

Review

New mechanisms and therapeutic potential of curcumin for colorectal cancer

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Curcumin is a polyphenol derived from *Curcuma longa*. Over the last few years, a number of studies have provided evidence of its main pharmacological properties including chemosensitizing, radiosensitizing, wound healing activities, antimicrobial, antiviral, antifungal, immunomodulatory, antioxidant and anti-inflammatory. More recent data provide interesting insights into the effect of this compound on cancer chemoprevention and chemotherapy. In fact, preclinical studies have shown its ability to inhibit carcinogenesis in various types of cancer including colorectal cancer (CRC). Curcumin has the capacity of interact with multiple molecular targets affecting the multistep process of carcinogenesis. Also, curcumin is able to arrest the cell cycle, to inhibit the inflammatory response and the oxidative stress and to induce apoptosis in cancer cells. Likewise, it has been shown to possess marked antiangiogenic properties. Furthermore, curcumin potentiates the growth inhibitory effect of cyclo-oxygenase (COX)-2 inhibitors and traditional chemotherapy agents implicating another promising therapy regimen in the future treatment of CRC. However, its clinical advance has been hindered by its short biological half-life and low bioavailability after oral administration. This review is intended to provide the reader an update of the bioavailability and pharmacokinetics of curcumin and describes the recently identified molecular pathways responsible of its anticancer potential in CRC.

Keywords: Colorectal cancer / Curcumin / Mechanisms

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1 Introduction

Curcumin is a polyphenol found in the dietary spice, derived from dried rhizomes of the perennial herb *Curcuma longa* Linn, a member of the ginger family. Fractions of turmeric known as curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin) are considered the active compounds and possess a yellowish orange colour. Curcumin, the primary curcuminoid studied in a host of areas, is an orange-yellow, crystalline powder and insoluble in water; however, it readily goes into solution in ethanol and DMSO. It is used as a spice to give the specific flavour and yellow colour to curry [1, 2].

As a traditional medicine, turmeric has also been extensively used for centuries to treat a diversity of disorders including rheumatism, bodyache, skin diseases, intestinal worms, diarrhoea, intermittent fevers, hepatic disorders, biliousness, urinary discharges, dyspepsia, inflammations, constipation, leukoderma, amenorrhoea and colic inflammatory disorders in its original countries such as arthritis, colitis and hepatitis [3].

Over the last few years, a number of studies have provided evidence of several pharmacological properties of curcumin including chemosensitizing, radiosensitizing, wound healing activities, antimicrobial, antiviral, antifungal and anti-inflammatory [4–16]. In fact, curcumin can

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Abbreviations: ACF, aberrant crypt foci; AOM, azoxymethane; AP, activator protein; APC, adenomatous polyposis coli; CEC, colon epithelial cells; COX, cyclo-oxygenase; CRC, colorectal cancer; DMH, 1,2-dimethylhydrazine; EGFR, epidermal growth factor receptor; ERK, extracellular signal-related kinases; GADD153, growth arrest and DNA damage-inducible gene 153; GJIC, gap-junction intercellu-

lar communication; GST, glutathione-S-transferase; HIF-1, hypoxia inducible factor-1; IGF-1R, insulin-like growth factor receptor; iNOS, inducible nitric oxide synthase; JAK, janus family of kinase; JNK, c-Jun NH₂-terminal kinase; LOX, lipoxygenase; M(1)G, adducts of malondialdehyde with DNA; MAPK, mitogen-activated protein kinase; MMP, metalloproteinase; NF-κB, nuclear factor kappa B; NO, nitric oxide; Nrf2, nuclear factor E2-related factor 2; PARP, poly(ADP-Ribose) polymerase; PG, prostaglandin; PPARγ, peroxisome proliferator-activated receptor gamma; ROS, reactive oxygen species; STAT, signal transducer and activator of transcription; TNF-α, tumour necrosis factor alpha; TPA, 12-O-tetradecanoylphorbol-13-acetate; TRAIL, TNF-α-related apoptosis-inducing ligand; VEGF, vascular endothelial growth factor

down-regulate various proinflammatory cytokines expression such as tumour necrosis factor alpha (TNF- α), IL-1, IL-2, IL-6, IL-8, IL-12 and chemokines, most likely through inactivation of the nuclear transcription factor-kappa B (NF- κ B). Likewise, there are recent reports which document that curcumin decreases the inflammation degree associated with experimental colitis [17–20], inducing a substantial reduction of the rise in myeloperoxidase (MPO) activity, an established marker for inflammatory cell (mainly polymorphonuclear leukocytes), and TNF- α . Besides, curcumin is able to reduce nitrites colonic levels and down-regulates cyclo-oxygenase (COX)-2, inducible nitric oxide synthase (iNOS) expressions and p38 mitogen-activated protein kinases (MAPK) activation [21].

Furthermore, curcumin has been reported in the last two decades as a potent antioxidant due to its ability to scavenge the mutagenic/carcinogenic reactive oxygen species (ROS; *e.g.* superoxide anions, hydroxyl radicals, peroxides and nitrite radicals) [12, 22–24]. Additionally, data have provided interesting insight into the immunomodulatory potential of curcumin. It can modulate the activation of T cells, B cells, macrophages, neutrophils, natural killer cells and dendritic cells. Nevertheless, curcumin at low doses can also enhance antibody responses. This suggests that curcumin's reported favourable effects in arthritis, allergy, asthma, atherosclerosis, heart disease, Alzheimer's disease, diabetes and cancer might be due in part to its ability to modulate the immune system [25].

Environmental factors such as medications or diet can modify colon cancer risk. Epidemiological, preclinical and clinical studies point to food components, including meat, fat, cereal, vegetables, *etc.* as the main and modifiable determinant of colorectal cancer (CRC) prevalence and tumour behaviour. Thus, a daily intake of antioxidative vitamins, and certain phytochemicals, which are present in fruits, vegetables, grains and some drinks have been proposed as primary chemopreventive agents. Because the proinflammatory and pro-oxidant states are closely linked to tumour promotion, dietary substances such as curcumin, with potent anti-inflammatory and or/antioxidant activities are anticipated to exert chemopreventive effects.

There are recent reports which document the anticarcinogenic potential of curcumin based on its inhibitory effects on the multistep process of carcinogenesis at various stages: tumour initiation, promotion and progression (for review see Surh and Chun [26] and Johnson and Mukhtar [27]). Preclinical studies of curcumin have shown the ability to inhibit carcinogenesis in various types of cancer including breast, cervical, gastric, hepatic, leukaemia, oral epithelial, ovarian, pancreatic, prostate and CRC [28]. As a result, there is extensive interest in the clinical development of this compound as a cancer chemopreventive and/or chemotherapeutic agent as evidenced by the development of phase I clinical trials and current enrolment in phase II clinical trials [27]. This review is intended to provide the reader an

update of the anticancer potential of curcumin as a chemopreventive agent in CRC and describes the recently identified molecular pathways responsible.

2 Molecular pathways of CRC: Role of inflammation and oxidative stress

Carcinogenesis is a multistep process typically occurring over an extended period as it takes many years to turn into complete malignancy, and comprises three major steps that reflect genetic alterations: initiation (normal cell \rightarrow transformed or initiated cell), promotion (initiated cell \rightarrow preneoplastic cell) and progression (preneoplastic cell \rightarrow neoplastic cell). These concepts although they represent a simplification of the real process, they are very useful to understand the natural history of cancer. Initiation process is moderately short and needs a chronic exposure to endogenous or exogenous genotoxic agents (chemical products, physical radiations or biological agents) that induces sporadic or inherited mutations. The tumour promotion stage is a long-term and irreversible process in which transformed cells can increase the genetic damage produced during the initiation and proliferate under the action of different stimuli (hormones, growth factors, some dietary lipids, *etc.*), influencing the later growth and clonal expansion of abnormal cells [29–31]. Finally, the third phase occurs when the cell undergoes additional genetic alterations that lead to the expression of the malignant phenotype. During this phase, the cells show a marked genomic instability and acquire the ability to infiltrate adjacent tissues and metastasizing power [30].

CRC is one of the most common gastrointestinal tract malignancies. It is the third cause of cancer-related death in the Western world [32] and affects about one million people every year throughout the world with a high mortality rate. CRC develops from a dysplastic precursor lesion, sporadically, in the context of high-risk hereditary conditions, or in the background of chronic inflammation.

Generally CRC arises as a result of sequential episodes of activating mutations in oncogenes, such as *ras*, and inactivating mutations, truncations or deletions in the coding sequence of several tumour suppressor genes, including p53 and adenomatous polyposis coli (*APC*), together with aberrant activity of molecules controlling genomic stability [33]. In particular, there are two main genomic instability that contribute to colon carcinogenesis: chromosomal instability (CIN) and microsatellite instability (MSI). The first results in abnormal segregation of chromosomes and abnormal DNA content (aneuploidy). The second involves loss of function genes that normally repair the mismatches between DNA base pairs that occur during the normal process of DNA replication in dividing cells [34].

Three genetic patterns have been categorized into the following groups of CRC: sporadic, inherited and familial CRC. In hereditary cancer, such as familial adenomatous

polyposis (FAP) and hereditary nonpolyposis colorectal cancer (HNPCC) some of these genetic mutations are inherited, while in sporadic cancer the mutations occur spontaneously. Particularly, the loss of a functional *APC* protein is one of the earliest events occurring in sporadic CRC suggesting that *APC* may act as a gatekeeper of the colonic epithelium [33]. Loss of *APC* function allows β -catenin, a protein which plays a role in both cell adhesion and intracellular signalling, to gain access to the cell nucleus, where it complexes with specific transcription factors to activate genes implicated in proliferation, apoptosis and cell adhesion. *APC* also plays a role in epithelial migration and can localize at kinetochores, thereby participating in chromosome segregation during mitosis, which may contribute to the genetic instability and loss of epithelial polarity during the malignant transformation of colonic epithelia [35].

In addition to hereditary factors, cancer risk appears to be markedly influenced by a number of different factors including chronic inflammation. In effect, patients with inflammatory bowel disease (IBD) are among the highest risk groups for developing CRC. Increased risk of CRC in these patients becomes greater with increasing extent and duration of the disease [34]. At present no genetic basis is able to explain the predisposition to CRC in those patients. Nevertheless, the main genomic instability that contribute to colon carcinogenesis in addition to MSI instability is CIN which results in damage of genetic material and consequently, loss of function of key tumour suppressor genes such as *APC* and *p53* which express proteins that regulate growth and apoptosis. Loss of *APC* function occurs later normally in colitis-associated CRC. In normal cells, *p53* is inactive through its joining to the MDM-2 protein, but several stimuli can activate it, resulting in anticancer responses: activation of genes involved in inhibition of cell cycle, apoptosis, chromosomal stability and inhibition of angiogenesis. Loss of *p53* gene function occurs early and is supposed to be the crucial event that drives the adenoma to carcinoma [34, 36].

Inflammation acts as a key regulator in all stages of carcinogenesis. Accumulating clinical and experimental evidence support a potent antitumorigenic efficacy of inhibiting the inducible isoform of COX-2 using nonsteroidal anti-inflammatory drugs and specific COX-2 inhibitors like celecoxib in the setting of high-risk hereditary conditions. COX-2 is expressed after proinflammatory cytokines stimulation, oncogenes such as *ras* or *scr* or after hypoxia situations. In several types of cancer, particularly, gastric carcinoma and colon adenoma, COX-2 is up-regulated generating prostaglandins (PGs) that can promote cell growth, angiogenesis and suppression of immunity. Overexpression of COX-2 is seen in up to 90% of colon carcinomas and 40% of colon adenomas. It is reasonable to assume that PGs produced by COX-2-expressing interstitial cells accelerate colon carcinogenesis [37, 38]. Among them, a direct procarcinogenic role of PGE₂ has been shown recently in various experimental animal models [35].

In addition to COX-2 inducible gene, iNOS and the interferon (IFN)-inducible genes are also increased in inflamed mucosa and remain elevated in colonic neoplasms [39]. iNOS is a calcium-independent nitric oxide (NO) synthase and responsible for production of large amounts of NO implicated in initiation, promotion and progression of tumours. The iNOS expression is induced by various inflammatory cytokines especially TNF- α , IFN γ and bacterial cell wall products like LPS [40]. Additionally, NO is able to cause DNA-damage and at the same time inhibits DNA repair mechanisms [41]. Indeed, in chronic inflammation NO stimulates COX-2 activity and increases *p53* mutations contributing to clonal cellular expansion and genomic instability.

Tumour cells are genetically unstable and produce structures which allow their cognition and destruction by the immune-surveillance system. Genetic and functional experiments indicate that tumour inflammatory infiltration primarily includes: T cells, specifically CD4+ and CD8+ and B cells, as well as, tumour-associated macrophages (TAMs), neutrophils, eosinophils, dendritic cells, natural killer cells and mast cells. They contribute to the development of carcinogenesis by producing a variety of cytokines such as TNF- α , proangiogenic factors and cytotoxic mediators including ROS and reactive nitrogen species (RNS) among others [42–44]. For instance, leukocytes produce cytokines, angiogenic factors as well as matrix-degrading proteases that allow the tumour cells to proliferate, invade and metastasize.

Tumour-infiltrating lymphocytes secrete matrix metalloproteinase (MMP)-9 generating growth-promoting signals, angiogenesis and invasion [45]. MMPs are members of the zinc-dependent endopeptidases which have been implicated in the degradation of extracellular matrix. MMP-2 and MMP-9 are often overexpressed in various cancers [46]. Their specific inhibitors also play a major regulatory role in matrix reorganization and the initiation of angiogenesis [47]. Thus, the overexpression of MMPs may be one part of the multistep process that leads to neoplastic cell proliferation and metastasis.

Gap-junction intercellular communication (GJIC) is essential for maintaining homeostasis *via* the modulation of cell proliferation and differentiation in multicellular organisms [48]. Normal fibroblasts and epithelial cells have functional GJIC, while the majority of tumour cells have dysfunctional homologous or heterologous GJIC. Thus, the augmentation of GJIC and inhibition of COX-2 and MMPs are considered examples of interesting biomarkers concerning in blocking tumour promotion and tumour progression in carcinogenesis.

It is logical to assume that factors associated with inflammation, such as oxidative stress, contribute to neoplastic transformation. ROS mainly the superoxide anion, which is converted to the secondary oxidant H₂O₂ by superoxide dismutase, and RNS, mostly NO and its metabolite peroxynitrite, can interact with DNA in proliferating epithelium

resulting in permanent genomic alterations including point mutations, deletions or rearrangements. In addition, ROS may contribute to the *p53* mutations and can functionally impair the protein components of the DNA mismatch repair system [34]. Likewise, exacerbating DNA injury induces the macrophage infiltration inhibitory factor (MIF) expression from macrophages and T lymphocytes. MIF is a potent cytokine that overcomes *p53* function by suppressing its transcriptional activity [49].

ROS can interfere with normal cellular signalling cascades by influencing the activation or expression of transcription factors and upstream kinase, especially NF- κ B and activator protein-1 (AP-1). NF- κ B can modulate the genes involved in tumour cell proliferation, invasion and angiogenesis; these include the cell adhesion molecules, COX-2, iNOS, MMP-9, MMP-2, chemokines and inflammatory cytokines among others. Also, the activation of NF- κ B can suppress apoptosis, thus promoting chemoresistance and tumourigenesis [50]. In relation to AP-1, it is known to play a main role in not only the proliferation of initiated cells but also in the metastasis of tumour cells. In fact, activation of AP-1 induces the expression of target genes for instance, COX-2, urokinase-type plasminogen activator, *Fos*, MMP-9, *cyclin D1* and vascular endothelial growth factor (VEGF) that are involved in neoplastic transformation, tumour progression, metastasis and angiogenesis [51]. The components of NF- κ B and AP-1 are activated by the three major groups of MAPKs in mammalian cells, the extracellular signal-related kinases (ERK), the c-Jun NH₂-terminal kinases (JNK) and p38 MAPK, that are activated by phosphorylation. NF- κ B, AP-1 and associated MAPK signal transduction pathways are believed to be crucial in cell transformation and tumour promotion, and hence have been proposed as targets for chemopreventive agents [52].

Recent investigations suggest the participation of the peroxisome proliferator-activated receptor gamma (PPAR γ) in the pathophysiology of inflammatory and immune responses possibly through inhibition of the MAPKs pathway or the activation of the NF- κ B. In colon tumour tissue, PPAR γ expression has been detected in several studies using clinical samples. Activation of PPAR γ leads to cell differentiation and apoptosis. Also, PPAR γ ligands have been shown to be potent inhibitors of angiogenesis, a process necessary for tumour growth and metastasis, and protect against cellular transformation. Further work is needed to establish in detail the antiproliferative and prodifferentiation mechanisms of PPAR γ activators and their expedient evaluation in the clinical management of IBD-associated CRC [53, 54].

3 Pharmacokinetics, bioavailability and toxicity of curcumin

Curcumin, a diferuloylmethane, is a lipophilic molecule with phenolic groups and conjugated double bonds which

is unstable at light and basic pH, degrading within 30 min [55, 56]. Since antioxidants such as glutathione, *N*-acetylcysteine and ascorbic acid could completely inhibit curcumin degradation at pH 7.4, oxidative mechanism is speculated to be responsible for polyphenol decomposition [57]. However, under acidic conditions its degradation is much slower, with less than 20% of total curcumin decomposed at 1 h [58]. For this reason, curcumin seems to be stable in the gastrointestinal tract where the pH is between 1 and 6.

A typical crude extract of *C. longa* rhizomes contain 70–76% curcumin, 16% demethoxycurcumin, 8% bisdemethoxycurcumin [59] and the recently identified cyclocurcumin [60]. Curcuminoids impart the characteristic yellow colour to turmeric. Nevertheless, the content of curcumin in turmeric is usually 4–5% [61–63].

The acceptable daily intake of this diferuloylmethane as an additive has been defined by the World Health Organization (WHO) as 0–1 mg/kg body weight [64]. A common therapeutic dose is 400–600 mg curcumin three times daily, corresponding up to 60 g fresh turmeric root or about 15 g turmeric powder [62].

Although the *in vitro* records showed curcumin to be a potential chemotherapeutic agent against a broad range of cancer cells, its clinical advance has been hindered by its short biological half-life and low bioavailability after oral administration [65–67].

Table 1 illustrates the pharmacokinetic profile of curcumin from preclinical and clinical data. The absorption, metabolism and tissue distribution of curcumin after an oral dosage have been analysed in different animal models and in several clinical trials. Results from pharmacokinetic assays in animals and humans showed low serum levels of the polyphenol following a single dose administration [65, 66, 68–72]. However, data from the curcumin absorption studies are in principle contradictory in rodent models, because although Holder *et al.* [68] demonstrated that approximately 60–66% of the dose was absorbed, another investigations found that the diferuloylmethane was poorly absorbed [69, 73, 74], revealing that about 75% of ingested curcumin was excreted unaltered in the faeces and negligible amounts appeared in the urine [69]. These data are in accordance with the results obtained in a more recent study performed in rats by Sharma *et al.* [75], where curcumin and its metabolites were analysed by HPLC in plasma, and its pharmacokinetics were compared following a diet containing 2% curcumin *versus* intragastric administration of curcumin suspended in an amphiphilic solvent. The investigators found that the plasma detectable polyphenol level was in the nanomolar range, between 0 and 12 nM. In addition, it has been shown that the quantifiable serum levels are not achieved until doses of up to 3.6 g [27]. Pan *et al.* [76] investigated the pharmacokinetic properties of curcumin in mice and found that about 2.25 μ g/mL of curcumin appeared in the plasma in the first 15 min, after intraperitoneally administration of the diferuloylmethane (0.1 g/kg).

In humans, doses of 2–8 g curcumin alone result in serum levels that were undetectable [72]. Moreover, Lao *et al.* [77] did not either detected plasma concentrations of curcumin

in subjects that intake dose level of 10 or 12 g. A cancer study performed by Cheng *et al.* [65] determined low concentration of curcumin by HPLC in 25 subjects with high

Table 1. Pharmacokinetic profile of curcumin from preclinical and clinical data

Ref.	Animal	Dose	Route	Pharmacokinetic data
<i>Preclinical studies</i>				
[66]	Rat	2 g/kg	–	Low serum levels Piperine increased bioavailability by 154%
[67]	Rat	500 mg/kg 40 mg/kg	p.o. i.v.	Detectable in plasma Biotransformed to curcumin glucuronide and sulphate Disappeared from plasma in 1 h
[68]	Rat	–	Oral i.p. i.v.	Mostly fecal excretion Excreted in the bile Major biliary metabolites: THC and HHC glucuronides
[69]	Rat	1 g/kg	Oral i.v.	75% excreted in the feces Negligible in urine Poorly absorbed in the gut No toxicity at 5 g/kg Transported into bile Major part metabolized
[73]	Rat	400 mg	Oral	60% absorbed None in urine; conjugated glucuronides and sulphates None in heart blood Less than 5 microgram/ml in portal blood Negligible in liver and kidney (<20 µg/tissue) for 24 h At 24 h, 38% in lower part of the gut
[75]	Rat	2% of diet 500 mg/kg	Oral i.g.	Plasma levels 12 nM Tissue concentration: 0.1–0.9 nmol/g in liver and 0.2–1.8 mmol/g in colon Increased hepatic GST (16%) Decreased colon MDH-DNA adduct (36%) More in plasma; less in colon
[76]	Mouse	0.1 g/kg	i.p.	2.25 µg/mL in plasma in first 15 min Levels of 177.04, 26.06, 26.90 and 7.51 µg/g in intestine spleen, liver and kidney, respectively, 1 h after administration; 0.41 µg/g in brain Biotransformed from DHC to THC, and then converted to monoglucuronide conjugates
<i>Clinical studies</i>				
[65]	Human	1–12 g/day	Oral	Well tolerated up to 8 g/day up to 3 months Serum levels peaked at 1–2 h and declined at 12 h Serum levels: 0.51 ± 0.11, 0.63 ± 0.06 and 1.77 ± 1.87 µM after taking 4.000, 6.000 and 8.000 mg of curcumin
[66]	Human	2 g/day	Oral	Serum level not detectable Piperine increased bioavailability by 2000%
[72]	Human	36–180 mg/day	Oral	Most in feces; none in blood or urine 59% decrease in lymphocytic GST after 14 days
[77]	Human	0.5–12 g/day	Oral	Low levels were detected in two subjects administered 10 or 12 g After 72 h, minimal toxicity not dose-related Excellent tolerance
[80]	Human	0.45–3.6 g/day	Oral	Concentrations in normal and malignant colorectal tissue: 12.7 ± 5.7 and 7.7 ± 1.8 nmol/g, respectively, after 3.6 g ingested Sulphate and glucuronide conjugates Trace levels in the peripheral circulation
[81]	Human	0.5–3.6 g/day	Oral	Administration for up to 4 months associated with mild diarrhoea as its only discernible toxicity Detectable levels of parent compound and conjugates in plasma and urine after ingestion of highest dose Inhibition of PGE ₂ production in blood leukocytes measured <i>ex vivo</i>

i.p., intraperitoneally; i.v., intravenous; p.o., per oral; i.g., intragastric; THC, tetrahydrocurcumin; HHC, hexahydrocurcumin; DHC, dihydrocurcumin.

risk of cancer lesions. The peak serum concentration after 4, 6 and 8 g were 0.51 ± 0.11 , 0.64 ± 0.06 and 1.77 ± 1.87 μM , respectively, but doses below 4 g were barely detectable.

Absorbed curcumin may be associated with serum albumin through hydrophobic interactions [78] for its tissue distribution and may, thereby, be transported to appropriate target cells, where it elicits its pharmacological effects. The polyphenol eagerly penetrates into the cytoplasm and then is able to mount up in membranous structures, such as plasma membrane, ER and nuclear envelope [79]. It has been reported that 1 h after intraperitoneally administration of the diferuloylmethane (0.1 g/kg) to mice, the curcumin levels in the intestines, spleen, liver and kidneys reached the values of 177.04, 26.06, 26.90 and 7.51 $\mu\text{g/g}$, respectively [76]. Only traces (0.41 $\mu\text{g/g}$) were observed in the brain at 1 h. Furthermore, a recent evaluation of the concentrations of the polyphenol in normal and malignant colorectal tissue of patients receiving 3.6 g of the diferuloylmethane showed levels of that 12.7 ± 5.7 and 7.7 ± 1.8 nmol/g, respectively while trace levels of curcumin were found in the peripheral circulation [80]. The results suggest that a daily dose of 3.6 g curcumin achieves pharmacologically efficacious levels in the colorectum with negligible distribution of the polyphenol outside the gut. On the other hand, Sharma *et al.* [75] found that tissue concentrations of the polyphenol in liver and colon mucosa of patients were 0.1–0.9 nmol/g and 0.2–1.8 $\mu\text{mol/g}$, respectively.

In spite of the therapeutic effects exerted by the diferuloylmethane appear to be limited to the lower gastrointestinal tract [72, 81] as consequence of its poor bioavailability, and subsequently prominent exposition to unmetabolized polyphenol, luminal curcumin may have topical activity on colonic epithelial cells independent of systemic absorption [10]. This fact, together with the evidence of that curcumin reaches levels up to 300 nmol/g tissue in the intestine after an intraperitoneal dose of 100 mg/kg of the drug, suggests a major exposition to curcumin than any other tissues (*i.e.* compared with that of 1 nmol/g in brain or 72 nmol/g in liver). All these tissues require systemic bioavailability for cancer prevention [67]. In comparison with dietary administration, suspended curcumin given intragastric resulted in more curcumin in the plasma but much less in the colon mucosa. The results show that curcumin mixed with the diet achieves drug levels in the colon and liver sufficient to explain the pharmacological activities observed and suggests that this mode of administration may be preferable for the chemoprevention of colon cancer.

After its accumulation, it is suggested that the diferuloylmethane undergoes extensive entero-hepatic recirculation [68, 70, 73] as well as a rapid intestinal metabolism and excretion, primarily in bile [68, 70] and to a lesser extent in urine [69, 73, 74]. The metabolism of curcumin in rodents involves successive reduction and conjugation [68, 76]. Thus, after an intraperitoneal administration of curcumin in

mice, the chemical structures of the obtained metabolites indicate a first biotransformation to dihydrocurcumin and tetrahydrocurcumin (the major metabolite of curcumin), followed by the conversion of both compounds to monoglucuronide conjugates [76]. In rats, curcumin oral administration demonstrated small quantity of the polyphenol in plasma with higher levels of curcumin glucuronide and curcumin sulphate in plasma, and small amounts of hexahydrocurcumin, hexahydrocurcuminol and hexahydrocurcumin glucuronide [67]. In humans, curcumin metabolism is poorly understood although there is evidence in fact that the diferuloylmethane is metabolized extensively in the human gut, being its metabolic conjugation and reduction greater in human intestine than rat intestine. This conclusion was obtained by Ireson *et al.* [71] who performed a study of the polyphenol metabolism in subcellular fractions of human and rat intestinal tissue, in the corresponding hepatic fractions as well as *in situ* intact rat intestinal sacs, detecting differences between both species. Curcumin conjugation was found to be much greater in intestinal fractions from humans than in those from rats, whereas curcumin conjugation was less extensive in hepatic fractions from humans when was compared with those from rats. Cytosol from human intestinal and liver tissue, exhibited 18 and 5 times, respectively, the curcumin-reducing ability as that observed with the corresponding rat tissue.

To the best of our knowledge, there are no comparative studies in terms of pharmacological potency among curcumin and its metabolites. The information at this respect is scarce. However, there is evidence that pharmacologic effects of curcumin exerted in the colorectum are likely to be caused by the parent compound and not by its metabolites [80]. On the other hand, certain curcumin metabolites, such as tetrahydrocurcumin, possess anti-inflammatory and antioxidant activities similar to those of their metabolic progenitor. However, recent data indicate that the anti-inflammatory property is lost when curcumin is reduced to tetrahydrocurcumin, although its antioxidant property is still intact [59]. On the other hand, a number of research groups have taken the natural product as a starting point to prepare and biologically evaluate a wide variety of curcumin analogues. One widely used structural modification truncates the central conjugated beta-diketone in curcumin to the monocarbonyl dienone. A diverse array of the latter compounds exhibit cytotoxicities against an equally diverse set of cancer-related cell lines. Importantly, these compounds still retain toxicity profiles in rodents comparable to the parent natural product, whereas some analogues (*e.g.* EF-24, 41) exhibit good oral bioavailability and good pharmacokinetics in mice [82]. Thiol conjugates of EF-24 analogues have been prepared that address stability and solubility issues while demonstrating cellular activities similar to the unmodified dienones. In parallel experiments, the factor VIIa-tissue factor complex (fVIIa-TF) has been exploited to develop a targeting strategy for the analogues. In particu-

lar, the EF24-FFRck-fVIIa protein conjugate is not only somewhat more effective relative to the drug alone against breast cancer and melanocyte cells. Both simple curcumin analogues and the protein conjugate evidence antiangiogenic activity in cell culture. The implication is that the fVIIa-TF targeting process, like the dienone drugs, permits a double-pronged attack with the potential to destroy a tumour directly by apoptosis [82].

Inasmuch as the polyphenol is relatively insoluble in water, its bioavailability from oral administration can be improved by dissolution in ambivalent solvents such as glycerol, acetone, DMSO and ethanol [75]. Furthermore, it is interesting to highlight that the coadministration of piperine, a component of pepper, results in a substantial increase of curcumin bioavailability in both rats and humans [66]. In fact, an elevation of 2000% was seen in availability of the polyphenol when it was administered in mixture with piperine compared to curcumin alone [66]. Indeed, the favourable health effects of curcumin alone may be further magnified in the context of a combination of dietary additives that are profusely consumed as part of Asian diets [83]. Similarly, the association with epigallocatechin gallate, a constituent of green tea, induced a synergistic effect on the growth of premalignant and malignant oral epithelial cells [84]. Then, use of carefully chosen combination of discrete dietary agents, formulated as “nutraceuticals”, might be beneficial to exploit the synergy among them.

Recently, some investigators have obtained different formulations employing drug delivery systems that integrate curcumin to enhance its bioavailability. These systems include liposomes used *in vitro* and *in vivo* studies (*i. e.* murine model) [85, 86], natural biodegradable polymers assayed in rats [87] or even a β -cyclodextrin formulation of the diferuloylmethane [88]. More recently, Marczylo *et al.* [89] have performed a comparative study of systemic availability of curcumin *versus* the polyphenol formulated with phosphatidylcholine in rats, concluding that the combination furnished higher systemic levels of parent agent than unformulated curcumin. These results are in agreement with those obtained by Liu *et al.* [90], who also found a significant improvement in the diferuloylmethane bioavailability in rats after administration of a complex of curcumin and soya phospholipid *versus* curcumin alone. Other researchers have synthesized polymeric nanoparticle encapsulated formulation of curcumin (nanocurcumin) utilizing the micellar aggregates of cross-linked and random copolymers of *N*-isopropylacrylamide, with *N*-vinyl-2-pyrrolidone and poly(ethyleneglycol)monoacrylate [91]. Nanoparticles circumvent the pitfalls of the polyphenol poor solubility.

Because little work has been done to develop curcumin into an intravenously injectable formulation and there is no report on the pharmacokinetic analysis of injected polyphenol, Ma *et al.* [92] have recently investigated the potential effect for using methoxy poly(ethylene oxide)-*b*-poly(ϵ -caprolactone) micelles as vehicles for intravenous injection

of curcumin. The results showed that encapsulation of the diferuloylmethane in the polymeric micellar formulation caused a profound change in the pharmacokinetics of the drug. There was a substantial increase in the distribution volume (70-fold), 3-fold decrease in clearance and 162-fold increase in the curcumin $t_{1/2}$, although further studies are required to evaluate the tissue distribution of the micellar formulation of curcumin.

Hence, although serum concentration of curcumin may be dependent on the dose administered, in human, a successful administration in terms of bioavailability and biologic activity might differ based on the formulation used. Consequently, the problem of bioavailability is not only of concern in trying to predict *in vivo* efficacy from *in vitro* studies.

Regarding toxicity, as curcumin is a constituent of the diet it is nontoxic in nature [93]. Although the experience of using curcumin in the diet for a long time inspires confidence in its safety, one cannot assume *a priori* that diet-derived agents are innocuous when administered as pharmaceutical formulations at doses that generally exceed those consumed in the dietary matrix [63]. Moreover, because several studies have demonstrated minimal toxicity with moderate doses of polyphenol given in various formulations [94–96] or even in very high doses [97, 98], curcumin has been found to be safe pharmacologically. In addition, different studies have indicated no dose-limiting toxicity when the diferuloylmethane is administered at doses up to 5 g/day in rats [93] or up to 8 g/day in humans [65]. Anecdotal reports suggest that dietary consumption of turmeric up to 1.5 g per person per day, equating to a probable maximum of 150 mg of curcumin daily, are not associated with adverse effects in humans [99]. Thus, humans appear to be able to tolerate high doses of curcumin without significant side-effects. This discrepancy could be explained because of the differences in metabolism of the polyphenol in humans as compared to susceptible species such as rats. However, in a study conducted by Lao *et al.* [77] to determine the maximum tolerated dose and safety of a single dose of standardized powder extract, doses up to 12 g were unacceptable to patients due to the bulky volume of the employed curcumin formulation. As well, at high-ingested dose and consequent substantial concentrations of the diferuloylmethane in organs accessible to it, its beneficial antioxidant properties may be masked by the unwanted corollary of pro-oxidation, although the balance of these properties depends on the presence of metal ions and cell type [100]. At this respect, it has been suggested that the ability of curcumin to function as an antioxidant depends on its concentration and the environment. Thus, at 10 μ M the polyphenol acts as an antioxidant while it functions as a pro-oxidant generating superoxide radicals and inducing apoptosis at 50 μ M [101].

Only a few clinical studies of oral curcumin and curcuminoids have reported perceptible adverse effects. As Tho-

masset *et al.* [102] recently indicated, participants in early clinical trials of polyphenolic phytochemicals have received a large variety of doses. Referring to curcumin, its daily doses have ranged from 180 mg to 8 g. The low dose was used in trials in which molecular biomarkers were evaluated in patients with CRC [72, 103], while the highest dose was employed in patients with premalignant disease in which histological changes were measured [65]. For example, in patients with advanced CRC, curcumin was well tolerated at all dose levels up to 3.6 g daily for up to 4 months. Two types of gastrointestinal adverse event were reported by patients in this study, which were probably related to curcumin consumption diarrhoea and nausea [81].

4 Preclinical studies of curcumin for CRC prevention and therapy

Table 2 includes some of the mechanistic properties of curcumin *in vitro*. Table 3 illustrates the effects and/or mechanisms of action of the polyphenol in experimental models *in vivo*.

Numerous *in vitro* assays using colon cancer cell lines have been performed showing that curcumin possesses anti-cancer activity, but the underlying mechanisms remain largely to be defined. **Moreover, animal model systems have revealed that the diferuloylmethane prevents tumours induced by a diversity of chemical carcinogens in tissues**

Table 2. Cell lines, effects and/or mechanisms of action and doses of curcumin in preclinical *in vitro* models of colon cancer

Ref.	Colon cancer cells	Doses (μ M)	Effects and/or mechanisms
[132]	Lovo cells	20–40	Arrest the cell cycle in S, G2/M phase and apoptotic cell death induction
[133]	HCT-116	20	Cyclins E and D reduction, increase of Cdc2 activity, cleavage of PARP and caspase-3, induction of caspase-8 activity and Bcl-XL expression reduced
[134]	HCT-116	20	Induction of caspase-3-mediated beta-catenin cleavage and c-Myc down-regulation resulting in G2/M phase arrest Degradation of beta-catenin, E-cadherin and APC resulting in apoptosis
[135]	HT-29 Caco-2	25–100	Modulation of cell cycle genes of which several have a role in transition through the G2/M phase Down-regulation of some cytochrome P450 genes expression
[140, 142]	HCT-116	10	GADD153 up-regulation, modulate by regulatory thiol redox-sensitive signalling cascade
[150]	HCT116 Bax+/- HCT116 Bax-/-	25	Bax is required for curcumin-induced apoptosis
[151]	Colo 205	20	Promotion of Bax-, cytochrome C-, p53- and p21-expression -Inhibition Bcl-2expression Production of ROS and Ca ²⁺ , decreases of mitochondrial membrane potential and induction of caspase-3 activity
[160, 162]	HCT-116	35	Activation and induction of JNK-dependent apoptosis Induction of AP-1 and NF- κ B
[161]	HCT-116	50	JNK activation <i>via</i> ROS-associated mechanism
[163]	HT-29 C4	1 and 25 35 and 50	Induction of AP-1 luciferase reporter gene and endogenous cyclic D1 protein Decrease of AP-1 luciferase reporter gene and endogenous cyclic D1 protein
[164]	HT-29 N9 HT29 SW480	10 0-50	Inhibition activity against NF- κ B and I κ B α Inhibition of PGE ₂ and down-regulation of COX-2 Inhibition of NF- κ B/p65
[174]	Moser cells (human colon cancer-derived cell line)	10–20	PPAR γ activation inhibiting tyrosine phosphorylation of EGFR and suppressing gene expression of EGFR and cyclic D1
[175]	HCT-116	5	Attenuation of tyrosine phosphorylation of EGFR and IGF-1R
[183]	HT-29		Inhibition of cell growth
[184]	HT-29	25	Decrease of COX-2 expression and inhibition of LOX catalytic activities by blocking the phosphorylation of (cPL)A ₂
[185]	Epithelial cells	20	Inhibition of COX-2 expression by inhibiting NF- κ B
[186]	HCT-116		Inhibition of cell growth
[187]	HT-29	0–75	Inhibition of COX-2 expression
[198]	HCT-116	5–25	Inhibition of IL-8 gene and protein by inhibiting neurotensin-induced AP-1 and NF- κ B induction
[201]	HCT116 and HT29	30	Up-regulation of DR5 proteins

Table 3. Dose, route and effects and/or mechanisms of action of curcumin in preclinical animal models of colon cancer

Ref.	Model	Daily dose	Route	Effects and/or mechanisms
[109]	B(a)P-induced forestomach tumourigenesis in A/J mice	0.5–2.0% of diet	Oral	Inhibition of the number of tumours <i>per</i> mouse Inhibition of the percentage of mice with tumours Reduction of tumour size
[109]	ENNG-induced duodenal tumourigenesis in C57BL/6 mice	0.5–2.0% of diet	Oral	Inhibition of the number of tumours <i>per</i> mouse Inhibition of the percentage of mice with tumours Reduction of tumour size
[109]	AOM-induced colon tumourigenesis in CF-1 mice	0.5–4.0% of diet 2% of diet	Oral	Inhibition of the number of tumours <i>per</i> mouse Inhibition of the percentage of mice with tumours Reduction of tumour size
[110]	B(a)P-induced forestomach tumourigenesis in A/J mice	2% of diet	Oral	Inhibition of BaP-induced forestomach cancer in mice by affecting both activation as well as inactivation pathways of BaP metabolism in the liver
[111]	Rat model of AOM-induced colon cancer	2000 ppm of diet	Oral	Inhibition of colonic ACF formation by 45% Inhibition of crypt multiplicity containing four or more crypts <i>per</i> focus to 46% Inhibition of AOM-induced colonic mucosal iNOS by ~40% Only a moderate effect on NOS, COX-1 and COX-2 activities
[112]	AOM-induced rat colon cancer model	8000 ppm of diet	Oral	Suppression of the total number of ACF <i>per</i> colon by ~52% Reduction of the ACF incidence by ~48% Inhibition of crypt multiplicity
[113]	AOM-induced rat colon cancer model	2000 ppm of diet	Oral	Induction of apoptosis <i>via</i> the mitochondrial pathway Inhibition of incidence of colon adenocarcinomas and the multiplicity of invasive, noninvasive and total (invasive plus noninvasive) adenocarcinomas Suppression of the colon tumour volume by >57% Disminution of colonic mucosal activities and tumour PLA2 (50%) and PLC gamma 1 (40%) and levels of PGE2 (>38%) Reduction of products from arachidonic acid, <i>via</i> COX and LOX pathways, in colonic mucosa and tumours
[114]	AOM-induced rat colon cancer model	0.2 or 0.6% of diet	Oral	Inhibition of colon tumourigenesis Suppression of the incidence and multiplicity of noninvasive adenocarcinomas Strongly inhibition of the multiplicity of invasive colon adenocarcinomas, in a dose-dependent manner
[116]	AOM-induced rat colon cancer model	0.6% of diet	Oral	Resistance of middle-aged rats to inhibition of AOM-induced colonic ACF
[117]	AOM-induced rat colon cancer model	0.6% of diet	Oral	Augmentation of COX-2 expression to compensate AOM-induced reduction of COX-1 expression
[118]	DMH-induced mouse colon cancer model	0.5% of diet THC (0.5 and 0.2% of diet)	Oral	Diminution of tumour incidence and the occurrence of histological lesions Reduction of the tumours size
[119]	DMH-induced rat colon cancer model	80 mg/kg BDMCA (a curcumin analog, 80 mg/kg)	i.g. i.g.	Diminution of the colon tumour incidence Oxidative stress modulation through its influence on lipid peroxidation and antioxidant status
[120]	DMH-induced rat colon cancer model	–	–	Cotreatment with curcumin and catechin caused greater inhibition of DMH-induced ACF and colon carcinogenesis than the single use of curcumin or catechin
[121]	C57BL/6J-Min/+ (Min/+) mice colon cancer model	0.1% of diet	Oral	Tumour prevention by curcumin was associated with increased enterocyte apoptosis and proliferation Decrease of the oncoprotein b-catenin expression
[124]	CHA-induced rat colon cancer model	2 and 4% of diet	Oral	Dose-dependent increases in CEC proliferation rate and pool size in normal rats
[75]	Rats	2% of diet 500 mg/kg	Oral i.g.	Increase of hepatic GST activity Decrease of colon M(1)G levels
[129]	Mice	2% of diet	Oral	Induction of Phase II enzymes activity, particularly GST and quinone reductase, in liver and kidney
[127]	Rats	1–500 mg/kg	i.g.	Activation of GST at low doses and inhibition of GST activity at high concentrations
[168]	Mice	1–25 μ M	Topical	Inhibition of TPA-mediated activation of Erk and p38 MAPK and subsequent activation of NF- κ B
[194]	DMH-induced rat colon cancer model	0.6% of diet	Oral	The combination curcumin/celecoxib augments the growth inhibitory effect of the COX-2 inhibitor

Table 3. Continued

Ref.	Model	Daily dose	Route	Effects and/or mechanisms
[197]	Apc ^(Min/+) mice model	0.1 or 0.2% of diet	Oral	Short-term feeding does not affect total adenoma number or COX-2 expression Long-term dietary curcumin on COX-2 protein levels appear to reflect retardation of adenoma development
[207] [85, 86]	Mice Xenograft mice model of pancreatic and colon cancer	– 40 mg/kg body weight, 3 times <i>per week</i>	Topical i.v.	Direct antiangiogenic activity Antitumour and antiangiogenesis effects, including attenuation of CD31 (an endothelial marker), VEGF and IL-8 expression

B(a)P, benzo(a)pyrene; ENNG, *N*-ethyl-*N*-nitro-*N*-nitrosoguanidine; ACF, aberrant crypt foci; PL, phospholipase; PG, prostaglandin; COX, cyclooxygenase; LOX, lipoxygenase; DMH, 1,2-dimethylhydrazine; THC, tetrahydrocurcumin; BDMCA, bis-1,7-(2-hydroxyphenyl)-hepta-1,6-diene-3,5-dione; i.g., intragastric; CHA, cholic acid; GST, glutathione *S*-transferase; M(1)G, adducts of malondialdehyde with DNA; TPA, 12-*O*-tetradecanoylphorbol-13-acetate, i.v., intravenous; VEGF, vascular endothelial growth factor.

such as skin [104, 105], breast [106, 107] and liver [108]. In the gastrointestinal, in particular for stomach and duodenum, anticancer properties of curcumin have been also documented [109, 110].

The development of strategies for the chemoprevention of CRC have been facilitated by the use of significant animal models mimicking the neoplastic processes that take place in humans, including similarities in histopathology and molecular and genetic lesions during both the early and promotion/progression stages of carcinogenesis, but have disadvantages of heterogeneity with regard to frequency, latency and growth of tumours. The *in vivo* efficacy of curcumin has been tested in several animal models of CRC, for instance, in azoxymethane (AOM) induced, curcumin repressed both AOM-induced aberrant crypt foci (ACF) [111, 112], and colonic tumour development [113, 114]. AOM-induced tumours share many histopathological similarities with human tumours and are characterized by the presence of ACF, a well-established biomarker of colon cancer development [115], which is thought to be the earliest identifiable preneoplastic lesions in the colon carcinogenic model. A recent study of AOM-induced colon cancer has been conducted to determine whether aging affects the inhibition of colon carcinogenesis by curcumin. The results showed that the addition of the polyphenol to the diet reduced the number of ACF 49% in young rats and by 55% in old rats. However, interestingly, no reduction of ACF was found in mature rats fed curcumin. Inhibition of large ACF was also affected by age, with the greatest reduction of large ACF occurring in old rats. These results indicate that age may play a significant role in the efficacy of chemoprevention of colon cancer by the polyphenol since middle-age animals are resistant to the chemopreventive activity of curcumin against AOM-induced colon cancer [116, 117].

In 1,2-dimethylhydrazine (DMH)-induced colon carcinogenesis curcumin markedly reduced tumour incidence and the occurrence of histological lesions, as well as the size of tumours [118]. In addition, using this experimental

model of CRC, a bisdemethoxy curcumin analogue also exerted significant chemopreventive effects by decreasing the colon tumour incidence as well as by modulating oxidative stress through its influence on lipid peroxidation and antioxidant status in male Wistar rats [119]. Similarly, in attempt to examine the effect of combined use of curcumin and catechin on the number of ACF, the DMH-induced rat's colon carcinogenesis model was used. The results showed that cotreatment with curcumin and catechins caused greater inhibition of DMH-induced ACF and colon carcinogenesis than the single use of curcumin or catechin [120], suggesting their potential value in the prevention of human colon cancers.

Anticancer properties of curcumin were also documented in C57BL/6J-*Min*⁺ (*Min*⁺) mice. These animals bear a germline mutation in the *Apc* gene and spontaneously develop numerous intestinal adenomas by 15 wks of age. Examination of intestinal tissue from the treated animals showed that tumour prevention by curcumin was associated with increased enterocyte apoptosis and proliferation [121].

Additionally, inclusion of curcumin to the conventional chemotherapeutic agent(s)/regimen could be an effective therapeutic strategy for CRC. Recent studies have shown a synergistic effect of curcumin with different traditional anticarcinogenic agents as such 5-fluorouracil, 5-FU plus oxaliplatin (FOLCOX) and celecoxib [122, 123] in CRC cell growth inhibition in a rat model. The superior effects of the combination therapy of curcumin and FOLCOX or curcumin and celecoxib were possibly due to attenuation of epidermal growth factor receptors (EGFRs) and insulin-like growth factor receptor (IGF-1R) signalling pathways and through COX-2 and non-COX-2 pathways respectively.

Although curcumin has preventive actions in animal models of CRC cancer, as mentioned above, recently it has been reported its chemopreventive effects on the proliferation rate of colon epithelial cells (CEC), using a recently developed stable isotope-mass spectrometric method for measuring DNA synthesis rate. Surprisingly, curcumin administration did not reduce but instead resulted in dose-

dependent increases in CEC proliferation rate and pool size in normal rats. The authors conclude that reduction of CEC proliferation therefore cannot explain the proposed chemopreventive actions of curcumin in colon cancer [124].

5 Influence of curcumin on the carcinogenesis stages: Cell cycle and apoptosis

5.1 Effects on signal transduction pathways

The antitumorigenic property of curcumin has been attributed partly to its ability to inhibit the processes of initiation and promotion in carcinogenesis. It has been suggested that the polyphenol may inhibit initiation by blocking the cytochrome P-450 enzyme activity and increasing the glutathione-*S*-transferase (GST) levels [110, 125]. Since curcumin may act as a bifunctional inducer of Phase I and II carcinogen-metabolizing enzymes, it is possible that the diferuloylmethane may inhibit mutagen bioactivation, carcinogen-DNA adduct formation and, subsequently, tumour initiation *in vitro* [125, 126] as well as *in vivo* [110, 127]. During this process, nuclear factor E2-related factor 2 (Nrf2) is believed to play a central role because phase II detoxification and antioxidant genes appear regulated by Nrf2/antioxidant response element (ARE) pathway, which is a major target of dietary compounds [128]. In an assay performed by Sharma *et al.* [75] in rats, the investigators measured total GST activity and adducts of malondialdehyde with DNA (M(1)G), which reflect endogenous lipid peroxidation, in colon mucosa, liver and blood leukocytes. They demonstrated that dietary curcumin induced an increase of hepatic GST and a decrease of colon M(1)G levels when was compared with controls, although did not alter any of the markers in the blood. However, although curcumin induces the activity of phase II enzymes in male mice, particularly GST and quinone reductase in liver and kidney [129], this effect seems to be concentration-dependent: the polyphenol activates GST at low doses while at high concentrations inhibits GST activity in rat [127]. Moreover this activation of GST could be induced through Nrf2/ARE pathway [127, 130, 131].

On the other hand, curcumin has been found to arrest the cell cycle and to induce apoptotic cell death in colon cancer, attenuating tumour progression. Chen *et al.* [132] reported that curcumin inhibited Lovo cells growth by arresting the cell cycle in S, G₂/M phase and induced apoptotic cell death. These results were corroborated in HCT-116 human colon cancer cells by Moragoda *et al.* [133], where curcumin was able to decline cyclins D and B expression, which are involved in regulating the G₂/M phase of the cell cycle, and increased activity of Cdc2, a cyclic-dependent kinase involved in regulating the late phase of the cell cycle. Similarly, Jaiswal *et al.* [134] demonstrated that curcumin treatment caused p53- and p21-independent G₂/M phase

arrest and apoptosis in human colon cancer HCT-116(p53(+/+)), HCT-116(p53(-/-)) and HCT-116(p21(-/-)) cell lines. Also, a G₂/M cell cycle arrest by curcumin was also confirmed in HT-29 human colon cancer cells [135]. Besides, it has been reported that curcumin modulates a number of cell cycle genes which exert a role in transition through the G₂/M phase after 3 or 6 h of curcumin exposition. Moreover, some cytochrome P450 genes expression was down-regulated by curcumin in HT29 and Caco-2 human colon cancer cells [135].

The janus family of kinase (JAK) and signal transducer and activator of transcription (STAT) also comprise an important signalling pathway in induction of growth-arrest and apoptosis.

Of the seven STAT proteins identified so far, constitutively activated STAT3 and STAT5 have been implicated in multiple myeloma, lymphomas, leukaemias and several solid tumours, making these proteins logical targets for cancer therapy [136]. Rajasingh *et al.* [137] have demonstrated that *in vitro*, treatment with curcumin induced a dose-dependent decrease in JAK and STAT phosphorylation resulting in the induction of growth-arrest and apoptosis in T cell leukaemia. This inhibition of constitutively active JAKs and STATs by curcumin is consistent with an earlier report showing the inhibition of IL-12-induced JAK–STAT pathway by curcumin in normal T cells [136, 138, 139]. However until the moment there are not studies that show this effect in CRC models.

Additionally, curcumin causes up-regulation of the growth arrest and DNA damage-inducible gene 153 (GADD153) in HCT-116 cells [140]. The complete function of this gene is not fully known, but one particular role is triggering the critical events leading to the initiation of apoptosis [141]. More recently, other results support the view that curcumin-induced GADD153 gene up-regulation is modulated by ROS generation through the ability of curcumin to neutralize glutathione. Moreover, some involvement of PI3K and PKC δ , key parts of an important thiol redox-sensitive cascade, has been also suggested [142].

Recently, evidence has shown that Ca²⁺/calmodulin (Ca²⁺/CaM) is a calcium binding protein implicated in a variety of cellular functions, including cell growth and proliferation and it has been recognized as a potential target for cancer therapy [143, 144]. HBC (4-{3,5-bis-[2-(4-hydroxy-3-methoxy-phenyl)-ethyl]-4,5-dihydro-pyrazol-1-yl}-benzoic acid) is a recently developed curcumin derivate which exhibits potent inhibitory activities against the proliferation of several tumour cell lines [145]. It has been demonstrated that HBC induces sustained phosphorylation of ERK1/2 and activates p21^{WAF1} expression, resulting in the suppression of the cell cycle progression of HCT15 colon cancer, being similar to other Ca²⁺/CaM antagonists [145].

Modulation of apoptosis provides a protective mechanism against intestinal neoplasia. Several authors [146–148] have shown that curcumin restrain apoptosis induced

by a variety of agents (*i. e.* UV, chemotherapeutic drugs or dexamethasone), being mediated by ROS production inhibition, DNA fragmentation and cytochrome *C* release. These events have been observed in several cell lines, which indicate that cell type as well as the nature of the apoptotic stimuli may determine the degree and the nature of biological curcumin effects.

At this respect, it has shown that the diferuloylmethane caused cleavage of poly(ADP-Ribose) polymerase (PARP) and caspase-3, induction of caspase-8 activity and reduced expression of Bcl-XL, showing that apoptosis is induced by curcumin *in vitro* [133]. Redox-mediated apoptosis induction by a novel synthetic curcumin analogue, EF24, was also shown in the wild-type and Bcl-XL overexpressing HT29 human colon cancer cells [149].

The role of Bcl-2 family proteins has also been studied in curcumin-induced apoptosis *in vitro*. Multiple apoptotic stimuli induce conformational changes in Bax, a proapoptotic protein belonging to Bcl-2 family and its deficiency is a frequent cause of chemoresistance in colon adenocarcinomas. To understand the role of Bax in curcumin-induced apoptosis, Rashmi *et al.* [150] used HCT116 human colon cancer cells with one allele of Bax gene (Bax^{+/-}) and Bax knockout HCT116 (Bax^{-/-}) cells in which Bax gene is inactivated by homologous recombination. In Bax^{-/-} cells curcumin-induced activation of caspases 9 and 3 was blocked and that of caspase 8 remained unaltered. This study demonstrated the role of Bax but not Bak as a critical regulator of curcumin-induced apoptosis and implied the potential of targeting antiapoptotic proteins like Bcl-XL or proapoptotic protein overexpression of like Smac (second mitochondria derived activator of caspase) as interventional approaches to deal with Bax-deficient chemo-resistant cancers for curcumin-based therapy [150]. More recently, curcumin induced cytotoxicity and apoptosis dose- and time-dependently through caspase-3 activity induction in human colon cancer colo 205 cells. Also, it promoted the Bax, cytochrome *C*, p53 and p21 expressions but inhibited the expression of Bcl-2 [151].

In a study performed by Volate *et al.* [112], using AOM-induced rat colon cancer model, diets treatment with curcumin significantly reduced the incidence of ACF. The ability of curcumin to modulate ACF was correlated well with its ability to induce apoptosis. Western blot analysis of caspase-9, Bax and Bcl-2 proteins from the colon scraping suggested that curcumin-induced apoptosis *via* the mitochondrial pathway [112].

Heat shock protein (Hsp) can function as antiapoptotic protein by suppressing the signalling events of apoptosis [152–154]. Caspase activation by curcumin was described to be blocked by Hsp [155]. It has been suggested that curcumin may not be effective in cancer that overexpress hsp70, but down-regulation of hsp70 remarkably sensitizes human colon cancer cells to apoptosis induced by curcumin [155–157].

Recently, several authors have been indicated that curcumin is able to increase the degradation of β -catenin, E-cadherin and adenomatous polyposis coli gen (*APC*), altering the cell–cell adhesion pathway and resulting in apoptosis [134]. In fact it has been demonstrated that curcumin may impair Wnt signalling and decreasing transactivation of β -catenin/T-cell factor (TCF)/lymphoid enhancer factor (LEF) signalling subsequently attenuating tumour progression [134]. This is an important chemoprevention mechanism by curcumin since it has been demonstrated that dysregulated β -catenin/TCF is implicated in cancer progression and poor prognosis [31]. Indeed, previous studies have indicated that β -catenin suppression by curcumin may inhibit tumour growth rate of SW480 colon carcinoma xenograft and other colon cancer cell lines [158, 159]. In C57BL/6J-Min/+ (Min/+) mice, curcumin decreased tumour formation by 63% at a dietary level of 0.15%. Examination of intestinal tissue from the treated animals showed that tumour prevention by curcumin was associated with increased enterocyte apoptosis and proliferation. The polyphenol also decreased the oncoprotein β -catenin expression in the enterocytes of Min/+ mouse, an observation previously associated with an antitumour effect [121].

Other mechanism related to apoptotic activity of curcumin involves generation of ROS and Ca²⁺, which has been demonstrated in colo 205 cells. Curcumin-induced ROS production leading to an increase in Chk phosphorylation and induced Ca²⁺ production, and the capture of Ca²⁺ by the chelator leading to a decrease in Ca²⁺ and MMP production levels in the examined cells [151].

Similarly, other apoptotic pathway implicated is the MAPK pathway. JNK is one of its members whose activation and induction of JNK-dependent apoptosis by curcumin treatment has been demonstrated in HCT-116 cells. However, p38 MAPK or ERK had not effect upon curcumin-induced apoptosis [160].

It is well known that ceramides are the lipid backbone of sphingomyelin and glycolipids, and are capable of influencing cell growth, viability and apoptosis. Moreover, ceramides activate stress-activated protein kinases such as JNK, which have also been implicated in the induction of apoptotic cell death as mentioned above. In a recent report, Moussavi *et al.* [161] demonstrated that curcumin-induced ceramide generation in colonic cancer cells. Afterwards, they investigated the role of ROS in this phenomenon. The data indicated that both ROS generation and JNK activation were important mechanisms in curcumin mediated apoptosis in colon cancer cell lines [161].

The modulatory effect of curcumin on the transcription activation of NF- κ B, which has been implicated in all the stages of carcinogenesis, and its role in apoptosis in colon cancer, seems controversial *in vitro* studies. It is widely believed that curcumin proapoptotic properties are mediated by down-regulation of NF- κ B. The p65/RelA subunit of NF- κ B may influence cell death, in part by activation of

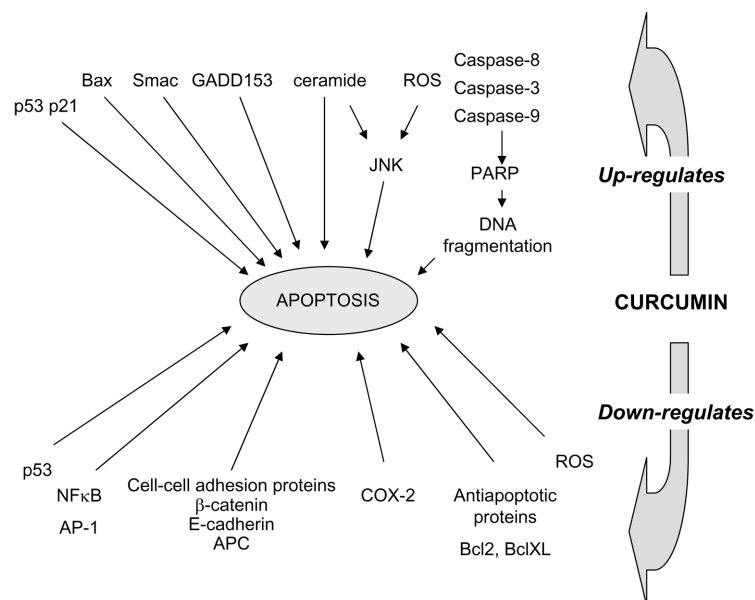


Figure 1. Modulation of apoptosis by curcumin in colon cancer models. AP-1, activator protein-1; APC, adenomatous polyposis coli; COX-2, cyclooxygenase-2; GADD153, growth arrest and DNA damage-inducible gene 153; JNK, c-Jun NH2-terminal kinase; NF- κ B, nuclear factor kappa B; PARP, poly(ADP-Ribose) polymerase; ROS, reactive oxygen species; Smac, second mitochondria derived activator of caspase.

NF- κ B antiapoptotic target genes including X-linked inhibitor of apoptosis (XIAP), A20, bcl-xL and inhibition of sustained activation of JNK. Collett and Campbell [162] have documented that curcumin inhibits NF- κ B, activates JNK and promotes apoptosis in HCT116 CRC cells. Additionally, the same authors have shown that curcumin-mediated activation of JNK or induction of apoptosis occurs independently of NF- κ B transcriptional activity inhibition. Overexpression of p53 had no effect on curcumin-induced JNK activation but potentiated curcumin-induced apoptosis. Furthermore, enhancement of curcumin-induced apoptosis by p53 overexpression could not be attributed to active repression of NF- κ B antiapoptotic target genes, XIAP, Bcl-XL or A20 [162].

Jeong *et al.* [163] shown that 10 mM of curcumin has potent inhibitory activity against NF- κ B-luciferase activity in HT-29 N9 cells (stable clone of HT-29 transfected with NF- κ B luciferase construct), as well as an inhibition of LPS-induced phosphorylation of inhibitory kappaB-alpha ($I\kappa$ B)- α . In other study, curcumin affected the levels of NF- κ B/p65 in a time-dependent manner but did not affect NF- κ B/p50 in colon 205 cells [164]. However, Collett and Campbell [162] showed that inhibition of NF- κ B by curcumin is not essential for its antiapoptotic effects in HCT-116 cells suggesting that other unidentified regulators of cell death may be implicated.

AP-1 also plays essential roles in diverse biological processes including biological apoptosis, proliferation, transformation and differentiation and cancer development, although the exact outcome may be highly dependent on tissue and developmental stage [165–167]. Jeong *et al.* [163], in a new model system using the human colon cancer cell line HT-29 stably transfected with AP-1-luciferase reporter gene (HT-29 C4), evaluated and compared the effects of

several chemoprevention agents on this signalling pathway. They showed that curcumin caused a significant induction of AP-1 luciferase reporter gene and endogenous cyclic D1 protein at the concentration range between 1 and 25 μ M and decrease at higher concentration (35 and 50 μ M). Moreover, curcumin at 50 μ M dose exhibited more potent inhibitory effect on cell viability of HT-29. In HCT116 cells, it has also been demonstrated stimulation of AP-1 transcriptional activity after curcumin treatment [160], although this effect may be dose- or cell type specific, indicating that such use of curcumin should be approached with precaution.

Inhibition of AP-1 and NF- κ B signalling pathways by curcumin has also been shown *in vivo* studies. For example, Chun *et al.* [168] reported that topical curcumin application to mouse skin inhibited 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-mediated activation of ERK and p38 MAPK and subsequent activation of NF- κ B.

Figure 1 shows the possible pathways by which curcumin may modulate apoptosis in colon cancer models.

5.2 Effects of curcumin on gene expression and protein activity

The tumour suppressor and transcription factor p53 binds to specific DNA sequences in promoter regions of its target genes after DNA damage. Activation of these genes by p53 is involved in the control of cell proliferation, and mutations in the p53 gene are commonly found to be associated with diverse types of human cancer [169, 170]. Moos *et al.* [171] demonstrated that curcumin impairs tumour suppressor p53 function in RKO colon cancer cells, which might confer risk that is inseparable from its potential benefits. However, as it has been commented above, curcumin down-regulated

p53 expression in HT29 cells [135] where mutated *p53* is highly expressed [172].

Inhibition of the *trans*-activation activity of the transcription factor early growth response-1 (Egr-1) by curcumin has also been reported in Moser, caco-2 and HT-29 cells, in which curcumin reduced EGFR expression [173]. Moreover, interruption of the ERK signal pathway was a necessity in curcumin inhibition of Egr-1 gene expression [173]. It is becoming increasingly evident that blocking of EGFR signalling pathways is an effective therapeutic approach for treatment and prevention of colorectal neoplasia.

In a recent study by Chen and Xu [174], incubation of curcumin in Moser cells, a human colon cancer-derived cell line, suppressed EGFR gene expression at least partially, by inhibition of EGFR tyrosine phosphorylation and suppressing EGFR and cyclic D1 expression [174]. In addition, other members of the receptor tyrosine kinases, especially IGF-1R, may play a role in its development and progression. For instance, it has been demonstrated that curcumin-induced inhibition of colon cancer HCT-116 cells growth by attenuation of tyrosine phosphorylation of IGF-1R [175].

It is known that PPAR γ activation induces growth arrest and differentiation markers of human colon cancer cells [176, 177], therefore effects of PPAR γ on controlling the expression of genes involved have attracted attention from the perspective of cancer prevention. Cell proliferation assays demonstrated that curcumin, in a dose-dependent manner, significantly inhibited cell growth of both HT-29 and Moser cells and stimulated the *trans*-activating activity of PPAR γ [174].

As mentioned above, the overexpression of MMPs may be one part of the multistep process that leads to neoplastic cell proliferation and metastasis. Curcumin inhibited MMP2 levels and promoted MMP9 levels, but not affected MMP7 levels in colon 205 cells [164].

5.3 Effects of curcumin on modulation of the immune and inflammatory responses

In recent years investigators have found that the human colon cancer cells overexpressing COX-2 due to alterations in transcriptional control [178] are more invasive than vector-transfected control cells [179]. Up-regulation of COX-2 expression occurs in 40–50% of colorectal polyps and in up to 85% of CRCs [180]. Up-regulation of Bcl-2 by COX-2 might provide a potential mechanism for resistance to apoptosis by diverse antineoplastic agents [181].

Jobin *et al.* [182] have shown that NF- κ B is critical for the induction of COX-2 gene expression by TNF- α in human colon tumour cells. In a study by Plummer *et al.* [183] curcumin reduced COX-2 expression by inhibiting physiologically relevant concentrations of TNF- α -induced NF- κ B activation in human CEC. Curcumin is able to exert this effect through inhibition of phosphorylation of NF- κ B

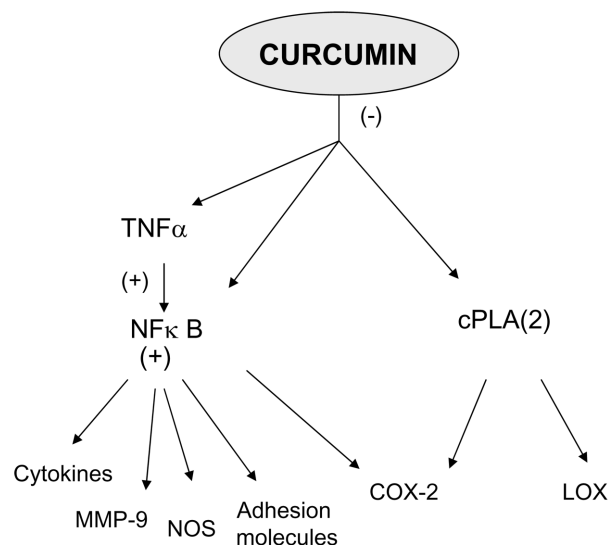


Figure 2. Modulation of the immune and inflammatory response by curcumin in colon cancer. cPLA(2), cytosolic phospholipase A₂; LOX, lipoxygenase; MMP, metalloproteinase; NF- κ B, nuclear factor kappa B; NOS, nitric oxide synthase; TNF- α , tumour necrosis factor alpha.

inducing kinase (NIK)/I κ B kinase (IKK) signalling complex. It has also been documented that curcumin affects arachidonic acid metabolism by blocking the phosphorylation of cytosolic phospholipase (cPL)A₂, decreasing the expression of COX-2 and inhibiting the catalytic activities of 5-lipoxygenase (LOX), contributing to the anti-inflammatory and anticarcinogenic actions of curcumin and its analogues [184] (Fig. 2).

More recently, it has been showed that curcumin inhibited COX-2 protein activity and expression in a dose-dependent manner in colorectal adenocarcinoma cell lines.

Indeed, induction of apoptosis by curcumin was higher in cells expressing COX-2 (HT29), whereas only weak effect was seen in cells that do not express COX-2 (SW480), and it was associated with the inhibition of PGE₂ and down-regulation of COX-2. The authors conclude that inhibition of cell survival and induction of apoptosis by curcumin in colorectal adenocarcinoma cell lines is associated with the inhibition of PGE₂ synthesis and down-regulation of COX-2 [185].

COX-2 down-regulation has also been described in colon 205 cells by Su *et al.* [164]. However, it has been also reported that curcumin inhibited the growth of non-COX-2 expressing HCT-116 cells and COX-2 expressing HT-29 CRC cells [186, 187]. These observations suggested that curcumin inhibits the colon cancer cells growth independent of COX-2 expression.

Multiple studies have indicated that specific COX-2 inhibitors may prevent CRC, since *in vivo* assays have shown that chemically induced colon tumours in rats as well as human colon tumours present high levels of COX-2

[184, 188, 189]. Furthermore, there is additional evidence supporting roles not only for COX-2 but also iNOS, which shows a marked reduction in colon carcinogenesis in rodents treated with highly selective COX-2 and iNOS inhibitors [190–193]. However, the long-term use of COX-2 inhibitors is not toxicity-free and may be limited due to its cardiovascular side effects. Animal models of colon carcinogenesis have shown that curcumin exerts chemopreventive activity by decreasing the overexpression of COX-2 and iNOS [111, 113]. Thus, Shpitz *et al.* [194] carried out an investigation to examine the chemopreventive effects of celecoxib and curcumin alone and in combination using the DMH rat model, and concluded that, *in vivo*, the diferuloylmethane augmented the growth inhibitory effect of celecoxib.

A synergistic inhibitory effect between curcumin and catechin on COX-2 mRNA expression was also observed by Xu *et al.* [120] in the early stage of rat colon carcinogenesis but not in colon tumour tissues in a similar rat-model assay. Beside the inhibition that the polyphenol is able to exert over PGE₂ production, curcumin also inhibit leukotrienes B₄ and C₄ synthesis by blocking LOX pathway [113, 195, 196]. A recent report has studied COX-2 protein expression and oxidative DNA adduct levels in intestinal adenoma tissue from Apc(Min+) mice to try and differentiate between curcumin's direct pharmacodynamic effects and indirect effects *via* its inhibition of adenoma growth employing different time periods, 4 or 14 wks. These results demonstrated that short-term feeding did not affect total adenoma number or COX-2 expression, while the effects of long-term dietary curcumin on COX-2 protein levels appear to reflect retardation of adenoma development [197].

Recently, Kwon and Magnuson [117] investigated the role of arachidonic acid metabolism as a potential mechanism of age-related differences in response to curcumin, middle-aged F344 male rats were given AOM injections after being fed, 0.6% curcumin. In middle-aged rats, dietary curcumin did not reduce the number of ACF and surprisingly increased COX-2 mRNA colonic levels. Colonic COX-2 and PGE₂ levels were also significantly increased in young rats fed curcumin after AOM injections. Interestingly, AOM did not affect COX-2 but decreased COX-1 expression in all ages. They conclude that during initiation, AOM inhibits colonic COX-1 expression without affecting COX-2 and dietary curcumin may increase COX-2 expression to compensate AOM-induced reduction of COX-1 expression.

Another important mechanism for the *in vitro* antitumour effect of curcumin may be through the suppression of chemokines. IL-8, regulated by AP-1 and NF- κ B transcription factor in various cancer cells, is becoming increasingly recognized as an important local factor in tumourigenesis and metastasis. Wang *et al.* [198] found that curcumin inhibited neurotensin-induced AP-1 and NF- κ B induction and subsequent IL-8 gene expression and protein secretion in HCT-116.

TNF- α -related apoptosis-inducing ligand (TRAIL), also known as Apo2L, is a member of the TNF family and can induce apoptotic cell death [199, 200]. TRAIL binds to four different types of membrane-bound death receptors (DR4, DR5, DcR1 and DcR2). Both DR4 and DR5 contain a conserved cytoplasmic region called the “death domain”, which is required for TRAIL-induced apoptosis. Up-regulation of DR5 proteins by curcumin was also observed in a variety of tumour cell types (HCT116 and HT29) [201].

5.4 Effects of curcumin on angiogenesis

Rigorous examination has strongly showed participation of angiogenesis in expansion of primary tumours and their metastasis to distinct organs [202, 203]. Among the factors that can stimulate angiogenesis, vascular endothelial growth factor has emerged as one of the most important, and inhibition of vascular endothelial growth factor has recently shown efficacy in the treatment of advanced CRC. Hypoxia develops within solid tumours and is one of the most potent stimuli of vascular endothelial growth factor expression. This effect is mediated primarily by hypoxia inducible factor-1 (HIF-1), often considered a master regulator of angiogenesis in hypoxia. Consequently, inhibition of HIF-1 has been proposed as a strategy to block tumour angiogenesis therapeutically. Nevertheless, accumulating data indicates that HIF-independent pathways can also control angiogenesis [204].

It has been shown that curcumin has direct antiangiogenic activity *in vitro* [205, 206] and *in vivo* [206–208], which may explain its chemopreventive effect at the level of tumour growth and metastasis. Possible antiangiogenic mechanisms are being postulated like the down regulation of proangiogenic genes such as VEGF and angiopoietin and a decrease in migration and invasion of endothelial cells [101]. Curcumin has been shown to suppress angiogenesis and metastasis in a variety animal tumour models such as the skin, for example in a wound-healing model by inducing transforming growth factor-beta, which induces both angiogenesis and accumulation of extracellular matrix, which continues through the remodelling phase of wound repair [208]. Also, inhibition of angiogenesis by curcumin has been demonstrated *in vivo* using a mouse corneal model [207]. Suppression of angiogenic growth factor production, integral to the formation of new vessels, has also been effected by curcumin in lung metastasis induced by B16F-10 melanoma cells were studied in female C57BL/6 mice [209].

Although to our knowledge there is only one paper about colon cancer and angiogenesis *in vivo* which confirms that hypothesis, Li and colleagues [85, 86] have demonstrated that liposomal curcumin inhibited pancreatic as well as colon carcinoma growth in murine xenograft models and these effects are accompanied by a potent antiangiogenic response.

Table 4. On-going phase II human clinical studies of curcumin in patients with cancer

Disease	Institution	Study design	Study start	Study completion
ACF in colon	Chao Family Comprehensive Cancer Center, USA	Phase II single-arm: curcumin	September 2006	Recruiting
ACF in colon	University of Medicine and Dentistry New Jersey, USA	Phase II: curcuminoids vs. sulindac	April 2004	Recruiting
Sporadic adenomatous colonic polyps, recently resected	University of Pennsylvania, USA	Phase II, placebo-controlled: curcuminoids	July 2005	Recruiting
FAP	Johns Hopkins University, USA	Phase II single-arm: curcumin	November 2005	Recruiting
Advanced pancreatic cancer	Rambam Health Care Campus, Israel	Phase II single-arm: curcumin + gemcitabine	July 2004	Recruiting
Advanced pancreatic cancer	M.D. Anderson Cancer Center, USA	Phase II single-arm: curcumin	November 2004	March 2007

6 Conclusions

Epidemiologic, preclinical and clinical studies point to food components, including meat, fat, cereal, vegetables, etc. as the main and modifiable determinant of CRC prevalence and tumour behaviour. Because the proinflammatory and pro-oxidant states are closely linked to tumour promotion, dietary substances such as curcumin, with potent anti-inflammatory and or/antioxidant activities are anticipated to exert chemopreventive effects. **Preclinical studies of curcumin have shown the ability to inhibit carcinogenesis in various types of cancer including breast, cervical, gastric, hepatic, leukaemia, oral epithelial, ovarian, pancreatic, prostate and CRC.** It has the capacity of interact with multiple molecular targets affecting the multistep process of carcinogenesis. Furthermore, curcumin induces apoptosis in cancer cells without cytotoxic effects on healthy cells and possesses marked antiangiogenic properties.

Furthermore, curcumin potentiates the growth inhibitory effect of COX-2 inhibitors and traditional chemotherapy agents implicating another promising therapy regimen in the future treatment of CRC. Although the experimental records showed curcumin to be a potential chemotherapeutic agent against a broad range of cancer models, its clinical advance has been hindered by its short biological half-life and low bioavailability after oral administration. Nevertheless, there is extensive interest in the clinical development of this compound as a cancer chemopreventive and/or chemotherapeutic agent as evidenced by the development of phase I clinical trials and current enrolment in phase II clinical trials (Table 4 shows on-going phase II trials in patients with ACF, polyps or pancreatic cancer. For details, please refer to www.ClinicalTrials.gov).

Regarding toxicity, since curcumin is a constituent of the diet it is nontoxic in nature. Moreover, several studies have demonstrated minimal toxicity with moderate doses of polyphenol given in various formulations. However, future studies are likely to focus on a deeper understanding and knowledge of the biology of this remarkable compound and

its analogues in order to predict its efficacy in the clinical settings.

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