Curcumin: A natural antiinflammatory agent

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ABSTRACT

Extensive scientific research on curcumin, a natural compound present in the rhizomes of plant *Curcuma longa* Linn., demonstrated its antiinflammatory action. Curcumin was found to inhibit arachidonic acid metabolism, cyclooxygenase, lipoxygenase, cytokines (Interleukins and tumour necrosis factor) Nuclear factor-κB and release of steroidal hormones. Curcumin was reported to stabilize lysosomal membrane and cause uncoupling of oxidative phosphorylation besides having strong oxygen radical scavenging activity, which was responsible for its antiinflammatory property. In various animal studies, a dose range of 100–200 mg/kg body weight exhibited good antiinflammatory activity and seemed to have negligible adverse effect on human systems. Oral LD_{50} in mice was found to be more than 2.0 g/kg body weight.

KEY WORDS: Cyclo-oxygenase (COX); free radical scavenger; inflammation.

The rhizome of turmeric is widely used in indigenous medicine.[1] A paste made from powdered rhizome of *Curcuma longa* Linn., mixed with slaked lime applied locally, is an ancient household remedy for sprains, muscular pain and inflamed joints. It is also applied in poultices to relieve pain and inflammation.[2] The volatile oil and curcumin obtained from *C. longa* exhibit potent antiinflammatory effect.[3]

Curcumin is yellow coloured phenolic pigment,[4] obtained from powdered rhizome of *C. longa* Linn. (Family-Zingiberaceae). It is the major constituent of the oleoresin of turmeric. In the crude extract of rhizomes of *C. longa* about 70–76% curcumin is present along with about 16% demethoxycurcumin and 8% bisdemethoxycurcumin. It is extensively used for imparting colour and flavour to the food and in the traditional Indian medicine, turmeric powder is used to treat a wide variety of diseases. Extensive scientific research on curcumin have demonstrated a wide spectrum of therapeutic effects such as antiinflammatory,[5] antibacterial,[6] antiviral,[7] antifungal,[8] antitumor,[9] antispasmodic[10] and hepatoprotective.[11] Recently, its potential utility in autoimmune deficiency syndrome (AIDS) has been demonstrated.[12]–[14] In this review, the findings on curcumin's antiinflammatory activity and its mechanisms are presented.

Preclinical studies

Curcumin and antiinflammatory activity

Arora *et al* reported antiinflammatory activity in different fractions of the petroleum ether extract of *C. longa*.[5] The total petroleum ether extract of the rhizome of turmeric and two of its fractions A and B were evaluated for their antiinflammatory activity in albino rats (180–200 g) and compared with that of hydrocortisone acetate and phenylbutazone. It was found that the antiinflammatory activity of the total petroleum ether extract was less than the individual fractions A and B. The fractions were almost as active as hydrocortisone acetate in the inflammation induced by cotton pellet method. Curcumin isolated from the alcoholic extract of turmeric has been shown to be a useful antiinflammatory agent. In subacute toxicity experiments, no significant toxic side effects were observed in rats when the extract was administered for 4 weeks at the dose level of 1–2 g/kg. Oral LD_{50} was found to be 12.2 g/kg.[5] Recently, antiinflammatory activity of curcumin has been demonstrated in acute and chronic models of inflammation in rats and mice.[15],[16] In rats with Freud's adjuvant-induced arthritis, administration of curcumin significantly reduced the inflammatory swelling compared to control.[16] Oral doses up to 160 mg/kg of curcumin failed to prevent phenylquinone-induced inflammation in mice. In instances of acute inflammation, oral administration of curcumin was found to be as effective as cortisone or phenylbutazone, whereas in chronic inflammation it was only half as effective.[17] Curcumin may also be applied topically to animal skin to counteract inflammation and irritation associated with inflammatory skin conditions and allergies.[17]

Natural analogues of curcumin

Two naturally occurring analogues of curcumin, Feruloyl

4-hydroxy cinnamoyl methane (FHM) and bis-(4-hydroxy cinnamoyl) methane (BHM) were isolated from the alcoholic extract of turmeric.[18] Both were screened for antiinflammatory activity using carrageenin-induced rat paw edema and compared with sodium curcuminate and phenylbutazone.[19] The FHM was found to be more potent and the activity with 30 mg/kg dose of FHM was found to be equivalent to that of 100 mg/kg of phenylbutazone.[19] Curcumin analogues revealed a dose-dependent effect up to the dose of 30 mg/kg. Further increase in dose resulted in decreased antiinflammatory activity.[19]

Semi-synthetic curcumin

As curcumin is insoluble in water, its water-soluble semi-synthetic derivatives were studied for antiinflammatory activity. Ghatak *et al* prepared sodium phenate of curcumin and demonstrated its antiinflammatory activity.[20] They found that sodium phenate of curcumin showed better antiinflammatory activity than curcumin and hydrocortisone acetate in experimental inflammation induced by carrageenin and formalin in albino rats. Mukhopadhayay *et al* have studied the structure-activity relationship (SAR) with respect to antiinflammatory activity in a series of curcumin analogues.[21] They reported that sodium salt of curcumin was found to be most effective in carrageenin-induced rat hind paw oedema among curcumin and some of its semi-synthetic analogues.

Clinical trials

Deodhar *et al* have studied the antiinflammatory action of curcumin in patients with rheumatoid arthritis. The study demonstrated a significant improvement in the duration of morning stiffness, walking time and joint swelling, with curcumin, which was almost comparable to phenylbutazone.[22] Satoskar *et al* evaluated the antiinflammatory property of curcumin in patients with postoperative inflammation. The effect of the drug on individual parameters revealed that phenylbutazone and curcumin had better antiinflammatory responses in these patients compared to the placebo. Curcumin was found better than phenylbutazone in reducing spermatic cord oedema and tenderness.[23]

Kuttan *et al* reported that an ethanolic extract of turmeric or a curcumin ointment provided symptomatic relief in patients with cancers of oral cavity, breast, vulva and skin. Out of 62 patients, only one showed adverse reaction.[24]

Lal *et al* studied the efficacy of curcumin in the management of chronic anterior uveitis (CAU).[25] Curcumin was administered orally to patients suffering from CAU at a dose of 375 mg three times per day for 12 weeks. Of the 53 patients enrolled, only 32 completed the 12 week study. They were divided into two groups: 18 patients received curcumin alone while 14 patients, who had a strong purified protein derivative (PPD) reaction, received antitubercular treatment in addition to curcumin. The patients from both the groups showed improvement after 2 weeks of treatment. All the patients who received curcumin alone improved well while those who received curcumin and antitubercular therapy showed a response rate of 86%. Follow-up of all the patients for the next 3 years indicated a recurrence rate of 55% in the first group and of 36% in the second group. About 4 of 18 patients (22%) in the first group and 3 of the 14 patients (21%) in the second group lost their vision in the follow-up period due to various complications such as vitritis, macular oedema, central venous block, cataract formation, glaucomatous optic nerve damage, etc. None of the patients reported any side effect during the treatment. Corticosteroid therapy is presently the only available standard treatment for this disease. Lack of side effects with curcumin is its greatest advantage compared with corticosteroids.

Lal *et al* described for the first time the clinical efficacy of curcumin in the treatment of patients suffering from idiopathic inflammatory orbital pseudotumours.[26] Curcumin was administered orally at a dose of 375 mg thrice a day orally for a period of 6–22 months in eight patients. They were followed up for a period of 2 years at three monthly intervals. Five patients completed the study; out of which four recovered completely and in one patient, the swelling regressed completely with some persistent limitation of movement. No side effect was noted in any of the patients and there was no recurrence. Though it was suggested that curcumin could be used as a safe and effective drug in the treatment of idiopathic inflammatory orbital pseudotumours, a large multicentric trial with adequate number of patients is required to confirm the beneficial effects of curcumin.

Mechanism of action of curcumin

Nonsteroidal antiinflammatory agents may act *via* single or combination of any of the mechanism involving inhibition of arachidonic acid metabolism, inhibition of cyclo-oxygenase (COX)/inhibition of the PG synthesis, inhibition of lipoxygenase (LOX), inhibition of cytokines (IL, TNF, etc.), release of steroidal hormones from the adrenals, stabilization of lysosomal membrane and uncoupling of oxidative phosphorylation, etc.

Srivastava *et al* demonstrated that curcumin inhibited the incorporation of [^{14}C]arachidonic acid (AA) into platelet phospholipids and inhibited the deacylation of AA-labelled phospholipids (liberation of free AA) on stimulation with calcium ionophore A23187.[27],[28] Rat peritoneal macrophages preincubated with 10 μM curcumin or capsaicin for 1 h inhibited the incorporation of AA into membrane lipids by 82 and 76%, respectively; prostaglandin E_2 by 45% and 48%; leukotriene B_4 by 61% and 46% and leukotriene C4 by 34% and 48%, respectively.[29] Curcumin appears to block the synthesis of certain prostaglandins through inhibition of COX enzyme.[30],[31] Ramsewak *et al*. demonstrated that curcumins I–III were active against COX-I enzyme with 125 μg/ml and showed 32%, 38.5% and 39.2% inhibition of the enzyme, respectively. Curcumins I–III also showed 89.7%, 82.5% and 58.9% inhibition, respectively, of the COX-II enzyme with 125 μg/ml.[32]

Curcumin reduces pro-inflammatory leukotriene synthesis via inhibition of LOX enzyme.[33]–[35] Flynn *et al* studied the inhibitory activities of curcuminoids and yakuchinones on the 5-hydroxy-eicosatetranoic acid (5-HETE). Various diaryl-heptonoids, including curcumin, were found to be potent inhibitors of 5-HETE productions by intact human neutrophils with IC_{50} values ranging from 4 to 8 μM.[36] Curcumin reduces the neutrophil infiltration in inflammatory conditions[37]–[39] and inhibit platelet aggregation.[40],[41] It is also a potent inhibitor of

pro-inflammatory cytokines (IL and TNF).[42]–[46] The oxygen radical scavenging activity[47],[48] of curcumin has also been implicated in its antiinflammatory effects.[49]

Molecular mechanism and biochemical changes

Inhibition of COX

Zhang *et al* investigated whether curcumin inhibited chenodeoxycholate (CD)-or phorbol ester (phorbol 12-myristate 13-acetate, PMA)-mediated induction of COX-2 in several gastrointestinal cell lines (SK-GT-4, SCC450, IEC-18 and HCA-7).[50] Treatment with curcumin suppressed CD- and PMA-mediated induction of COX-2 protein and synthesis of prostaglandin E_2. Curcumin also suppressed the induction of COX-2 mRNA by CD and PMA.[50] To investigate the effect of curcumin on COX-2 expression, HT-29 human colon cancer cells were treated with various concentrations of curcumin. Curcumin inhibited the cell growth of HT-29 cells in a concentration and time-dependent manner. There was a marked inhibition of mRNA and protein expression of COX-2, but not COX-1.[51]

Kim *et al* demonstrated that the inhibitory action of curcumin on Janus kinase (JAK)-STAT signalling could contribute to its antiinflammatory activity in the brain.[52] In both rat primary microglia and murine BV2 microglial cells, curcumin effectively suppressed the ganglioside, Lipopolysaccharide (LPS) or interferon (IFN-γ)-stimulated induction of COX-2 and inducible NO synthase, important enzymes that mediate inflammatory processes. Curcumin markedly inhibited the phosphorylation of STAT1 and 3 as well as JAK1 and 2 in microglia activated with gangliosides, LPS, or IFN-gamma thus attenuating inflammatory response of brain microglial cells.[52]

Inhibition of prostaglandin synthesis

Effect of some biochemical changes produced during sub-acute inflammation in rats has been studied and compared with ibuprofen.[53] Curcumin in the doses of 100 and 200 mg/kg inhibited the granuloma formation by 21.7 and 30.8%, respectively, while ibuprofen in 15 and 20 mg/kg doses inhibited by 26.6 and 32.2%, respectively. Thus, ibuprofen was found to be 10 times more potent than curcumin on weight basis. In an *in vitro* study, curcumin (20 μg/ml) as well as ibuprofen (2 μg/ml) caused complete inhibition of the spontaneous contraction of the isolated pregnant rat uterus.

In an *in vivo* study, PGE_2 content in the inflammatory exudates of control rats with inflammation was 7.29 μg/ml. Treatment of the animals with curcumin (200 mg/kg) and ibuprofen (20 mg/kg) for 4 days reduced the PGE_2 content of the exudates by 45% and 61%, respectively. Thus, curcumin was found to be less effective than ibuprofen in inhibiting PG synthesis in inflammatory exudates as well as in the *in vitro* system. *In vitro* studies revealed that curcumin decreased phorbol ester-induced PGE_2 production down to almost preinduction level.[54] Tetrahydrocurcumin, hexahydrocurcumin and curcumin sulfate reduced it by 31%, 37% and 22%, respectively. Hexahydrocurcuminol was found to be devoid of inhibitory activities. In a confirmatory Western analysis using a COX-2 monoclonal antibody, curcumin was shown to reduce phorbol ester-induced COX-2 protein expression consistently by 60–70%. In contrast, curcumin metabolites interfered with COX-2 protein inhibition only weakly.[54]

Inhibition of cytokines

The pleiotropic cytokine-tumour necrosis factor-alpha (TNF) induced the production of interleukin-1 beta (IL-1), and together, they play significant roles in many acute and chronic inflammatory diseases. Gupta *et al*. demonstrated that curcumin inhibited TNF-α induced expression of adhesion molecules (ICAM-1, VCAM-1 and E-selectin) on human umbilical vein endothelial cells.[55] As diferuloylmethane significantly blocks the cytokine-induced transcript levels for the leukocyte adhesion molecules, it may be interfering at an early stage of signalling event induced by TNF-α.[55] Curcumin produced significant inhibition of IL-1β and IL-8 but minimal inhibition of TNF-α expression by preterm lung inflammatory cells at 20 μM concentrations. Adult PBMC expression of IL-8 was significantly inhibited by curcumin at 20 μM concentrations. Therefore, curcumin inhibits pro-inflammatory cytokine production (TNF-α, IL-1β and IL-8) by lung inflammatory cells and this is evidenced by a large number of experiments.[56]–[60] It was also shown that curcumin inhibited experimental allergic encephalomyelitis by blocking IL-12 signalling through JAK-STAT pathway in T lymphocytes.[61]

NF-κB inhibition

Binding of plasma factor VII (a) to tissue factor (TF) initiates the coagulation cascade. In normal condition, TF is not expressed in endothelial cells. However, endothelial cells express TF in response to LPS, TNF and other biological stimuli. Pendurthi *et al* studied the inhibition of TF gene activation in cultured endothelial cells by curcumin.[62] They demonstrated that curcumin inhibited PMA, LPS, TNF-α and thrombin-induced TF activity and TF gene transcription in human endothelial cells by impairing the proteolytic degradation inhibitor protein Iκ Bα. Thus antiinflammatory and anticarcinogenic activity of curcumin may be related to its ability to inhibit cellular gene expression regulated by transcription factors NF-κ B, AP-1 and Egr-1.[54] Bierhaus demonstrated that curcumin inhibited TNFα-induced Iκ Bα degradation and the nuclear import of NF-αB. In contrast, inhibition of AP-1 was due to a direct interaction of curcumin with AP-1-binding to its DNA binding motif. Thus, curcumin inhibits NF-κB and AP-1 by two different mechanisms and reduces expression of endothelial genes controlled by both transcription factors *in vitro*.[55] Curcumin also blocks cytokine-mediated NF-κB activation and pro-inflammatory gene expression by inhibiting inhibitory factor I-κB kinase activity and it has been confirmed by a large number of experiments.[60]–[70] The COX-2 inducible and nitric oxide synthase (iNOS) are important enzymes that mediate inflammatory responses. Improper up-regulation of COX-2 and iNOS has been associated with pathophysiology of certain types of human cancers as well as inflammatory disorders.

Recent studies have demonstrated that eukaryotic transcription factor nuclear factor κB (NF-κ B) was involved in regulation of COX-2 and iNOS expression. Surh studied the molecular mechanism underlying antiinflammatory activity of curcumin.[71] They suggested the down-regulation of COX-2 and iNOS through suppression of NF-κ B. Repression of degradation of the inhibitory unit I-κ Bα, which hampers subsequent nuclear translocation of the functionally active subunit of NF-κ B,

may be responsible for inhibition of NF-κ B by curcumin.[71] Pan *et al* comparatively studied suppression of nitric oxide synthase by curcumin and its hydrogenated metabolites through down-regulation of I-κB kinase and NF-κB activation in macrophages.[72]

Han *et al* demonstrated that curcumin inhibited the 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced NF-κB activation by preventing the degradation of the inhibitory protein I-κB α and the subsequent translocation of the p65 subunit in cultured human promyelocytic leukemia (HL-60) cells.[73] Alternatively, curcumin repressed the TPA-induced activation of NF-κB through direct interruption of the binding of NF-κB to its consensus DNA sequences.[73]

Chun *et al* demonstrated the effect of curcumin on TPA-induced expression of COX-2) in female mouse.[66] Immuno histochemical analysis of TPA-treated mouse skin revealed enhanced expression of COX-2 localized primarily in epidermal layer, which was markedly suppressed by curcumin pretreatment. Curcumin treatment attenuated TPA-stimulated NF-κB activation in mouse skin, which was associated with its blockade of degradation of the inhibitory protein I-κB α and of subsequent translocation of the p65 subunit to nucleus.[74]

Inhibition of platelet aggregation

Shah *et al* studied the mechanism of platelet aggregation by curcumin.[40] They showed that curcumin-inhibited platelet aggregation mediated by the platelet agonists epinephrine (200 μM), ADP (4 μM), platelet activating factor (PAF, 800 nM), collagen (20 μg/ml) and AA (0.75 mM). Curcumin preferentially inhibited PAF and AA-induced aggregation (IC-50; 20–25 μM) whereas much higher concentration of curcumin is required to inhibit aggregation induced by other platelet agonists. Pretreatment of platelets with curcumin resulted in inhibition of platelet aggregation induced by calcium ionophore A-23187 (IC_{50}:100 μM), but curcumin up to 250 μM had no inhibitory effect on aggregation induced by the proteinkinase C (PKC) activator phorbol myrisitate acetate (1 μM). Curcumin (100 μM) inhibited the A-23187-induced mobilization of intracellular Ca^{2+} as determined by using fura-2 acetoxymethyl ester. Curcumin also inhibited the formation of thromboxane A_2 (TX A_2) by platelets (IC_{50}: 70 μM). These results suggest that the curcumin-mediated preferential inhibition of PAF and AA-induced platelet aggregation which involved inhibitory effects on TXA_2 synthesis and Ca^{2+} signalling but without the involvement of PKC.

Stabilization of lysosomal enzymes

A number of NSAIDs like ketoprofen, suprofen have been reported to inhibit the release of lysosomal enzymes from the neutrophils.[75] The role of lysosomal enzymes, i.e. acid phosphatase and cathepsin D as mediator of inflammation is well documented.[76],[77] Stabilization of lysosomal enzymes by curcumin and ibuprofen was compared. Serum phosphatase activity increased from 7.26 to 15.4 units (+112%) due to inflammation. Curcumin (200 mg/kg) prevented the increase by 50% while ibuprofen (20 mg/kg) prevented it by 61%. In an *in vitro* study, curcumin was found to have greater lysosomal membrane stabilization effect than ibuprofen.[53] Joe *et al* demonstrated that curcumin and capsaicin lower the release of lysosomal enzymes and eicosanoids in rat peritoneal macrophages.[29]

Release of hormones

The release of endogenous corticosteroids by curcumin may also help indirectly in stabilizing lysosomal membrane, because glucocorticoids are known to have stabilizing effect on the lysosomal enzymes as evidenced by several experiments.[78],[79] Inflammation caused a significant increase in adrenal ascorbic acid and cholesterol level. A dose of 200 mg/kg of curcumin significantly decreased the adrenal ascorbic acid without affecting the cholesterol level.[53] Lower dose of curcumin (100 mg/kg) as well as ibuprofen had no effect.[53]

Antioxidative effect

Curcumin was found to be a very potent antioxidant.[80]–[83] Curcumin was found to generate hydroxyl radicals through the Fenton reaction by reducing Fe^{3+} to Fe^{2+}.[84] Effect of curcumin as superoxide scavenger was studied and curcumin was found to be a potent scavenger of superoxide.[85] They also reported a better correlation between antiinflammatory activity and superoxide scavenging property.

Balasubramanyam *et al* demonstrated that curcumin abolished both PMA and thapsigargin-induced ROS generation in cells from control and diabetic subjects. The pattern of these ROS inhibitory effects as a function of dose-dependency suggest that curcumin mechanistically interferes with PKC and calcium regulation.[86]

Priyadarsini *et al* tested the antioxidant activity of curcumin and dimethoxy curcumin by radiation-induced lipid peroxidation in rat liver microsomes.[87] They found that at equal concentration, the efficiency to inhibit lipid peroxidation is changed from 82% with curcumin to 24% with dimethoxy curcumin. These results suggested that, although the energetics to remove hydrogen from both phenolic OH and the CH (2) group of the beta-diketo structure were very close, the phenolic OH was essential for both antioxidant activity and free radical kinetics. This was further confirmed by density functional theory (DFT) calculations where it was shown that the –OH hydrogen was more labile for abstraction compared to the –CH (2) hydrogen in curcumin suggesting that phenolic OH plays a major role in the activity of curcumin.

Inhibition of monocyte chemoattractant protein-1 (mcp-1) by curcumin

Nakayama *et al* described a novel effect of proteosome inhibitors on the expression of the monocyte chemoattractant protein 1 (MCP-1) in mesangial cells. They found that proteosome inhibitors MG 132 dose-dependently induced the expression of MCP-1 at the transcriptional level. The 5'-flanking region of the MCP-1 gene contains multiple AP-1 sites. A reporter assay showed that AP-1 activity was up-regulated after treatment with MG 132 and kinase assay revealed that c-jun-N-terminal kinase (JNK) was rapidly activated by MG132. Curcumin, a pharmacological inhibitor of the JNK-AP-1 pathway, abrogated the induction of MCP-1 by MG132. These data revealed that proteosome inhibition triggered the expression of MCP-1 and other genes via the multistep induction of the JNK-c-Jun/AP-1 pathway.[88]

Inhibition of acidic glycoprotein (gp a 72) by curcumin

Joe *et al*. observed an increased level of acidic glycoprotein Gp A 72 in the sera of arthritic rats.[89] The appearance of Gp A 72 in the serum preceded the onset of the paw inflammation in the arthritic rats and persisted in the chronic phase. They found that oral administration of antiinflammatory spices like capsaicin and curcumin lowered the levels of Gp A72 by 88% and 73%, respectively, with concomitant lowering of paw volume in the arthritic rats.

Zsila *et al* demonstrated binding of curcumin molecule to human alpha1-acid glycoprotein (AGP), an acute phase protein in blood.[90] Oppositely signed induced circular dichroism (CD) bands measured in the visible spectral region in pH 7.4 phosphate buffer indicated that the protein bounded curcumin molecule in a left-handed chiral conformation. Curcumin-induced changes in the tertiary structure of AGP, which lead to the decreased binding affinity.

Conclusion

A large number of studies have revealed that curcumin has wide therapeutic actions such as antiinflammatory, antispasmodic, antimicrobial, anticancer, hepatoprotection and neuroprotection etc. Its antiinflammatory activity is mainly due to inhibition of AA metabolism, COX, LOX, cytokines (ILs and TNF) and NF-κB. Curcumin is reported to stabilize lysosomal membrane and causes uncoupling of oxidative phosphorylation besides having strong oxygen radical scavenging activity. The most interesting feature of curcumin is lack of gastrointestinal side effects despite being an antiinflammatory agent. Thus curcumin may prove as a useful drug for treatment of diseases such as arthritis, cancer, HIV etc. More research work is needed in order to explore its new areas of therapeutic applications.

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