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Review
Biological actions of curcumin on articular chondrocytes

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Summary

Objectives: Curcumin (diferuloylmethane) is the principal biochemical component of the spice turmeric and has been shown to possess potent anti-catabolic, anti-inflammatory and antioxidant, properties. This article aims to provide a summary of the actions of curcumin on articular chondrocytes from the available literature with the use of a text-mining tool. We highlight both the potential benefits and drawbacks of using this chemopreventive agent for treating osteoarthritis (OA). We also explore the recent literature on the molecular mechanisms of curcumin mediated alterations in gene expression mediated *via* activator protein 1 (AP-1)/nuclear factor-kappa B (NF-κB) signalling in chondrocytes, osteoblasts and synovial fibroblasts.

Methods: A computer-aided search of the PubMed/Medline database aided by a text-mining tool to interrogate the ResNet Mammalian database 6.0.

Results: Recent work has shown that curcumin protects human chondrocytes from the catabolic actions of interleukin-1 beta (IL-1β) including matrix metalloproteinase (MMP)-3 up-regulation, inhibition of collagen type II and down-regulation of β1-integrin expression. Curcumin blocks IL-1β-induced proteoglycan degradation, AP-1/NF-κB signalling, chondrocyte apoptosis and activation of caspase-3.

Conclusions: The available data from published *in vitro* and *in vivo* studies suggest that curcumin may be a beneficial complementary treatment for OA in humans and companion animals. Nevertheless, before initiating extensive clinical trials, more basic research is required to improve its solubility, absorption and bioavailability and gain additional information about its safety and efficacy in different species. Once these obstacles have been overcome, curcumin and structurally related biochemicals may become safer and more suitable nutraceutical alternatives to the non-steroidal anti-inflammatory drugs that are currently used for the treatment of OA.

Key words: Articular cartilage, Osteoarthritis (OA), Curcumin, Nuclear factor kappa B (NF-κB) cell signalling, Pro-inflammatory cytokine, Inflammation, Inflammatory mediator, Cytotoxicity, Apoptosis.

Introduction

It is now generally accepted that osteoarthritis (OA) is not only the final common pathway for aging and injuries of the joint, but it is also an active joint disease with a prominent inflammatory component. As medical advances lengthen average life expectancy, OA will become a larger public health problem – not only because it is a manifestation of aging but because it usually takes many years to reach clinical relevance. OA is already one of the ten most disabling diseases in industrialized countries. OA is rare in people under 40 but becomes more common with age – most people over 65 years of age show some radiographic evidence of OA in at least one or more joints. OA is the most frequent cause of physical disability among older adults globally. More than 8 million people in the UK and over 20 million Americans are estimated to have OA.[b] It is also anticipated that by the year 2030, 20% of adults will have developed OA in Western Europe and North America. OA is not only a common problem among the elderly population, but also it is becoming more widespread among younger people. In the United States, rheumatoid arthritis (RA) and OA combined affect as many as 46 million people. This amounted to a healthcare cost of over $128 billion in 2003.[c] This huge financial burden emphasizes the acute need for new and more effective treatments for articular

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[b]http://www.niams.nih.gov/.
[c]http://www.arthritis.org/.

cartilage defects especially since there are few disease modifying drugs or treatments for OA. Existing pharmaceuticals include analgesics, steroids and non-steroidal anti-inflammatory drugs (NSAIDs) which only treat the symptoms of OA by reducing pain and inflammation. Therefore, OA represents a major opportunity for research innovation in the development and testing of new nutraceutical therapies.

In this concise review article we explore the anti-inflammatory properties of the turmeric derived polyphenol curcumin and its nutritional biochemistry in the context of its potential for treating OA patients. Curcumin, also known as diferuloylmethane (Fig. 1), is the best-characterized chemopreventive agent extracted from the roots of *Curcuma longa* (Turmeric). Curcumin possesses potent antioxidative, anti-inflammatory, anti-septic and anti-cancer properties. Curcumin has been described as an anti-inflammatory agent[1]. It has long been used as an anti-inflammatory treatment in traditional Chinese and Ayurvedic medicine[2]. Since OA and related osteoarticular conditions of synovial joints are characterized by inflammation, a better understanding of the biochemistry of curcumin and its biological actions in joint tissues may facilitate the development of clinically safe, orally administered therapeutic agents for treating joint diseases.

Methods

The review results of a computer-aided search of the PubMed/Medline for the period 1977 through September 2009 [Keywords: turmeric, curcumin, cartilage, bone, synovial membrane, inflammation, OA, arthritis]. Four reviewers (YH, AM, AC and MM) selected the *in vitro* and *in vivo* studies included in this review. To aid the traditional literature search for this review, a text-mining tool was employed to establish relationships between important protein molecules, biological processes and pathways in chondrocytes. The text-mining was performed by DA and EML. The ResNet Mammalian database 6.0 within Pathway Studio™ (Ariadne Genomics, Rockville, US) consists of entities (for example, a protein, a cellular process or a disease process) and predefined functional relations that have been reported between these entities (for example, regulation, binding, expression). These relations are derived by automated text-mining of scientific literature from PubMed abstracts and 47 full text journals using human, rat and mouse-based data[3]. The output can be displayed and explored through a visual and tabular interface. To identify proteins, small molecules, cell processes and diseases associated with curcumin relevant to this review, the ResNet database (updated October 2008) was searched using the term ''curcumin''. The introduction and discussion sections were collectively written by all the authors.

All the experimental papers carried out by the authors and reviewed herein were performed with commercially available research grade curcuminoids containing at least 80% of curcumin.

Results

CONVENTIONAL LITERATURE REVIEW

Turmeric: a major component of Indian, Chinese, Middle Eastern and ayurvedic herbal medicine

Turmeric is an ancient spice and a traditional remedy that has been widely used as a medicine, condiment and flavoring. It has been used for centuries to treat indigestion and many other ailments in the East. The use of turmeric in the Indian subcontinent dates back nearly 4000 years, to the Vedic culture in India, when turmeric was the principal spice and also held religious importance. In modern India, turmeric is added to most meat and vegetarian dishes, predominantly curries. Turmeric also features in Arabic, Hebrew, Turkish and Persian culinary traditions. Turmeric was only considered as culinary spice in many other parts of the world until the early 1970s and mid 1980s, when laboratory researchers discovered anti-inflammatory compounds called curcuminoids in the spice[1,4,5]. The most important of these compounds and the most intensively studied by far, is curcumin. Turmeric contains approximately 5% curcumin, which is the main biologically active phytochemical compound[6]. It is extracted and investigated for its range of health-related and disease-preventing medicinal properties.

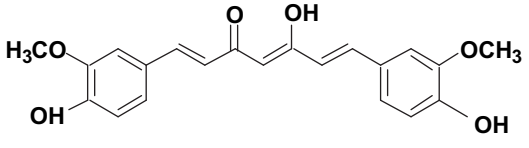

Fig. 1. The chemical structure of curcumin. Chemical names: diferuloylmethane or 1,7-bis (4′-hydroxy-3′-methoxyphenyl)-1,6-heptadiene-3,5-dione.

Curcumin and other curcuminoids

Curcumin is the principal curcuminoid component of turmeric. The other major curcuminoids are demethoxycurcumin and bis-demethoxycurcumin[7]. Curcumin can exist in at least two tautomeric forms, keto and enol. Early studies showed that curcumin exists predominantly as a keto−enol tautomer. The enol form is more energetically stable in the solid phase and in solution. More recent Nuclear Magnetic Resonance (NMR) work has demonstrated that curcumin exists in solution as keto−enol tautomers[8]. Further research is required to determine the fate of curcumin and other curcuminoids as they are absorbed across the intestine and chemically modified by the liver in laboratory rodents, other animal models and human subjects.

Nutritional biochemistry of curcumin: absorption, metabolism and bioavailability in vivo

There are two major problems associated with the metabolism and bioavailability of curcumin. The bioavailability of naturally occurring curcumin is low − the absorption and metabolism of the molecule in the gastrointestinal system results in the rapid loss of its unique properties *in vivo*. Also, when curcumin is ingested orally, very little may actually reach the systemic circulation. Researchers have vigorously debated what 'absorption' actually constitutes − in the context of this paper 'absorption' correlates with systemic bioavailability in serum. Oral clinical trials in human subjects have demonstrated that the systemic bioavailability of orally administered curcumin is relatively low[9]. Curcumin is rapidly converted to curcumin glucuronides and curcumin sulfates or reduced to hexahydrocurcumin in the intestine and the liver[10]. Metabolic derivatives of curcumin do not possess the same biological activity as the original compound. In one study, conjugated or reduced metabolites of curcumin were less effective inhibitors of prostaglandine E_2 (PGE_2) production in cultured human colon cells than curcumin itself[11]. The serum concentration of curcumin peaks 1−2 h after an oral dose in human subjects with peak serum concentrations of 0.5, 0.6 and 1.8 μM at massive doses of 4, 6 and 8 g/day, respectively[12]. More recently, clinical trials have found that plasma curcumin, curcumin sulfate and curcumin glucuronide concentrations were in the range of 10 nM (0.01 μM) 1 h after a large 3.6 g dose of oral curcumin[13]. Curcumin and its glucuronidated and sulfated metabolites were also measured in urine at a dose of 3.6 g/day[14]. Curcumin and its metabolites could not be detected in

plasma at lower doses than 3.6 g/day. There is some evidence that orally administered curcumin accumulates in gastrointestinal tissues. Colorectal cancer patients administered 3.6 g/day of curcumin orally for 7 days prior to surgery, have detectable curcumin levels in both malignant and normal colorectal tissue[15]. In contrast, curcumin was not detected in the liver tissue of patients with liver metastases of colorectal cancer after the same dose of oral curcumin suggesting that oral curcumin administration may not effectively deliver curcumin to tissues outside the gastrointestinal tract[16].

Interestingly, if curcumin is dissolved in oil before ingestion, it might bypass some of the intestinal metabolic enzymes that are thought to modify its structure. It is thought to be absorbed directly into the chylomicrons and subsequently, the lymphatic system, which bypasses the liver in the 'first pass phenomenon'[17]. Numerous other approaches have been undertaken to improve the bioavailability of curcumin. These include, firstly, the use of adjuvant like piperine which is an inhibitor of hepatic and intestinal glucuronidation[18]; secondly, the use of liposomal curcumin[19]; thirdly, polymeric nanoparticle-encapsulated curcumin called "nanocurcumin"[20]; fourthly, the use of curcumin phospholipid complex[21]; fifthly, by the use of self microemulsifying drug delivery systems[22]; and lastly, complexed with cyclodextrin[23].

Solubility of curcumin in vitro

Curcumin is insoluble in water and other aqueous solutions, thus in order to get curcumin into suspension, it must be dissolved in organic solvents such as ethanol, dimethyl sulfoxide (DMSO), acetone or dimethyl formamide[24–26]. The majority of *in vitro* studies performed by the authors and many other investigators have been performed with curcumin dissolved in DMSO. In our own experiments, the experimental controls always contain equivalent concentrations of DMSO. Curcumin solubilized in this manner for *in vitro* testing must be gradually introduced into culture medium, else it precipitates out of solution. The half life of curcumin in solution can vary from minutes to 6 h depending on variables such as pH, temperature and media[27–29]. These factors may influence the efficacy of curcumin *in vitro* and must be considered when extrapolating information from these studies.

Apoptotic, anti-apoptotic and anti-proliferative activity of curcumin

Most of the papers that report apoptotic, anti-apoptotic and anti-proliferative effects of curcumin have been published in the cancer literature. It is therefore inappropriate to cite these papers in a review that focuses on chondrocytes, cartilage and OA. Curcumin at concentrations up to 10 μM has been shown to have both anti-proliferative and apoptotic effects on synoviocytes. Curcumin used in the concentration range of 10–50 μM has been shown to dose-dependently decrease cell viability [estimated by tetrazolium salt reduction (MTT) assay] in cultured synoviocytes isolated from normal synovium[30–32]. In addition, curcumin (20 μM) has been shown to enhance celecoxib's apoptotic effects on synovial cells from OA joints, whereas curcumin added alone had no apoptotic effect[32]. The relevance of this inhibitory effect on cell growth in OA is that synovial fibroblasts secrete mediators of inflammation and joint destruction and are recognized as important factors in the pathogenesis of OA. Therefore, induction of apoptosis of these cells to induce long-term remission is an attractive therapeutic goal. However, the curcumin concentration needed to induce cell apoptosis is at least three times more elevated than the Cmax recorded in plasma after oral administration[17]. One possible solution to this problem could be intra-articular injection of high doses of curcumin. However, the safety implications of this approach will need to be investigated.

In cultured chondrocytes, the effect of curcumin on cell viability depends on the culture system used. Using the transformed chondrocyte cell line C28/I2, Toegel and colleagues observed a cytotoxic effect at the concentration of 50 μM[33]. In primary equine chondrocytes, we have not observed any cytotoxic effects at concentration below 12 μM (unpublished observations). The effects of different concentrations of on the viability of chondrocytes in equine cartilage explants *in situ* is currently under investigation in our laboratories. It would be valid to note that these concentrations are super-physiological and will not be achieved *in vivo*.

We have recently tested the hypothesis that curcumin protects human chondrocytes from the cellular and morphological alterations induced by interleukin-1 beta (IL-1β). We investigated the *in vitro* effects of curcumin on apoptotic signalling proteins in IL-1β-stimulated human chondrocytes. Human articular chondrocytes were pre-treated with 10 ng/ml IL-1β alone for 30 min before being co-treated with IL-1β and 50 μM curcumin for 5, 15 or 30 min, respectively. The production of activated caspase-3, a marker of apoptosis marker was analyzed by immunohistochemistry, immunoelectron microscopy and immunoblotting. Transmission electron microscopy of chondrocytes stimulated with IL-1β revealed early degenerative changes, which were reversed by curcumin co-treatment. Additionally, curcumin antagonized IL-1β-induced caspase-3 activation in a time-dependent manner[34]. These studies have clearly demonstrated that curcumin exerts anti-apoptotic effects on IL-1β-stimulated human articular chondrocytes.

Antioxidant activity of curcumin

The degradation of articular cartilage in OA results from a combination of inappropriate mechanical stresses on joints with instability, inflammatory mediators and biochemical factors, mainly matrix metalloproteinases (MMPs) and reactive oxygen species (ROS)[35]. The principal ROS involved in the pathogenesis and progression of OA are nitric oxide (NO), peroxynitrite ($ONOO^-$) and superoxide anion radicals ($O_2^{\bullet}$)[36]. These factors are not only deleterious agents involved in cartilage degradation, but also they act as catabolic cell signalling molecules[37]. The activity of ROS is balanced by enzymatic and non-enzymatic antioxidants, that act by inhibiting oxidative enzymes, scavenging free radicals or chelating ion metals[38]. A number of antioxidant supplements or drugs with antioxidant properties have been developed to reinforce the cellular antioxidant status. However, so far, there is no consistent or convincing evidence to support the notion that additional antioxidant supply is efficacious in relieving the symptoms of OA or preventing structural changes in OA cartilage[35,38,39]. Although further investigation is required to support the concept of antioxidant therapy in the management of joint diseases, basic research to find new and safe scavengers of ROS for the treatment of OA is justified.

Curcumin has been shown to be an effective scavenger of ROS and reactive nitrogen species *in vitro*[40,41]. However, it is still not established whether curcumin acts directly as an antioxidant *in vivo*. Curcumin may function indirectly as

an antioxidant by inhibiting the activity of inflammatory enzymes, such as MMP-9[42] or by enhancing the synthesis of glutathione, an important intracellular antioxidant[43].

Anti-inflammatory activity of curcumin

Arachidonic acid in cell membranes plays an important role in inflammatory responses by generating potent chemical messengers known as eicosanoids. Membrane phospholipids are hydrolyzed by phospholipase A_2 (PLA_2), releasing arachidonic acid, which may be metabolized by cyclooxygenases (COX), to form prostaglandins and thromboxanes; or by lipoxygenases (LOX), to form leukotrienes. Curcumin has been found to inhibit PLA_2, COX-2 and 5-LOX activity in cultured cells[44]. Although curcumin has been shown to inhibit the catalytic activity of the enzyme 5-LOX directly, it inhibited PLA_2 by preventing its phosphorylation and COX-2 mainly by inhibiting its transcription. Nuclear factor-kappa B (NF-κB) is the transcription factor that enhances the transcription of the COX-2 gene and other pro-inflammatory genes, such as inducible nitric oxide synthase (iNOS). Curcumin has been found to inhibit NF-κB-dependent gene transcription in articular chondrocytes[34,45] (see subsequent sections). Curcumin also inhibits the induction of COX-2 and iNOS in cell culture and in animal studies[46,47].

Anti-catabolic activities of curcumin

Studies of synovial fibroblasts cultured from human RA patients have shown that macrophage migration inhibitory factor (MIF) up-regulates messenger RNAs encoding MMPs, a process which is inhibited by curcumin[48]. Oncostatin M (OSM), a member of the IL-6 superfamily of cytokines, is elevated in patients with OA and, in synergy with IL-1β, promotes cartilage degeneration by MMPs[49]. Curcumin is able to suppress IL-1β and OSM-induced MMP-1, MMP-3, MMP-9 and MMP-13 gene expression by human chondrocytes *via* inhibition of NF-κB activation and nuclear translocation[34,45,50]. The following section reviews the literature that has established the importance of the curcumin/activator protein 1 (AP-1)/NF-κB axis and the finding that curcumin's potent effect on cell activity is primarily through its inhibition of the transcription factors AP-1 and NF-κB and modification of binding of transactivating factors.

Mechanisms of curcumin mediated alterations in gene expression in chondrocytes, osteoblasts and synovial fibroblasts; functional roles of the AP-1/NF-κB signalling pathways

There is considerable interest in the use of nutrigenomics and plant derived phytopharamaceuticals as new therapies for various arthritic and rheumatic conditions that affect cartilage, bone and synovium. Inflammatory processes play a fundamental role in the damage of articular tissues and many *in vitro* and *in vivo* studies have examined the contribution of components of subcellular signalling pathways to the pathogenesis of various rheumatic diseases such as OA and RA[51]. The inhibition of MMP activity by phytopharmaceuticals such as curcumin is also thought to be mediated by inhibition of subcellular signalling pathways. The NF-κB transcription factor is intimately involved in the regulation of expression of numerous genes in the setting of inflammatory diseases. NF-κB is a protein complex that controls gene transcription in almost all animal cells. It is involved in cellular responses to cytokines, free radicals, and bacterial or viral antigens[52]. The AP-1 is a transcription factor belonging to the c-Fos, c-Jun, activating transcription factor (ATF) and Jun dimerization protein (JDP) families and controls a number of cellular processes including differentiation, proliferation, and apoptosis. It regulates gene expression in response to a variety of extracellular stimuli, including cytokines and growth factors. AP-1 up-regulates transcription of genes containing the 12-O-tetradecanoylphorbol-13-acetate DNA response element (TRE; 5′-TGAG/CTCA-3′)[53] AP-1 binds to this DNA sequence *via* a leucine zipper[54]. The AP-1 and NF-κB transcription factors jointly regulate many important biological and pathological processes. Inflammation, cartilage degradation, cell proliferation, angiogenesis and pannus formation are processes in which the role of NF-κB is prominent[55]. Consequently, the identification of inhibitors of NF-κB signalling should pave the way for the development of novel therapeutics for the treatment of chronic joint diseases[55,56]. However, the AP-1 and NF-κB transcription factors also play crucial homeostatic roles in the normal physiology of joints which justifies further research on these signal transduction pathways.

Suppression of AP-1 activation by curcumin was observed by Huang and co-workers in mouse fibroblast cells in 1991[57]. Their early *in vitro* experiments provided the molecular evidence to suggest that inhibition of c-Jun/AP-1 binding to its cognate motif by curcumin may be responsible for the inhibition of AP-1-mediated gene expression. Onodera and colleagues[48] expanded the work done on synovial fibroblasts by showing that the up-regulation of MMP-1 and MMP-3 mRNAs in cultured synovial fibroblasts derived from RA patients in response to macrophage MIF can be inhibited by curcumin. However, the IL-1 receptor antagonist, a specific antagonist of the IL-1 receptor failed to inhibit the transcriptional regulation of these MMPs. This study led to the conclusion that MIF plays an important role in joint destruction in RA *via* induction of MMPs. Later studies by the same group showed that MIF also up-regulates MMP-13 mRNA in rat calvaria-derived osteoblasts. The pharmacological inhibitors genistein, herbimycin A and 4-amino-5-(4-chlorophenyl)-7-(t-butyl)pyrazolo [3,4-d] pyrimidine significantly suppressed the up-regulation of MMP-13 mRNA[58]. The selective mitogen-activated protein kinase (MAPK) kinase (MEK)1/2 inhibitor PD98059 and curcumin both suppressed the up-regulation of MMP-13 mRNA induced by MIF[58].

Subsequent work by Zafarullah's laboratory demonstrated that the inhibition of IL-1β-stimulated MAP kinases, AP-1 and NF-κB transcription factors down-regulates MMP gene expression in articular chondrocytes[49]. Their work was the first to show that inhibition of Jun N-terminal kinase (JNK), AP-1 and NF-κB by curcumin achieved 48–99% suppression of MMP-3 and 45–97% of MMP-13 in human and 8–100% (MMP-3) and 32–100% (MMP-13) in bovine articular chondrocytes[49]. Their important observations also highlighted the involvement of MAPKs, AP-1 and NF-κB transcription factors in the IL-1β induction of MMPs in chondrocytes. Later work from the same group using primary human chondrocytes derived from OA femoral heads or chondrosarcoma cells demonstrated that inhibitors of extracellular signal-related kinases (ERK) (U0126, PD98059, and ERK1/2 antisense phosphorothioate oligonucleotide), JNK (curcumin, SB203580, and SP600125), and p38 (SB203580 and SB202190) pathways down-regulate the tumor necrosis factor-stimulated expression of MMP-13[59].

Human wee1 kinase is a protein kinase that down-regulates the M-phase promoting factor, a complex of cell division cycle 2 (cdc2) and cyclin B kinase, which controls mitotic cell

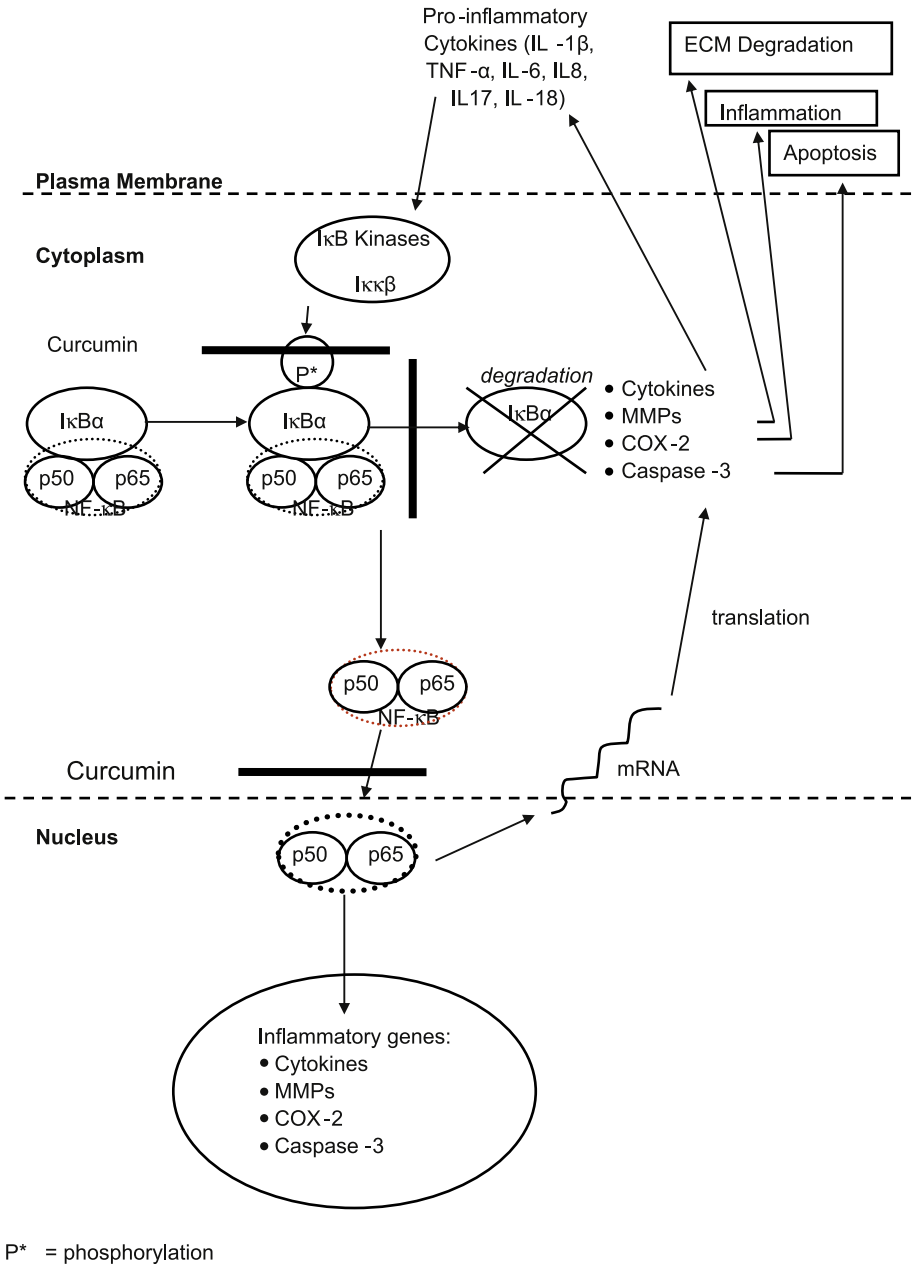

Fig. 2. The curcumin/AP-1/NF-κB axis in inflammatory cell signalling responses in joint cells. This schematic illustrates the potential sites of action of curcumin in the NF-κB signalling pathway in a typical joint cell. Curcumin (C) can prevent IκBα phosphorylation, as well as preventing the translocation of active NF-κB into the nucleus, thereby preventing it from activating gene expression of MMPs, stimulators of MMP synthesis (cytokines), inflammatory mediators (cytokines and COX-2) and apoptotic executioners (caspase-3).

division. Wee1 kinase is directly transactivated by and upregulated in association with c-Fos/AP-1. Work on rheumatoid synovial cells has shown that the wee1 kinase gene promoter region contains an AP-1-binding motif (5′-CGAGTCA-3′; -823/-817) through which wee1 kinase gene is directly transactivated by c-Fos/AP-1[60]. In rheumatoid synovial cells wee1 kinase increases in conjunction with the increase of c-Fos/AP-1 but this increase is inhibited by curcumin[60].

Other studies have used osteosarcoma cells and curcumin as an inhibitor of AP-1 to inhibit the biological actions of OSM and provide evidence for the activation of the MEK/ERK and signal transducer and activator of transcription (STAT) pathways in OSM signalling[61].

Recent work on IL-17 and IL-18 has shown that these cytokines have the capacity to promote cartilage breakdown by inducing MMPs, aggrecanases and growth factors in arthritic joints. Curcumin and Bay-11-7085 have been shown to block IL-17 signalling pathways[62]. Similarly curcumin has been shown to dose-dependently abrogate the effect of IL-18 on vascular endothelial growth factor (VEGF) production[63]. Thus IL-17 and IL-18 mediated MMP, a disintegrin-like and metalloproteinase with thrombospondin motifs (ADAM-TS) and VEGF up-regulation may be targeted *via* AP-1 and NF-κB inhibition using curcumin.

These focussed studies in joint derived cells enforce the well-established finding from other cells and tissue that

Table I
Summary of the biological actions of curcumin on joint tissues

Cell viability

- Decreases cell viability of adherent synoviocytes
- No effect on cell viability of adherent chondrocytes, chondrocytes in alginate beads or in cartilage explants (at concentration below 30 μM)
- Antagonizes IL-1β-induced caspase-3 activation in a time-dependent manner

Antioxidant effects

- Scavenger of reactive oxygen and nitrogen species *in vitro*
- Inhibits IL-1β-induced NO production by bovine and human chondrocytes and human cartilage explants
- Inhibits IL-1β-induced superoxide dismutase activity in bovine chondrocytes in monolayer

Anti-inflammatory effects

- Inhibits NF-κB-dependent gene transcription in chondrocytes
- Inhibits COX-2, but not COX-1, gene expression in IL-1β-treated bovine chondrocytes in monolayer
- Inhibits IL-6 and IL-8 gene expression by bovine and human chondrocytes
- Inhibits IL-6, IL-8 and PGE_2 production by human chondrocytes and cartilage explants

Anti-catabolic effects

- Inhibits IL-1β-induced glycosaminoglycan (GAG) release from canine and human OA cartilage explants
- Decreases MMP-3 synthesis in chondrocytes in alginate beads and in human cartilage explants
- Suppresses IL-1β and OSM-induced MMP-1, MMP-3, MMP-9 and MMP-13 gene expression by human chondrocytes *via* inhibition of NF-κB activation and nuclear translocation

Anabolic effects

- No effect on aggrecan production by human chondrocytes in alginate beads
- Decreases proteoglycan mRNA expression in bovine chondrocytes in monolayer
- Reverses the IL-1β-induced inhibition of type II collagen and β1-integrin gene expression in human chondrocytes

curcumin's potent effect on cell activity is achieved through its inhibition of transcription factor AP-1 and NF-κB mediated gene expression. The results of these studies are summarized in Fig. 2.

Potential anabolic effects of curcumin

Any anabolic effects mediated by curcumin may be attributed to its anti-catabolic effects. Direct evidence for its involvement as a pro-anabolic compound is still lacking. IL-1β is well known for down regulating type II collagen expression in chondrocytes; we have shown that this process is reversed by curcumin treatment. In addition to its effects on type II collagen expression, curcumin also reverses the IL-1β induced down-regulation of β1 integrin[64]. Recently, Jackson *et al.* showed that micromolar concentrations of curcumin (10 μM) decreased and inhibited collagenase and stromelysin expression in HIG-28 rabbit chondrocytes[30].

TEXT-MINING REVIEW

Text-mining as a tool for investigating inflammatory pathways in chondrocytes

The application of advanced information retrieval systems can assist in reviewing scientific data[65]. In addition to the traditional literature search for this review, a text-mining/entity relationship tool was employed as described in the Methods section.

An initial search for curcumin across all cell types identified 113 cell processes with direct relations to curcumin. The same search restricted to chondrocytes identified only two cell processes (pathogenesis and translation). Searching for direct relations between curcumin and protein/protein classes across all cell types reported relations with 426 protein/protein classes. Restricting the search to chondrocyte as a cell type, curcumin has relations with eight proteins and three protein classes. These analyses indicate that the scientific literature reporting the action of curcumin in chondrocytes has been focused to comparatively few biological processes and proteins. Based on these analyses curcumin may have many other effects on chondrocyte cellular function beyond those investigated to date.

Whilst direct relations between curcumin and other entities/processes in chondrocyte retrieved limited information, it was considered that further analysis could provide insight into the potential influence of curcumin in chondrocyte. Extending the search to include direct relations between "curcumin-related proteins" within chondrocytes and "cell processes" and "diseases" indicated the potential range of processes and diseases related to chondrocyte function that could be affected by the actions of curcumin.

Text-mining revealed the central roles that are played by NF-κB, MMP-1, MMP-3, MMP-13 and caspase-3 in articular chondrocytes and the inter-relationships between these proteins and various processes (for example, cartilage degeneration, arthritis, OA, bone growth, bone fracture healing, apoptosis). The contribution of curcumin to these processes is important as a direct relation exists between curcumin and MMP-1, -9 and -13 as well as caspase-3, NF-κB and STAT.

Whilst an automated text-mining search cannot replace the expert reviewer it does support the traditional approach

and provides a relatively simple and rapid visual tool to aid knowledge management, interrogating the literature in PubMed/Medline and undertake knowledge gap analysis. This approach has helped us sharpen our focus on inflammatory pathways that are specifically activated in chondrocytes in response to pro-inflammatory mediators and investigate the contribution of curcumin to this area of the literature on articular chondrocytes.

In summary, text-mining has proved to be a powerful for interrogating the literature in PubMed. This approach has helped us sharpen our focus on pathways that are specifically activated in chondrocytes in response to pro-inflammatory mediators and investigate the contribution of curcumin to this area of the literature on articular chondrocytes.

Conclusions and future perspectives

In modern developed countries, public concern about synthetic drugs and their negative side effects is growing. Consequently, there is increasing interest in natural remedies, particularly those of botanical origin. Plant derived extracts, which possess therapeutic properties, such as curcumin, offer a potentially safer and cheaper alternative to conventional drugs. Curcumin has beneficial effects on numerous cell types *in vitro* and has already begun clinical trials in humans (Table I). However, the bioavailability of curcumin and its metabolites is debatable and has raised the question of whether these *in vitro* effects can be replicated *in vivo*.

We have used text-mining and a conventional literature search based review of the literature to summarize the research that has been done in this area. Although the majority of papers quoted in this review may support the scientific rationale that curcumin benefits OA patients, much more research is needed to gain a better understanding of its potential side effects and contra-indications associated with long-term consumption of curcumin and curcuminoids. This review also highlights the fact that the effects of curcumin on articular chondrocytes are relatively limited. However, the existing published research suggests that it may offer considerable potential as an aid to preventing or at least delaying the onset of OA. In fact, its anti-catabolic effects, namely reducing degradative enzyme expression and activity, and its positive influence on anabolic gene expression suggests that it may be a suitable adjunct to conventional pharmaceutical therapy. Curcumin is also a powerful inhibitor of inflammatory mediators, indicating that it could be an alternative to NSAIDs. In contrast to NSAIDs, curcumin has no gastrointestinal side effects, and can even protect the gastric mucosa. Therefore, curcumin could be beneficial in the management of chronic inflammatory-related joint disease, including OA. However, despite this optimistic view, it must be recognized that there is a paucity of data regarding possible adverse effects of curcumin at concentrations that are biologically effective *in vitro*. The absence of systemic adverse effects after oral administration of curcumin is probably the result of its poor bioavailability and chemical modification by the gut and liver. Whilst some evidence exists for toxicity, at super-physiological concentrations, these are unlikely to be experienced or achieved *in vivo*. Nevertheless, we cannot exclude the possibility that increasing curcumin absorption, by chemical or natural process, could have unsuspected deleterious effects. It is now documented that curcumin at concentrations in excess of 50 μM shows cytotoxicity in a chondrocyte cell line[33]. In addition, there is no published information about the possible side effects of the metabolites of curcumin. Further work is therefore required to address the issues of bioavailability and tissue accumulation in order to calculate appropriate dose formulations to assess whether curcumin can be convincingly considered as an aid to treating OA in both human and veterinary medicine.

Conflict of interest and funding disclosure

The Bone and Cartilage Unit (BCRU-University of Liège) has received an educational grant to evaluate the anti-inflammatory properties of a turmeric extract containing min. 90% of curcumin. This extract is not a product proprietary to *bio*Xtract. This is a commercially available extract, produced according to the European specifications for food additive E100 (Commission Directive 95/45/EC of 26 July 1995 laying down specific purity criteria concerning colours for use in foodstuffs). Therefore, the effects reported by BCRU are not specific to a product commercialized by *bio*Xtract. Further, Prof Henrotin did not received consulting fees to perform these *in vitro* investigations.

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