

Curcumin suppresses p38 mitogen-activated protein kinase activation, reduces IL-1 β and matrix metalloproteinase-3 and enhances IL-10 in the mucosa of children and adults with inflammatory bowel disease

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Inflammatory bowel disease (IBD) is a major source of morbidity in children and adults. Its incidence is rising, particularly in young people. IBD carries a lifelong risk of cancer, which is proportional to disease duration. Drug and surgical treatments rarely offer cure and often carry a high side effect burden. Dietary therapy is highly effective in Crohn's disease. For these reasons, there is much interest in developing novel dietary treatments in IBD. Curcumin, a component of the spice turmeric, and an anti-inflammatory and anti-cancer agent, shows preclinical and clinical potential in IBD. Its mechanisms of action are unknown. Our aim was to assess the effect of curcumin on key disease mediators p38 mitogen-activated protein kinase (MAPK), IL-1 β , IL-10 and matrix metalloproteinase-3 (MMP-3) in the gut of children and adults with IBD. Colonic mucosal biopsies and colonic myofibroblasts (CMF) from children and adults with active IBD were cultured *ex vivo* with curcumin. p38 MAPK, NF- κ B and MMP-3 were measured by immunoblotting. IL-1 β and IL-10 were measured by ELISA. We show reduced p38 MAPK activation in curcumin-treated mucosal biopsies, enhanced IL-10 and reduced IL-1 β . We demonstrate dose-dependent suppression of MMP-3 in CMF with curcumin. We conclude that curcumin, a naturally occurring food substance with no known human toxicity, holds promise as a novel therapy in IBD.

Curcumin: Inflammatory bowel disease: IL-10: Matrix metalloproteinase-3: p38 Mitogen-activated protein kinase

Inflammatory bowel disease (IBD) is a source of considerable morbidity in children and adults, and nutritional therapy holds attractive possibilities. The two main forms are Crohn's disease (CD) and ulcerative colitis (UC). It is characterised by bloody diarrhoea, abdominal pain and poor growth, and follows a lifelong relapsing and remitting course. IBD carries a long term risk of colorectal cancer⁽¹⁾ proportional to extent of colonic involvement and disease duration⁽²⁾. Thus, the probability of developing cancer is cumulative over decades, a fact of clear relevance to people diagnosed in childhood. The cause of IBD is unknown, but involves interplay between genetic predisposition, defective mucosal immune regulation and environmental (including nutritional) factors. The incidence of IBD in children in the UK is 5.2 per 100 000 per year⁽³⁾. In adults in northern Europe, it is 10–20 per 100 000 per year⁽⁴⁾. The incidence of IBD is rising, and new cases presenting in teenage years account for a significant portion of this rise⁽⁴⁾. IBD is less common in developing countries than in the industrialised world⁽⁵⁾, and individuals emigrating from East to West take on the western risk of IBD^(5,6). This holds further relevance to the importance of nutrition in IBD.

Current treatments for IBD are dietary, drug or surgical. CD responds well to polymeric or elemental feed, which brings about remission in 80 % of patients^(7,8). This option is

particularly valuable in children and adolescents, in whom avoiding steroids, which have negative effects on growth and bone development, is especially important. Its mechanism of action remains obscure, although theories include reduction of dietary antigen load, enhancement of immunosuppressive mechanisms or alteration in gut bacterial flora^(9,10). Treatments are generally not curative and many carry a high side effect burden. For these reasons, and because of the clear relationship between nutrition and CD, keen interest continues in new dietary treatments for IBD.

Curcumin, a major constituent of the kitchen spice turmeric, has long been used in Ayurvedic and other traditional medicines. Curcumin has antioxidant, anti-inflammatory and anti-cancer properties. The mechanisms by which curcumin mediates these effects include suppression of NF- κ B^(11,12), signal transducer and activator of transcription-3⁽¹³⁾, cyclooxygenase-2⁽¹⁴⁾, TNF- α , IL-1 and IL-6⁽¹⁵⁾, activation of PPAR- γ ⁽¹⁶⁾ and alteration of p38 mitogen-activated protein kinase (MAPK) signalling^(17,18). Curcumin is also an inhibitor of acetylation, acting on p300 acetyl transferase^(19,20). Many proteins are subjected to acetylation, initiating events which regulate for example transforming growth factor- β signalling⁽²¹⁾ and insulin-like growth factor binding protein-3 expression^(22,23). Curcumin is non-toxic to human subjects

Abbreviations: CD, Crohn's disease; CMF, colonic myofibroblasts; IBD, inflammatory bowel disease; MAPK, mitogen-activated protein kinase; MMP-3, matrix metalloproteinase-3; TSA, trichostatin A; UC, ulcerative colitis.

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even at doses many fold higher than those found in the diet, and it is showing early promise as a treatment for CD and UC^(24,25). Its mechanism of action in inflamed human gut mucosa is not known. The present work examines the *in vitro* effects of curcumin on the key inflammatory signalling pathway p38 MAPK, as well as on major pro- and anti-inflammatory gut cytokines in mucosal biopsies from children with active IBD. We also explore the response to curcumin of colonic myofibroblasts (CMF) from patients with active IBD, in terms of matrix metalloproteinase-3 (MMP-3) expression, p38 MAPK activation and NF- κ B signalling. To our knowledge, this is the first work to examine the effect of curcumin in human *ex vivo* intestinal cells and tissues.

Materials and methods

Intestinal mucosal biopsies

Colonic mucosal biopsies were obtained with consent from children and adolescents with CD or UC undergoing ileo-colonoscopy at the Royal London Hospital. Ethics approval for the study was granted from East London and The City Health Authority Research Ethics Committee. Biopsies were taken from areas showing clear macroscopic disease changes and histopathological diagnosis and active inflammation subsequently confirmed. Since the purpose of the study was to examine the potential of a novel therapeutic agent in IBD, we chose only to study its effects in subjects with disease. Biopsies were collected into ice-cold Roswell Park Memorial Institute medium 1640 + Glutamax (Invitrogen, Paisley, UK) supplemented with fetal bovine serum, penicillin/streptomycin and gentamicin (all Sigma, Gillingham, UK). Biopsies were immediately placed in overnight culture in HL-1 medium (Invitrogen), penicillin/streptomycin and gentamicin, with graded doses of curcumin (Sigma). In other experiments, biopsies were similarly cultured with the p38 inhibitor SB203580 (Glaxo Smith Kline, Brentford, UK). Dimethyl sulphoxide (Sigma) was used as a vehicle control.

Colonic myofibroblasts

Intestinal resection specimens were obtained with consent from children, adolescents and adults undergoing surgery for active CD or UC at the Royal London Hospital or the Home-ron Hospital. Tissue was collected onto ice-cold complete Roswell Park Memorial Institute medium as above. The mucosal layer was removed, washed in Hanks' balanced salt solution and incubated in EDTA, followed by collagenase (all Sigma). The resultant suspension was then passed through a cell strainer, washed in complete Roswell Park Memorial Institute and centrifuged. The pellet was resuspended in complete Roswell Park Memorial Institute and further purified by Ficoll density gradient separation. Finally, the cells were washed once again, centrifuged and resuspended in Dulbecco's modified Eagle's medium supplemented with non-essential amino acids (both Invitrogen), fetal bovine serum, penicillin/streptomycin and gentamicin, and placed in incubation. CMF adhering to the flask were grown in successive passages until sufficient numbers resulted. For experiments CMF were incubated over 30 min (for p38 MAPK and

NF- κ B estimation) or 24 h (for MMP-3 estimation) in the presence of curcumin, anacardic acid (Merck Biosciences, Nottingham, UK) or trichostatin A (TSA; Sigma), alongside dimethyl sulphoxide vehicle control.

ELISA for cytokines

Supernatants from biopsy cultures were subjected to ELISA for IL-1 β (R&D Systems, Abingdon, UK) and IL-10 (Immunotools, Friesoythe, Germany). Each sample was tested in duplicate against the appropriate standard and optical densities measured by microplate reader (BioRad, Hemel Hempstead, UK). Results were analysed and presented using Microsoft Excel and Prism software.

Western blot for p38 mitogen-activated protein kinase, NF- κ B and matrix metalloproteinase-3

Biopsies were snap-frozen and solubilised in ice-cold radioimmunoprecipitation assay lysis buffer containing protease and phosphatase inhibitors. Where separate nuclear and cytosolic extracts were required, cells were fractionated using a commercial fractionation kit (Biovision, Mountain View, CA, USA). Protein estimation was performed using bicinchoninic acid/copper sulphate assay against bovine serum albumin standard (all Sigma). Protein samples were resolved on 10% SDS-PAGE, transferred onto nitrocellulose membrane and probed overnight with primary antibody against phosphorylated p38 MAPK (R&D Systems), non-phosphorylated p38 MAPK (Cell Signalling Technology, Danvers, MA, USA), NF- κ B p65 subunit (Santa Cruz Biotechnology, Santa Cruz, CA, USA), I κ B (Santa Cruz Biotechnology), β -actin (Abcam, Cambridge, UK) or histone H1 (AbD Serotec, Kidlington, UK). CMF supernatants were resolved and transferred in the same way and membranes were probed for MMP-3 (The Binding Site, Birmingham, UK). Membranes were then reprobed with horseradish peroxidase-conjugated secondary antibodies (Dako, Ely, UK) and chemiluminescent substrate applied for photographic visualisation. Membranes were stripped and reprobed as appropriate.

Immunofluorescent staining for NF- κ B

At the end of the experiments as described above, CMF were fixed in 4% paraformaldehyde, permeabilised with 0.1% Triton, washed then blocked in 10% donkey serum (Sigma). The cells were next incubated with a rabbit polyclonal antibody against NF- κ B p65 subunit (Santa Cruz), washed again and then incubated with a secondary donkey anti-rabbit antibody conjugated to Alexa488 (Invitrogen). Nuclei were counterstained with 4',6-diamidino-2-phenylindole (Molecular Probes, Invitrogen), and slides were mounted with ProLong antifade reagent (Invitrogen) and observed under a Leica DM5000 epifluorescence microscope with an attached digital camera using $\times 63$ magnification.

Statistics

All biopsy data were expressed as a pair of results for each patient; untreated (vehicle control) and treated (curcumin). Gaussian distribution could not be assumed, therefore data

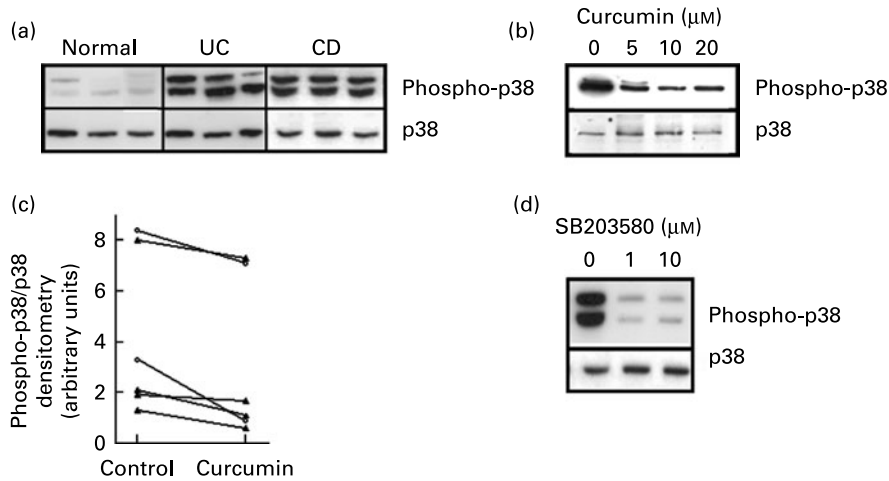


Fig. 1. Curcumin decreases p38 mitogen-activated protein kinase (MAPK) phosphorylation in *ex vivo* intestinal mucosal biopsies from children with active inflammatory bowel disease. (a) Phospho-p38 and p38 loading control in normal (*n* 3), ulcerative colitis (UC) (*n* 3) and Crohn's disease (CD) biopsies (*n* 3). (b) Western blot from a single representative experiment in biopsies from one individual with CD cultured with curcumin, v. vehicle control (dimethyl sulphoxide). (c) Data from all curcumin experiments (median control, 2.7; median curcumin, 1.4; *P*=0.031; *n* 6 (4 CD + 2 UC)). (d) UC biopsy cultured with p38 MAPK inhibitor SB203580. Where two bands are seen, these correspond to isoforms of phospho-p38; either or both may be found upregulated in disease. ▲, CD; ○, UC.

were analysed as non-parametric paired differences using the Wilcoxon signed rank (matched pairs) test, 95 % CI applied and two-tailed *P* values were calculated.

Results

Curcumin decreases p38 mitogen-activated protein kinase phosphorylation in ex vivo intestinal mucosal biopsies from children and adolescents with active inflammatory bowel disease

p38 MAPK activation (phosphorylation) is characteristically greatly increased in mucosal biopsies from patients with active CD and UC, compared to biopsies from normal mucosa (Fig. 1(a)). Because of the low p38 MAPK activity in non-inflamed tissues, we studied the effects of curcumin on inflamed tissues, using a biopsy cultured in vehicle (dimethyl sulphoxide) as a negative control for each patient. A 24-h treatment with curcumin caused a median 48 % reduction in phosphorylated p38 MAPK in *ex vivo* mucosal tissue culture from patients with active IBD (*P*=0.031; Fig. 1(b) and (c)), in comparison to the near total inhibition observed when biopsies were cultured with the specific p38 MAPK inhibitor SB203580 in the same experimental system (Fig. 1(d)). The numeric variability in p38 MAPK between individuals seen in Fig. 1(c) is explained by the necessary use of arbitrary units to express this densitometry data.

There is no evidence of reduced NF-κB activation with curcumin in ex vivo intestinal mucosal biopsies from children and adolescents with active inflammatory bowel disease

As curcumin has been shown in some cell types to suppress the NF-κB signalling pathway^(11,12), we next proceeded to examine whether NF-κB suppression also played a role in the anti-inflammatory effects of curcumin in our experimental gut mucosal system. We found no evidence to suggest a change in nuclear p65 or cytosolic inhibitor of κB in *ex vivo* tissue cultures from paediatric patients with active IBD (Fig. 2).

Curcumin suppresses IL-1β and enhances IL-10 expression in ex vivo intestinal mucosal biopsies from children and adolescents with active inflammatory bowel disease

Curcumin caused a modest but consistent reduction in IL-1β production in *ex vivo* cultured mucosal biopsies from children with active IBD (*P*=0.0098; Fig. 3(a)). We then moved on to assess the effect of curcumin on the important anti-inflammatory cytokine IL-10. Overall, curcumin caused a large median rise of 237 % in IL-10 expression in *ex vivo* tissue cultures from paediatric patients with active IBD (*P*=0.002; Fig. 3(b)). Thus, curcumin favourably modulated the intestinal mucosal cytokine profile, markedly enhancing anti-inflammatory IL-10 and suppressing the key pro-inflammatory cytokine IL-1β.

Curcumin decreases matrix metalloproteinase-3 production in ex vivo colonic myofibroblasts from patients with active inflammatory bowel disease

While an intestinal biopsy is a good model of disease, it consists of many different cell types. We wished to study the effect of curcumin on a single component, while still retaining the active IBD phenotype and not reverting to

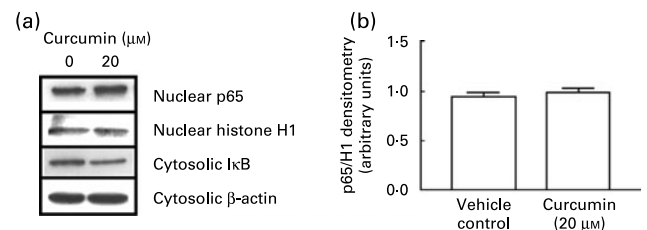


Fig. 2. There is no evidence of reduced NF-κB activation with curcumin in *ex vivo* intestinal mucosal biopsies from children with active inflammatory bowel disease. (a) Western blot from a single representative experiment showing nuclear p65 subunit and histone H1 loading control with corresponding cytosolic inhibitor of κB (IκB) and β-actin loading control in a Crohn's disease (CD) biopsy. (b) Data from all nuclear p65 experiments (*n* 4 (3 CD + 1 UC)). Error bars represent standard errors of the mean.

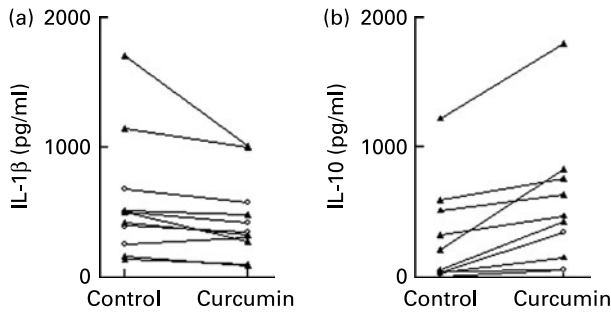


Fig. 3. Curcumin suppresses pro-inflammatory and enhances anti-inflammatory cytokine expression in *ex vivo* intestinal mucosal biopsies from children with active inflammatory bowel disease. (a) IL-1 β (median control, 463 pg/ml; median curcumin, 384 pg/ml; $P=0.0098$; n 10 (6 Crohn's disease (CD) + 4 ulcerative colitis (UC))). (b) IL-10 (median control, 135 pg/ml; median curcumin, 455 pg/ml; $P=0.002$; n 10 (7 CD + 3 UC)). \blacktriangle , CD; \circ , UC.

more distant cell lines. We therefore next chose to examine CMF, key stromal effector cells in IBD, and amenable to *in vitro* culture directly from a patient with active disease. Myofibroblasts do not express IL-1 β or IL-10, but play an active role in IBD, expressing MMP-3. Curcumin suppressed MMP-3 production in TNF- α -stimulated CMF from patients with active IBD and the response was dose-dependent (Fig. 4).

p38 mitogen-activated protein kinase is unaffected by curcumin in ex vivo colonic myofibroblasts from patients with active inflammatory bowel disease

To seek a mechanistic explanation for the MMP-3 suppression observed with curcumin (Fig. 4), we examined early (30 min)

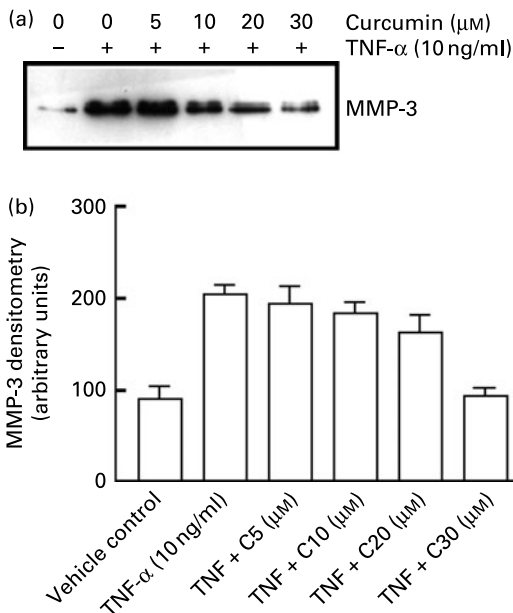


Fig. 4. Curcumin decreases matrix metalloproteinase-3 (MMP-3) production in *ex vivo* colonic myofibroblasts (CMF) from patients with active inflammatory bowel disease. (a) Western blot from a single representative experiment in Crohn's disease (CD) CMF. (b) Data from all experiments (n 7 (5 CD + 2 ulcerative colitis)). Error bars represent standard errors of the mean. Because MMP-3 is measured in CMF supernatants, equal loading is controlled through the seeding of equal cell numbers in each culture well (3×10^5) and equal volume supernatant (15 μ l) in each electrophoresis well.

and late (24 h) p38 MAPK activation in the CMF. No early or late changes in p38 MAPK activation with curcumin were observed in TNF- α -stimulated *ex vivo* CMF from patients with active IBD (Fig. 5). This suggests that, in contrast to the mucosal cytokine system, curcumin exerts its effect on stromal cells via a p38 MAPK-independent process.

NF- κ B signalling is not significantly inhibited by curcumin in ex vivo colonic myofibroblasts from patients with active inflammatory bowel disease

In light of previous reports on the mechanism of action of curcumin in other cell types^(11,12), we next proceeded to investigate whether the response of CMF to curcumin was NF- κ B-dependent. To this end we employed two separate methods: immunofluorescent staining; Western blotting. By immunostaining, we confirm successful *ex vivo* activation of CMF using TNF- α with translocation of NF- κ B p65 from the cytoplasm into the nuclei. Similarly to our earlier data on NF- κ B in biopsies (Fig. 2), we found no clear difference in NF- κ B nuclear translocation with curcumin in CMF (Fig. 6(a)). By Western blot, we show only a small decrease in nuclear p65 with curcumin (Fig. 6(b) and (c)). We conclude that while NF- κ B signalling may be marginally inhibited by curcumin, this is not the primary mechanism through which it inhibits MMP-3 expression in CMF.

The acetylation inhibitor anacardic acid suppresses matrix metalloproteinase-3 production by colonic myofibroblasts in a dose-dependent fashion, which mirrors that seen with curcumin

In further pursuit of a mechanistic explanation for the MMP-3 suppression observed with curcumin (Fig. 4), we next considered curcumin's known potency as an inhibitor of acetylation. We treated TNF- α -stimulated CMF with a different inhibitor of acetylation, anacardic acid. Anacardic acid is, like curcumin, a naturally occurring plant-based substance, in this case found in cashew nut shell liquid. Like curcumin, it is a non-competitive inhibitor of p300 acetyl transferase, a ubiquitous catalyst of acetylation⁽²⁶⁾. Anacardic acid suppressed MMP-3 production in TNF- α -stimulated CMF from patients with active IBD, in a dose-dependent manner (Fig. 7(a) and (b)), which closely mirrored that seen with curcumin. This suggests that in CMF both compounds are acting via a mechanism dependent on their ability to inhibit acetylation.

The pro-acetylating agent trichostatin A enhances matrix metalloproteinase-3 production by colonic myofibroblasts, and this is abrogated by both curcumin and anacardic acid

To examine this further, we used the well-established inhibitor of histone deacetylase TSA, which is therefore a pro-acetylating agent⁽²⁷⁾. In agreement with our earlier data (Fig. 4), we confirmed the upregulation of MMP-3 with TNF- α , and the suppression of this effect by curcumin; we also demonstrated that TSA upregulated MMP-3. This upregulation was almost totally abrogated by both curcumin and anacardic acid (Fig. 7(c)).

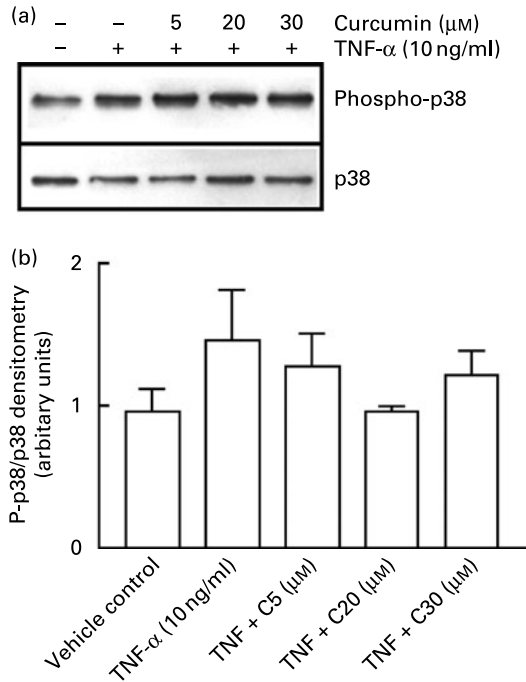


Fig. 5. p38 mitogen-activated protein kinase is unaffected by curcumin in *ex vivo* colonic myofibroblasts (CMF) from patients with active inflammatory bowel disease. (a) Western blot from a single representative experiment in ulcerative colitis (UC) CMF. (b) Data from all experiments (n 5 (4 Crohn's disease + 1 UC)). Error bars represent standard errors of the mean.

Discussion

Dietary factors that regulate cell signal transduction processes may have important therapeutic implications. The MAP kinases, when activated by external signals, initiate phosphorylation

cascades culminating in events such as transcription, differentiation and apoptosis. They are central to the coordination of inflammatory responses and highly conserved, suggesting critical functions for survival. They are classified into three families: extracellular signal-related kinases; c-Jun N-terminal kinases; p38 MAPK. p38 MAPK regulates production of MMP⁽²⁸⁾, inflammatory enzymes such as cyclooxygenase-2⁽²⁹⁾, and key inflammatory cytokines including TNF-α, IL-1, IL-8 and interferon-γ⁽³⁰⁾. The MAPK and molecules in their signalling pathways therefore present interesting therapeutic targets in inflammatory disease. p38 MAPK is the most markedly elevated MAPK in IBD⁽³¹⁾, implying an important role in pathogenesis. The p38 MAPK inhibitor SB203580 blocks the enzyme by competing for ATP in its active pocket⁽³²⁾. The inhibition of p38 MAPK by curcumin in mucosal biopsies may involve upstream elements in the pathway. p38 MAPK inhibition is a likely mechanism by which curcumin suppresses downstream pro-inflammatory cytokines such as IL-1β.

IL-1β is a central effector of the inflammatory response. It is produced by immune cells in response to stimuli including p38 MAPK activation⁽³⁰⁾ and mediates wide ranging inflammatory consequences. It is raised in the serum and tissues of patients with IBD compared to controls⁽³³⁾. For this reason, and because we are testing a potential therapeutic agent, in the present work we studied the effect of curcumin on diseased samples, employing an internal negative control for each experiment. Curcumin is known to suppress IL-1β in various cell types^(15,34,35), including in the intestinal mucosa in mouse models of colitis⁽³⁶⁾. This is the first work to our knowledge demonstrating suppression of IL-1β by curcumin in human intestinal tissue. Proposed mechanisms include inhibition of MAPK⁽³⁷⁾ and prevention of recruitment of IL-1 receptor-associated kinase to the IL-1 receptor I⁽³⁸⁾. It seems that curcumin has a complex mode of action involving

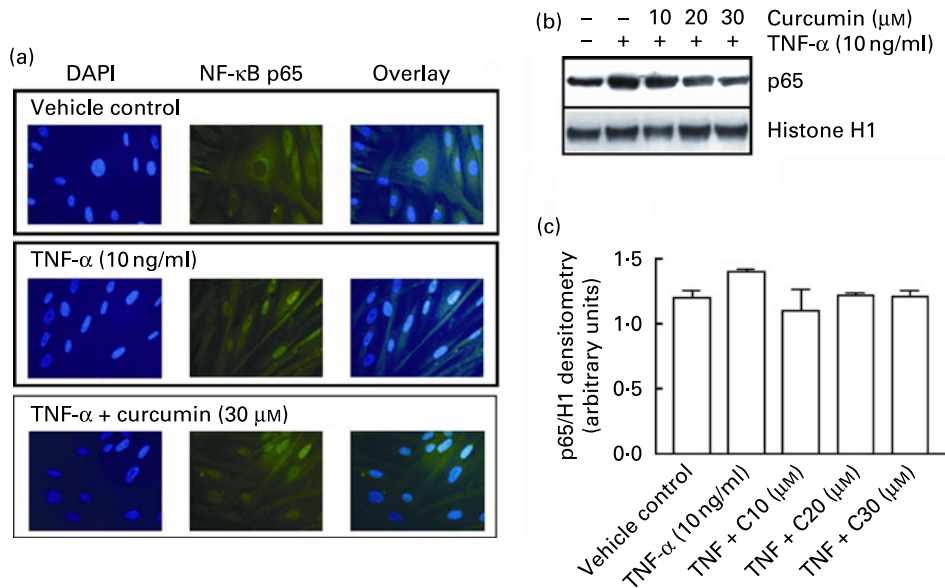


Fig. 6. NF-κB signalling is not significantly affected by curcumin in *ex vivo* colonic myofibroblasts (CMF) from patients with active inflammatory bowel disease. (a) Immunofluorescent staining of Crohn's disease (CD) CMF for 4',6-diamidino-2-phenylindole (DAPI) (nuclei) and NF-κB p65 subunit; single representative experiment shown. (b) Western blot from a single representative experiment showing nuclear p65 and histone H1 loading control. (c) Western blot data from all experiments (n 3 (2 CD + 1 UC)). Error bars represent standard errors of the mean.

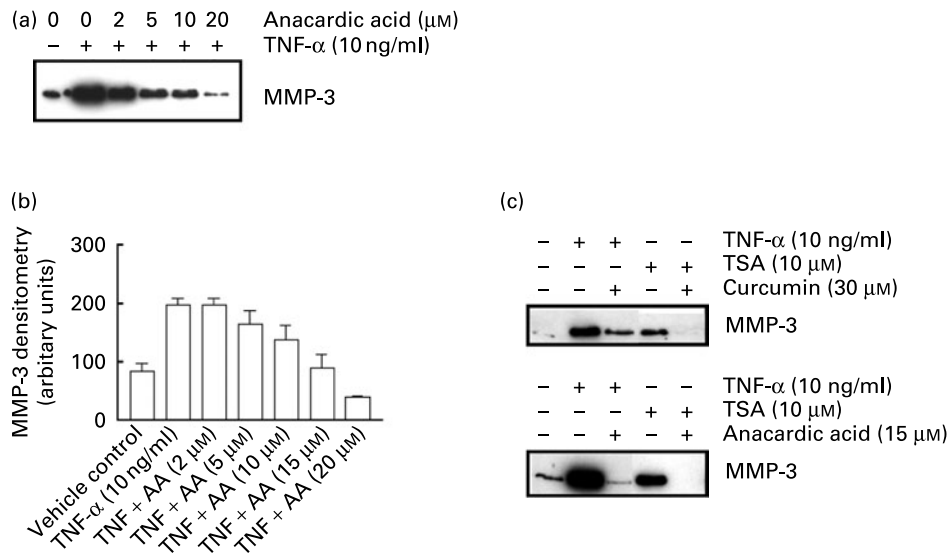


Fig. 7. Anacardic acid (AA) suppresses matrix metalloproteinase-3 (MMP-3) production in *ex vivo* colonic myofibroblasts (CMF) from patients with active inflammatory bowel disease. Trichostatin A (TSA) enhances MMP-3 production and this is abrogated by both curcumin and AA. (a) Western blot from a single representative experiment with AA in Crohn's disease (CD) CMF. (b) AA data from all experiments (n 7 (5 CD + 2 UC)). Error bars represent standard errors of the mean. (c) Responses of CD CMF to TNF- α , TSA, curcumin and AA.

multiple targets. We conclude that the suppression of IL-1 β by curcumin in the gut is at least in part p38 MAPK-dependent and holds biological and future clinical importance in the treatment of IBD.

IL-10 is the major anti-inflammatory cytokine released by T and B cells⁽³⁹⁾. It is synthesised late after a stimulus compared to other cytokines⁽⁴⁰⁾ and inhibits production of pro-inflammatory cytokines⁽⁴¹⁾. It downregulates MHC II molecules, inhibiting antigen presentation⁽⁴²⁾ and induces production of cytokine inhibitors such as IL-1 receptor antagonist^(43,44). It inhibits development of T-cell clones⁽⁴⁵⁾ and has a role in generating regulatory T cells^(46–48). It inhibits MMP activity, limiting tissue damage⁽⁴⁹⁾. The IL-10 knock-out mouse is one of the few animal models with inflammation affecting the small intestine as well as the colon^(50,51). This is entirely dependent on exposure to bacteria. Thus, IL-10 is important in maintaining tolerance to intestinal flora. There is some existing work showing that curcumin increases IL-10 production, including in human T cells⁽⁵²⁾ and in the colonic mucosa in animal studies of experimentally induced colitis⁽³⁶⁾. This is the first study of curcumin and IL-10 in human intestinal mucosa. We show a significant increase in IL-10 expression with curcumin. Since IL-10 expression is normally a (late) consequence of p38 MAPK activation^(53–55), and we have shown that curcumin inhibits p38 MAPK, the mechanistic explanation for the increase in IL-10 is not through p38 MAPK. Curcumin's potency as an inhibitor of acetylation provides an alternative explanation. The IL-10 gene shares with insulin-like growth factor binding protein-3 a binding sequence in its promoter for the transcription factor specificity protein 3⁽⁵⁶⁾. On binding to this promoter, specificity protein 3 downregulates the expression of insulin-like growth factor binding protein-3⁽²²⁾, and acetylation of specificity protein 3 potentiates this effect⁽²³⁾. Curcumin may decrease binding of specificity protein 3 to this promoter thus restoring IL-10 expression. This hypothesis is currently under further study.

CMF are stromal cells which in health produce low levels of MMP that remains in latent form and effects physiological cell turnover. CMF, although responsive to cytokines⁽⁵⁷⁾, do not themselves produce IL-1 β or IL-10. Instead we examined MMP-3 (stromelysin-1) as a measure of CMF activation. In IBD, CMF overexpress MMP, which become activated in cascades causing unchecked tissue destruction, fibrosis and further increasing immune cell activation and homing to the gut⁽⁵⁸⁾. Our group has previously shown that MMP-3 recruits neutrophils into inflamed gut by proteolytically cleaving platelet basic protein to produce CXCL7, a potent neutrophil chemokine, and that myofibroblasts are required to maximise this epithelial chemokine signalling process⁽⁵⁹⁾. By inhibiting p38 MAPK and cytokines in the lamina propria, we would expect curcumin to have the added effect of reducing platelet basic protein production by the epithelium. Curcumin downregulates MMP production in various cell types^(60,61). This work shows for the first time this effect in human intestinal stromal cells, where we demonstrate dose-dependent suppression of MMP-3 with curcumin. As well as limiting tissue destruction this could reduce influx of activated leukocytes into inflamed gut. In further support of this, curcumin has recently been shown to suppress TNF- α and lipopolysaccharide-induced vascular cell adhesion molecule-1 expression in human intestinal microvascular endothelial cells, and to attenuate leukocyte adhesion to stimulated-human intestinal microvascular endothelial cells⁽⁶²⁾.

Unlike in our mucosal tissue system, in CMF curcumin did not affect p38 MAPK activation. Therefore, its suppression of MMP-3 occurs through a p38 MAPK-independent mechanism. To explain this discrepancy between biopsies and CMF, we postulate that the inhibition of p38 MAPK signalling by curcumin in IBD occurs largely in immune cells such as lymphocytes, macrophages, monocytes and dendritic cells, rather than in fibroblasts. These cell types (which cannot without transformation be grown in successive passages *in vitro*) are richly found in intestinal mucosal biopsies.

Indeed, the contradictory effects of curcumin, on MAPK and other targets, epitomise the complexity and paradoxical nature of the compound and are well documented in the literature. Under some circumstances curcumin suppresses MAPK signalling, as in a recent study, where it inhibits p38 MAPK activation⁽⁶³⁾ in human intestinal microvascular endothelial cells; similarly curcumin inhibits c-Jun N-terminal kinases in Jurkat T cells (a human T-cell line)⁽⁶⁴⁾. Paradoxically other investigators show activation of MAPK by curcumin, for example of c-Jun N-terminal kinases in human colon cancer HCT116 cells⁽⁶⁵⁾ and of p38 MAPK in primary human neutrophils⁽⁶⁶⁾. While the effect of curcumin on MAPK signalling varies with environment, the ultimate biological consequences are pro-apoptotic, anti-inflammatory and anti-angiogenic.

In light of reports that curcumin inhibits NF- κ B signalling in human cell lines (myeloid leukaemia and embryonic kidney)^(11,12), we examined the NF- κ B pathway. Curcumin did not significantly affect NF- κ B either in biopsies or CMF from patients with active IBD; therefore, in the gut mucosa the actions of curcumin do not appear to be NF- κ B-dependent.

Lastly we considered that curcumin's effect on MMP-3 was due to its properties as an acetylation inhibitor. There is evidence that MMP production is p300 acetyl transferase-dependent. This is shown (for MMP-9) in rat astrocytes⁽⁶⁷⁾ and mouse macrophages⁽⁶⁸⁾. The substrate for acetylation remains obscure but in this latter model the authors show evidence of histone acetylation. Further evidence for MMP-9 production requiring histone acetylation comes from a human tracheal smooth muscle cell model⁽⁶⁹⁾, a process which interestingly in this work is blocked by curcumin. Furthermore, our group has previously shown upregulation of MMP-3 in human fetal intestinal mesenchymal cells by butyrate, a product of colonic bacterial fermentation and a pro-acetylating agent⁽⁷⁰⁾. To examine this, we first tested another inhibitor of acetylation anacardic acid in the same system. Anacardic acid has the same mode of action as curcumin, in that both compounds are reversible non-competitive inhibitors of p300 acetyl transferase, acting at a site remote from the active site of the enzyme⁽²⁶⁾. This is the only known biological property of anacardic acid and the only shared property of the two compounds. The finding of dose-dependent MMP-3 suppression with anacardic acid, which parallels that seen with curcumin, supports the hypothesis that the effect is acetylation-dependent. Finally, we show upregulation of MMP-3 by pro-acetylating agent TSA, which is abrogated by both curcumin and anacardic acid. Taken together, these findings strongly suggest that MMP-3 production in CMF occurs by an acetylation-dependent mechanism, and that its suppression by curcumin is due to curcumin's known potency as an inhibitor of p300 acetyl transferase^(19,20).

The safety, tolerability and non-toxicity of curcumin at doses many fold higher than dietary are well established, and it is classified 'generally recognized as safe' by the United States Food and Drug Administration. Oral doses up to 12 g/d are well tolerated in human subjects⁽⁷¹⁾. There is also good evidence at a population level of the safety of life-long curcumin ingestion up to about 100 mg/d from India, where there is a very high natural dietary curcumin content⁽⁷²⁾. The curcumin concentrations used in this and other work correspond to much higher doses than those found even in

Asian diets. Therefore, while the potential benefit, and safety, of curcumin at therapeutic dose is clear, whether the findings presented here hold dietary or population relevance, is uncertain. It is at least intriguing to note that the incidence of IBD in Asia is lower than in the western world⁽⁵⁾. Furthermore, concurrent with the trend towards 'Westernisation' of traditional Asian diets, its incidence in Asia is rising over recent decades^(73,74).

Conclusions

Curcumin holds promise as a novel therapy for children and adults with IBD. Curcumin is a complex compound whose precise modes of action remain obscure, and it seems likely that its molecular targets differ according to cell and disease system. In the present work, we show evidence that its effects are at least partially dependent on its power to inhibit p38 MAPK and protein acetylation (p300 acetyl transferase) in the intestinal mucosa.

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