thought to be involved in carcinogenesis. Consequently, mopping up of activated oxygen species is an important mechanism invoked in the prevention of cardiovascular disease and cancer. Impairment of reactive oxygen species generation in rat peritoneal macrophages by 10 μM curcumin has been shown [93], and similar effects have been observed in red blood cells at similar concentrations [94]. More specifically, curcumin has been shown to scavenge superoxide anion radicals and hydroxyl radicals [95,96]. It should be noted, however, in keeping with other dietary phytochemicals, that curcumin may possess pro-oxidant activity as well as antioxidant effects, dependent on dose and chemical environment, *e.g.*, availability of free Cu^{2+} ions [97]. The balance between anti-oxidant and pro-oxidant activity is an important consideration in planning intervention trials in healthy volunteers, particularly if its activity results in potentially deleterious effects as suggested by biomarkers such as DNA adduct levels [98].

Nitric oxide (NO) is a short-lived, lipophilic molecule generated from L-arginine by various NADPH-dependent enzymes called NO synthases (NOS) [99]. NO is involved physiologically in vasorelaxation, neurotransmission, inhibition of platelet aggregation, immune defence and intracellular signalling. NO has an unpaired electron, and is therefore a free radical species; its bioactivity is related to production of many reactive intermediates, but many of these reactive nitrogen species are capable of damaging DNA or hindering DNA repair [100–102]. Peak inducible NOS (iNOS) activity may relate to the transition of colonic adenomas to carcinomas [103]. Upregulation of COX-2 *via* NF-κB or AP-1 pathways, or increasing intracellular concentrations of reduced glutathione, appears to confer resistance to NO-induced apoptosis in malignant cells *in vitro* [104,105]. *Ex vivo* studies have suggested that the inducibility of macrophage NOS activity is inhibited by 1–20 μM concentrations of curcumin [106]. Subsequently, it has been claimed that administration of an aqueous alkaline solution of curcumin in the drinking water at a dose of as little as 92 ng per g BW significantly inhibits murine hepatic lipopolysaccharide-induced *iNOS* gene expression [36]. Since inhibition of iNOS activity may represent a mechanism of intervention during carcinogenesis, if this effect is reproducible, curcumin's activity at such low concentrations would have considerable implications for cancer chemoprevention.

5.6. Effects on angiogenesis and cell adhesion

Curcumin also affects carcinogenic processes associated with the growth and dissemination of established malignancy. Angiogenesis, the formation of new blood vessels from host vasculature, has been associated with neoplasia since the experiments of Greene published in

1941 [107]. Angiogenesis is now regarded as critical to the transition of premalignant lesions in a hyperproliferative state to the malignant phenotype, thus facilitating tumour growth and metastasis [108]. The intensity of angiogenesis, as assessed by counting microvessels in malignant tissue, acts as a prognostic factor for many solid tumours such as breast, prostate and ovarian cancers and very early cancers of the endometrium and lung (reviewed in [109]). Similarly, expression of angiogenic growth factors correlates with prognosis for lung and other cancers. Inhibition of angiogenesis by curcumin (10 μM and above) has been demonstrated *in vivo* using a mouse corneal model [110]. Inhibition of angiogenic growth factor production, integral to the formation of new vessels, has also been effected by curcumin in non-malignant and malignant cells [111,112].

Similarly, curcumin has been shown to affect proteins related to cell–cell adhesion, such as β-catenein, E-cadherin and APC, and to inhibit the production of cytokines relevant to tumour growth, *e.g.*, tumour necrosis factor-α and interleukin-1, and to reduce the expression of membrane surface molecules that play a role in cellular adhesion [113–116]. These results hint at the possibility that curcumin may affect the behaviour of established malignancy *in vivo*.

6. Pharmacodynamics in humans

6.1. Dose–effect relationships

Although any substantial data in favour of a dose–response relationship for any biomarker of curcumin's activity is currently lacking, several observations in human volunteers and patients suggest that curcumin may possess systemic biological activity at low oral doses. In a small study performed in Taiwan, a single oral dose of 20 mg curcumin appeared to induce contraction of the gall bladder assessed by ultrasound scanning in human volunteers, compared to amylum placebo [117].

In a pilot study performed in Leicester, England, doses up to 180 mg of curcumin per day were administered to patients with advanced colorectal cancer for up to 4 months [28]. Two potential biomarkers of curcumin's systemic efficacy were evaluated. In three patients taking 36 mg of curcumin daily, lymphocytic activity of the detoxification enzymes, GST, decreased gradually with time from a pre-treatment GST value of 64 ± 19 nmol/min/mg protein to 26 ± 13 nmol/min/mg protein on day 29 of treatment. This decline was not observed at the higher dose levels and was not reproduced in a subsequent study of higher doses in the patients with the same disease [29]. Similarly, consumption of curcumin did not affect blood leukocyte levels of the oxidative DNA adduct, pyrimido-[1,2α]purin-10(3H)-one-2′-deoxyguanosine (M_1G), described for the first time in patients with colorectal cancer, although interesting observations were made regarding *GST* isoenzyme genotypes and baseline leukocytic M_1G adduct levels.

In contrast to leukocyte M_1G and GST, the inducibility of prostaglandin (PG) E_2 production in whole blood *ex vivo* may represent a surrogate biomarker for assessing the pharmacological activity of curcumin at a systemic level. As discussed above, COX-2 is an important target for chemoprevention and its pharmacological modulation holds implications for cancer treatment. At least part of curcumin's effect on inducible PGE_2 production in human blood can be attributed to inhibition of *COX2* transcription, which may be due to the inhibition of the NFκB-activating enzymes IKK-α/β [77,78,80]. The effect of curcumin described in an *ex vivo* assay developed using blood from healthy volunteers [118] was associated with plasma levels detected in the 10^{-8} M range in patients with advanced colorectal cancer [29], less than a hundredth of the concentration of curcumin shown *in vitro* to elicit an effect in blood or colon cells [22,80]. Blood was taken immediately pre-dose or 1 h post-dose on days 1, 2, 8 and 29 of treatment with 3.6 g of curcumin daily [29]. Following addition of acetylsalicylic acid (200 μM) to eliminate COX-1 activity, whole blood was incubated for 24 h in the presence of lipopolysaccharide (LPS, 10 μg/ml) [118]. In the trial described above, oral administration of curcumin did not impact on basal PGE_2 levels in leukocytes, nor did doses of 0.45–1.8 g daily alter LPS-induced PGE_2. In contrast, consumption of 3.6 g of curcumin daily affected LPS-induced PGE_2 levels [29]: When values obtained immediately pre- or 1 h post-dose on days 1, 2, 8, and 29 were pooled for the six patients consuming this dose, PGE_2 levels observed post-dose were 46% lower ($P = 0.028$) than those measured immediately pre-dosing. The difference reached significance on the first day and 29th day of treatment, but not on day 2 or day 8 [29]. Although these results suggested that consumption of 3.6 g of curcumin daily was linked with inhibition of PGE_2 induction in blood taken post-dose compared to blood taken pre-dose, overall time-dependent trends were not identified and no dose-response has been demonstrated for this biomarker. Although the *ex vivo* assay described using human blood is limited in its clinical application by the high inter-individual and high intra-individual variability [118], the results suggest the feasibility and potential utility of measurement of PGE_2 levels in target tissue as a biomarker reflecting potential anticancer activity of curcumin. It should also be noted that curcumin sulphate and products of metabolic reduction of curcumin also inhibited PGE_2 production in colon cells grown *in vitro*, although their inhibitory potency appeared lower than that of parent curcumin in these cells [22].

In parallel with studies in which potential changes in the blood from patients with advanced colorectal cancer were analysed, exploratory investigations have also been performed in patients undergoing operations for resectable colorectal cancer in whom colorectal and hepatic tissues have been analysed to study potential pharmacodynamic effects [30,31]. Twelve patients with confirmed colorectal cancer received oral curcumin at 0.45, 1.8 or 3.6 g *per diem* for 7 days prior to surgery [30]. Whereas ingestion of 3.6 g of curcumin daily for a week did affect M_1G levels in patients' colorectal tissue, it did not decrease COX-2 protein expression in this tissue [30]. Interestingly, M_1G adduct levels were 2.5-fold higher in malignant colorectal tissue than normal colorectal mucosa. Whilst administration of curcumin did not affect M_1G levels in normal colorectal mucosa, it caused a decrease in adduct levels in malignant colorectal tissue from 4.8 ± 2.9 adducts per 10^7 nucleotides to 2.0 ± 1.8 adducts per 10^7 nucleotides ($P < 0.05$). The effect was only observed at the highest dose level and requires replication in larger studies; a dose–response relationship could not be established. A similar study of hepatic tissue with the same oral dosing regime suggested that levels of curcumin in normal and malignant liver tissue were insufficient to exert biological activity [31].

6.2. Anti-inflammatory effects

Data from preclinical models would suggest that curcumin's suppression of the inflammatory response may involve inhibition of the induction of COX-2, iNOS and production of cytokines such as interferon-γ, at least in part due to its suppression of the Janus kinase (JAK)-STAT signalling cascade *via* its effect on the Src homology 2 domain-containing protein tyrosine phosphatases (SHP)-2 [119]. In myeloma cells, curcumin has also been shown to inhibit STAT3 phosphorylation and thus suppress interleukin-6 production [120]. Compatible with these immunological effects, data from a chemical model of inflammatory bowel disease suggest that curcumin may be of value in the treatment of this disease [121].

A number of teams have studied the effect of oral curcumin on inflammatory diseases in humans. Satoskar and colleagues [122] found a significant anti-inflammatory effect objectively and subjectively from 400 mg thrice daily for 5 days in post-operative patients. In a double-blind study, Deodhar and colleagues [35] administered 1200 mg curcumin four times daily to 18 patients with rheumatoid arthritis for 2 weeks; they reported a significant improvement in the patients' inflammatory symptomology without apparent toxicity. Two teams have studied the effects of oral curcumin on ophthalmological conditions. In one study, 375 mg of curcumin was administered thrice daily to patients with chronic anterior uveitis for 12 weeks, resulting in a suggestion of improvement in the condition [123]. In a subsequent study, the same dose of curcumin was administered to eight patients with idiopathic inflammatory orbital pseudotumours for 6–22 months [124]. Complete response was observed in half the patients up to 2 years of follow-up. Although histopathological details were not presented in this report, inflammatory orbital pseudotumour is now generally attributed to low-grade non-Hodgkin's lymphoma; hence this result suggests potential anti-cancer activity.

6.3. Anti-cancer effects

Curcumin's induction of apoptosis in cancer cells by a variety of mechanisms described above, as well as its inhibition of DNA topoisomerase II at micromolar concentrations [125], hints at its potential for chemotherapeutic activity in the treatment of cancer. Published anecdotes of curcumin's activity as a topical treatment for cancer can be found, most notably Kuttan's [126] report of turmeric as a topical treatment for oral cancers and leukoplakia. This research group reported a reduction in the size of the lesions in 10% of the 62 patients treated, but there was no control group, no assessment of anti-inflammatory activity and no chemical analysis of the preparation applied.

In one of the pilot studies performed in Leicester, UK, low doses (36–180 mg) of curcumin were administered daily to patients with progressive advanced colorectal cancer, refractory to standard chemotherapies, for up to 4 months [28]. Five out of fifteen patients treated in this study experienced radiologically stable disease for three months or longer, and a significant decrease in venous levels of a tumour marker, carcino-embryonic antigen, was observed in one patient. In a subsequent study performed in patients with progressive advanced colorectal cancer, doses of 0.45–3.6 g of curcumin were administered daily: radiologically stable disease was observed in 2 out of 15 patients for up to 4 months of treatment [29]. The variable natural history of colorectal cancer makes these results from pilot studies difficult to interpret, but there is perhaps a hint of cytostatic activity using macroscopic measures in this patient group.

Cheng and colleagues [26] in Taiwan investigated curcumin's potential anticancer activity in patients with pre-invasive malignant or high-risk pre-malignant conditions of the bladder, skin, cervix, stomach or oral mucosa. They administered doses of 1–8 g of curcumin (500 mg of curcumin per capsule, 99% pure) daily for 3 months; they noted that doses above 8 g per day were not tolerated by patients on account of the bulky volume of the number of capsules that had to be consumed daily. Histological improvement was noted in one of two patients with presumed bladder carcinoma *in situ*, two of seven patients with oral leukoplakia, one of six patients with stomach metaplasia, one of four patients with cervical intra-epithelial neoplasia (CIN) and two of six patients with Bowen's disease of the skin. Conversely, in one of four patients with CIN and one of seven patients with oral leukoplakia, the treatment failed to prevent the development of invasive malignancy during the 3-month study period. The small numbers of patients with each condition studied and the lack of blinding of the interpreting pathologists make definite conclusions impossible, but the results, particularly the photographic representations presented, re-emphasise the biological activity that curcumin might possess in a range of human tissues.

7. Conclusions

Curcumin possesses wide-ranging anti-inflammatory and anti-cancer properties. Many of these activities can be attributed to its potent antioxidant capacity at neutral and acidic pH, its inhibition of cell signalling pathways at multiple levels, its diverse effects on cellular enzymes and its effects on angiogenesis and cell adhesion. In particular, curcumin's ability to affect gene transcription and induce apoptosis in preclinical models advocates its potential utility in cancer chemoprevention and chemotherapy. Although curcumin's low systemic bioavailability following oral dosing seems to limit the tissues that it can reach at efficacious concentrations to exert beneficial effects, the attainment of such levels in the gastrointestinal tract, particularly the colon and rectum, has been demonstrated in animals and humans. In view of the peer-reviewed reports of the pharmacological properties of curcumin, its phase II clinical evaluation in individuals at risk of developing cancer, especially of the gastrointestinal tract, appears opportune.

Conflict of interest statement

None declared.

Acknowledgements

We thank the patients who have participated in the clinical trials reviewed in this article, our many postdoctoral and student collaborators who have conducted laboratory work on curcumin over the past decade, and Jo Arden, Sue Spriggs and Sara Jones for help with the manuscript.

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