Published in final edited form as:
Life Sci. 2010 June 19; 86(25-26): 907–918. doi:10.1016/j.lfs.2010.04.013.

Green tea polyphenol epigallocatechin-3-gallate: inflammation and arthritis

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Abstract

A number of factors including inflammation and oxidative stress are believed to play a role in the development of chronic joint diseases. Green tea has become a popular drink and is consumed throughout the world. Extracts of green tea and polyphenols present therein have been shown to inhibit the inflammatory responses in vitro in different cell types and the development of arthritis in animal model studies. There is considerable evidence that (-)-epigallocatechin-3-gallate (EGCG), the predominant green tea polyphenol which mimic its effects, inhibits enzyme activities and signal transduction pathways that play important roles in inflammation and joint destruction in arthritis. After oral consumption EGCG become bioavailable and proteomic studies suggest that EGCG may directly interact with a large set of protein targets and alter the physiological response of the cells. Taken together these and other studies identify and support the use of EGCG as a possible chemopreventive agent with a potential to inhibit the development of arthritis. Here we review the biological effects of EGCG in an attempt to understand its pivotal molecular targets that directly affect the inflammation and joint destruction process for prevention and/or for the development of new therapeutics for arthritis in humans.

Keywords

Green Tea; EGCG; Osteoarthritis; Rheumatoid arthritis; MAPK; NF-κB

Introduction

Arthritis represents a major health problem and its global burden is rising at an alarming rate. Arthritis affects nearly 46 million people in the USA and by 2030 the number of people with arthritis is expected to rise to 67 million (Helmick et al. 2007; Lawrence et al. 2007). In the Western world and USA, arthritis and related conditions have been identified as the third largest contributor to direct health expenditure (behind cardiovascular disease and

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Conflict of interest statement
The authors declare that there is no conflict of interest.

neurological disorders) (Penm et al. 2001). Osteoarthritis (OA) and rheumatoid arthritis (RA) are a group of diseases with different profiles and unknown etiology but similar outcome- joint destruction. OA involves the erosion of articular cartilage, inflammation of synovial membrane, and resorption of the underlying sub-chondral bone (vander-Kraan and vanden-Berg 2000; Arden and Nevitt 2006). On the other hand, RA is a chronic inflammatory and systemic disorder characterized by cellular infiltration and proliferation of synovium, leading to progressive destruction of the joints through the interaction between infiltrating cells and mediators they produce (Lee and Weinblatt 2001). However, pathological changes in both diseases are associated with an excessive production of pro-inflammatory molecules, which shift the balance between the synthesis and degradation of matrix components resulting in progressive destruction of the joint tissue (Kraan and Berg 2000). Pharmacologic treatment options for arthritis are diverse both in terms of mechanisms of action and delivery formulations; however, no single agent has been demonstrated to consistently offer both a high level of tolerability and a sustained degree of efficacy across a broad patient population. Current treatment options are mostly symptomatic and include Non Steroidal Anti-Inflammatory Drugs (NSAIDs) (Altman 2009) and cyclooxygenase-2 (COX-2) inhibitors (rofecoxib) (Hsiao et al. 2009) for pain relief but fail to block the progression of the disease. Unfortunately, these agents are also associated with gastrointestinal (Chan et al. 2010) and cardiovascular adverse events (Hsiao et al. 2009). Intra-articular therapies like glucocorticoids and hyaluronans injections have been used for pain relief in OA patients but recent observations suggest that intra-articular hyaluronic acid injections may accelerate cartilage breakdown in patients with symptomatic knee osteoarthritis (Gonzalez-Fuentes et al. 2010). Matrix Metalloproteases (MMPs) inhibitors have been studied as possible drugs for prevention of cartilage degradation but their clinical use has been limited by severe side-effects (Nuti et al. 2009). Recent reports showed that by blocking aggrecanase mediated cleavage in the aggrecan interglobular domain abrogates cartilage erosion and promotes cartilage repair (De Rienzo et al. 2009). However, despite ablation of a disintegrin and metalloproteinase with thrombopondin motif-5 (ADAMTS-5) activity (major factor for aggrecan loss), aggrecanolysis can still occur at two preferred sites in the chondroitin sulfate-rich region (De Rienzo et al. 2009; East et al. 2007) indicating the need for more effective inhibitors. Disease modifying anti-rheumatic drugs (DMARDs) are used to treat the clinical and radiological course of RA but have serious side effects (Feist and Burmester 2009). The use of biologics (antibodies or soluble receptors for interleukin (IL)-1, IL-6 and tumor necrosis factor (TNF)-α) has recently been introduced for treating RA, but these are also not universally effective (Feldman and Maini 2001; Choy et al. 2002; Braddock and Quinn 2004). Additionally, higher costs and increased risk of malignancies limit the use of such agents in many populations (Brown et al. 2002). Because of these limitations, there is a growing interest to use botanicals as an adjunct therapy to prevent the development of arthritis and other chronic diseases. There are many reasons why people use herbal products: conventional treatment may not be working as well as they would like; they have issues with side-effects of pharmaceutical treatment; they wish to reduce some of the stress that comes from living with a chronic illness and want to cope better; they believe that herbal products are safe, because these are ‘natural’. The objective of this review is to discuss the potential arthritis preventive and anti-inflammatory effects, as well as possible harmful effects, of green tea and its prominent polyphenol EGCG in animals and humans. This knowledge may be useful for the development of EGCG-based novel strategies for targeting pathways associated with joint destruction in arthritis.

Green tea catechins

Tea is a widely consumed beverage throughout the world and reported to possess significant health promoting effects (reviewed in Cabrera et al. 2006). Depending on the manufacturing

processes, teas are classified into three major types: 'non-fermented' green tea (produced by drying and streaming the fresh leaves to inactivate the polyphenol oxidase); 'semi-fermented' oolong tea (produced when the fresh leaves are subjected to a partial fermentation stage before drying); and 'fermented' black and red teas which undergo a post-harvest fermentation stage before drying and streaming (Cabrera et al. 2003). Non-fermented green tea consists of more polyphenolics (30%) than black (5%) and oolong (4.5%) tea (Belitz and Grosh 1997; Zuo et al. 2002). Green tea contains proteins (15%), amino acids (4%), fiber (26%), other carbohydrates (7%), lipids (7%), pigments (2%), minerals (5%), and phenolic compounds (30%) (Cabrera et al. 2006; Belitz and Grosh 1997). The main flavonoids present in the green tea include catechins (flavan-3-ols). The principal catechins found in the green tea are epicatechin (EC; 6.4%), epicatechin-3-gallate (ECG; 13.6%), epigallocatechin (EGC; 19%), and epigallocatechin-3-gallate (EGCG; 59%), and account for 30–40% of its dry weight. The strong antioxidant potential of green tea catechins has been widely demonstrated in in vitro and in animal studies (Cabrera et al. 2003, 2006; Frei and Higdon 2003; Nakagawa and Yokozawa 2002). Green tea catechins especially EGCG has been reported to have anti-mutagenic (Cheng et al. 2009), anti-cancer (Johnson et al. 2010), anti-diabetic (Zhang et al. 2010), anti-inflammatory (Danesi et al. 2010), anti-bacterial (Osterburg et al. 2009), anti-viral (Xiao et al. 2008), anti-obesity (Moon et al. 2007) and neuro-protective effects (Smith et al. 2010). A proteomics study of human vascular senescent endothelial cells treated with EGCG, has demonstrated altered expression of various proteins associated with cytoskeleton and cell cycle (Lee et al. 2006). In addition, a combined proteomics and gene expression analysis showed that EGCG affected the expression level of diverse proteins, including proteins related to cytoskeletal components, metabolism, binding proteins and heat shock proteins associated with neuro-protective effects (Weinreb et al. 2007). The potential disease-modifying effects of green tea on arthritis came to light through our study, when in a mouse model of RA induction and severity of arthritis was ameliorated by the prophylactic administration of green tea polyphenols (GTPs) in drinking water (Haqqi et al.1999). Subsequent studies suggested that EGCG possesses remarkable potential to prevent chronic diseases like OA and RA (Ahmed et al. 2004, 2005, 2008; Singh et al. 2002; Yun et al. 2008). The anti-inflammatory and anti-arthritic effects of EGCG are supported by in vitro and in vivo data indicating that EGCG or EGCG containing green tea can regulate the expression of cytokines, chemokines, MMPs, aggrecanase, reactive oxygen species (ROS), nitric oxide (NO), COX-2, and PGE_2 in cell type relevant to the pathogenesis of OA and RA (Haqqi et al. 1999; Ahmed et al. 2004, 2005, 2008; Singh et al. 2002; Yun et al. 2008). Further, EGCG is known to inhibit osteoclasts differentiation, expression of receptor activator for nuclear factor-κB (RANKL), epithelial neutrophil activating protein-78 (ENA-78), growth regulated oncogene (GRO)-α, monocyte chemotectic protein (MCP)-1 and myeloid cell leukemia sequence (Mcl)-1 in an inflamed RA joint (Morinobu et al. 2008; Ahmed et al. 2006 and 2009; Lin et al. 2008). These in vitro and in vivo observations point out the efficacy of EGCG and demonstrate that it can modulate multiple signal transduction pathways in a fashion that suppresses the expression of inflammatory mediators that play a role in the pathogenesis of arthritis.

Structure-activity relationship

Structural activity relationship with EGCG indicates that there is a linear increase of the rate constants with OH radical which correlates with the number of reactive hydroxyl group (i.e. the number of catechols or pyrogallol moieties). This suggests the importance of structure of EGCG linked to gallic acid for its antioxidant activity (Plumb et al. 1998). EGCG is reported to inhibit the IKK activity in intestinal epithelial cells and this is correlated with the presence of the gallate group because the polyphenols lacking gallate group failed to inhibit the IKK activity (Yang et al. 2001). Studies have shown that EGCG selectively inhibits the phosphorylation of c-Jun and DNA binding activity of AP-1 in human OA chondrocytes

(Ahmed et al. 2002). The inhibition of AP-1 activity by EGCG and related tea catechins was also attributed to the gallate moiety (Chung et al. 1999). Structural and functional analyses have identified the galloyl and a hydroxyl group present at the 3' position on EGCG molecule as responsible for its strong anti-inflammatory properties in articular chondrocytes (Andriamanalijaona et al. 2005). EGCG has been reported to enhance the susceptibility of TNFα-induced RA-synovial fibroblasts to apoptosis (Ahmed et al. 2009). It has been suggested that catechins without a pyrogallol-type structure showed no inhibition of apoptosis (Saeki et al. 2000). Proteasome is the major machinery in cells for protein degradation. Protein ubiquitination is a mechanism that enables proteasome to specifically degrade proteins that are destined to be destroyed (Pujol 1999). In eukaryotes, proteasome contains at least three types of catalytic activities: chymotrypsin-like, trypsin-like and caspase-like (Seemuller et al. 1995). Proteasome mediated degradation of IκB facilitate the NF-κB activation and translocation to nucleus (Saklatvala et al. 2007). Thus, proteasome mediated degradation pathway is considered an important target for the treatment inflammation and arthritis. EGCG inhibits potently and specifically the chymotrypsin-like activity of proteasome *in vitro* (IC_{50}86–194 nM) and ester bond within EGCG play a critical role in this inhibitory activity (Nam et al. 2001). We have shown that EGCG-mediated inhibition of NF-κB was via inhibition of proteosome activity in human chondrocytes (Singh et al. 2002).

Anti- arthritic potential of EGCG

Recent evidences based on the molecular and cellular evaluation of green tea effects in different systems have brought to the forefront the value of green tea catechins as agents for modulating inflammation and arthritis (Haqqi et al. 1999; Ahmed et al. 2004, 2005, 2006, 2008, 2009; Singh et al. 2002; Yun et al. 2008; Morinobu et al. 2008). EGCG is a potent anti-oxidant and can alter the redox status of joint and activity of inflammatory cells involved in disease pathogenesis. EGCG has been characterized as a ligand for the 67 kDa laminin receptor (LR) as experiments performed in human lung cancer cells demonstrated that EGCG inhibit their growth via 67 kDa LR with a k_d value of 39.9 nM (Tachibana et al. 2004). Beside the role of LR in cell adherence to the extracellular matrix (ECM), there is growing evidence that 67kDa LR activation induce functional changes within the cells (Nelson et al. 2008). The extracellular matrix is an "information rich" environment and interactions between the chondrocyte and ECM regulate many biological processes important in cartilage homeostasis and repair including cell attachment, growth, differentiation, and survival. Chondrocytes have been shown to express laminin β1 integrin receptor (Loeser 2000, 2002) and in synovial tissues of RA patients laminins and integrins co-localize with increased expression of inflammatory cytokines (Warstat et al. 2010; Poduval et al. 2010). These results suggest the possible interactive relationship between EGCG receptor present on chondrocytes and synovial cells for its possible anti-arthritic effect. Further in depth studies of this receptor and signaling in arthritis would help to clarify the specific effects of EGCG on chondrocytes and synoviocytes and may enable the better utilization of EGCG as a preventive or therapeutic agent.

Effect of EGCG on nuclear factor-kappa B (NF-κB) signaling pathway

NF-κB acts as a controlling switch for the regulation of genes important in cellular response, inflammation, innate immunity, and arthritis (Karin and Ben-Neriah 2000). It is well known that inflammation and cartilage degeneration is a result of increase in catabolic mediators and decrease in anabolic activity (Westacott and Sharif 1996). The anti-arthritic effects of EGCG are important not only in reducing the pro-inflammatory mediators production but also enhancing anabolic activity (Singh et al. 2002; Andriamanalijaona et al. 2005; Zheng et al. 2009; Yang et al. 2001;Wheeler et al. 2004). We have previously shown that EGCG inhibits NF-κB activity by blocking the phosphorylation of IκB-α in human OA

chondrocytes (Singh et al. 2002). Recently, it has been reported that EGCG inhibits the expression of NF-κB in RAW 264.7 macrophages (Lin et al. 2009). EGCG is also reported to inhibit TNF-α and LPS mediated activation of NF-κB via inhibiting IκB-α phophorylation (Ahmad et al. 2000). Of relevance to arthritis suppressive are the studies showing effects of EGCG, that EGCG inhibits IL-1 induced protein kinase Cδ phosphorylation and NF-κB activation and nuclear translocation to suppress chemokines and collagenases production in RA synovial fibroblasts (Ahmed et al. 2006). Also EGCG not only induces apoptosis but enhances the susceptibility of RA-synovial fibroblasts to TNFα-induced apoptosis by supressing Akt and NF-κB pathways (Ahmed et al. 2009). EGCG has been shown to inhibit IκB Kinase (IKK) activity, IκB-α phophorylation and NF-κB activation in intestinal epithelial cells (Yang et al. 2001), respiratory epithelial cells (Wheeler et al. 2004), endothelial cells (Hong et al. 2007), and in mast cells (Shin et al. 2006). We have also shown that EGCG inhibits IKK-α and IKK-β activity in SAOS-2 cells with concominant down regulation of NF-κB and induction of apoptosis (Hafeez et al. 2006). This inhibition of IKK activity by EGCG related to the presence of the gallate group because the polyphenols lacking gallate group did not inhibit IKK activity (Yang et al. 2001). EGCG was also found to inhibit IKK-β kinase activity and DNA binding activity of NF-κB in human articular chondrocytes (Andriamanalijaona et al. 2005; Rasheed et al. 2009). Basically, this may occur as a direct effect on IKK protein or by interfering with the interaction of IKK with its substrate IκB. EGCG may also block NF-κB activation by inhibiting signaling events upstream of IKK or its unique structure may inhibit IKK enzyme activity directly. Both mechanisms would lead to inhibition of NF-κB activation. As EGCG becomes bioavailable after oral consumption, modulation of NF-κB pathway by EGCG in vivo could contribute to its reported anti-arthritic and anti-inflammatory activity in the joints.

Effects of EGCG on mitogen activated protein kinases (MAPKs) and activator protein-1 (AP-1) pathways

The MAPKs are an essential part of signal transduction machinery involved in the regulation of inflammation associated with gene expression, cell survival, proliferation, inducible nitric oxide synthase (iNOS) and cytokine expression, and collagenase production (Hommes et al. 2003). MAPKs have three major classes c-Jun-N terminal kinase (JNK), extracellular signal regulated kinase (ERK), and p38 MAPKs (Johnson and Lapadat 2002). Of note, all three classes of MAPKs have been shown to be expressed and activated in chondrocytes and synovial tissue of RA and OA patients (Chowdhary et al. 2008; Schett et al. 2000). Thus inhibition of MAPKs may represent legitimate therapeutic targets for the treatment of arthritis. EGCG exerted a marked inhibition of both basal and IL-1 stimulated MAPKs phosphorylation at 50μM concentration in chondrocytes (Andriamanalijaona et al. 2005). EGCG was also reported to suppress the RANKL induced activation of JNK pathway without affecting p38 and ERK (Lee et al. 2010). We have shown that EGCG selectively inhibits IL-1β-induced activation of JNK, without significantly inhibiting the phosphorylation of p38-MAPK or ERK p44/p42 in human OA chondrocytes (Singh et al. 2003). Other studies have shown that EGCG treatment inhibited TNF-α induced phosphorylation of ERK1/2, p38 MAPK, and JNK in RA synovial fibroblasts (Yun et al. 2008). Tokuda et al., have reported that EGCG enhanced prostaglandin F2alpha-induced vascular endothelial growth factor (VEGF) synthesis and reduced TGF-β-stimulated HSP27 induction through the suppression of stress activated protein kinases (SAPK)/JNK (Tokuda et al. 2007; Hayashi et al. 2008). EGCG was also reported to suppresses endothelin-1-induced IL-6 synthesis via MEK1/2 in osteoblast-like MC3T3-E1 cells (Tokuda et al. 2008). EGCG has also been reported to interfere with the activation of MAPKs to regulate various inflammatory genes in human dermal fibroblasts via inhibition of p38, JNK and ERK phosphorylation (Bae et al. 2008), in endothelial cells via p38 MAPKs (Hong et al. 2007) and in HMC cells via ERK (Shin et al. 2006). EGCG has also been shown to inhibit the

oxidative stress-mediated phosphorylation of MAPKs in human epidermal keratinocytes (Katiyar et al. 2001). The down-regulation of IL-12 production by EGCG has been suggested as a mechanism for the amelioration of RA and possibly other diseases (Goodridge et al. 2003). This effect of EGCG was mediated via inhibition of phosphorylation of ERK and p38 MAPK with concomitant down-regulation of IL-12p40 production (Ichikawa et al. 2004).

AP-1 transcription factor is a heterodimer of Jun (c-Jun, Jun B, Jun D) and Fos (cFos, Fos B, Fra-1 and Fra-2) proteins and plays an important role in inflammatory response (Okamoto et al. 2008). EGCG was found to inhibit the DNA binding activity of AP-1 in human OA chondrocytes (Andriamanalijaona et al. 2005). Studies from our laboratory have also shown that EGCG selectively inhibits the IL-1β-induced phosphorylation of JNK p46 isoform resulting in the lower levels of phospho-c-Jun and DNA binding activity of AP-1 in human OA chondrocytes (Ahmed et al. 2002). EGCG mediated suppression of TNF-α-induced production of MMPs in RA synovial fibroblasts was dependent on the inhibition of AP-1 pathway (Yun et al. 2008). Overall, anti-arthritic effects of EGCG have been attributed to its ability to modulate signaling pathways controlling inflammatory gene expression and this may be of value for inhibiting bone and cartilage degeneration associated with OA and RA.

Effects of EGCG on signal transducers and activators of transcription (STAT) pathway

STAT proteins are involved in modulating cellular responses to pro-inflammatory cytokines, such as IL-1, TNF- α, as well as growth factors and immunomodulatory proteins, including interferon (IFN)- γ (Igaz et al. 2001). Some investigators have reported preferential activation of STAT-1, and others have reported preferential activation of STAT-3, in response to endogenous IL-6 in RA patients (de Hooge et al. 2004; Kasperkovitz et al. 2004). Inappropriate activity of STAT-3 is closely associated with experimental arthritis in vivo (Richards et al. 2006). Further, STAT-1 deficiency in mice resulted in exacerbation of the chronic inflammation and granuloma formation (de Hooge et al. 2004). However, STAT-1 has been shown to be strongly activated in synovium of RA patients (Yokota et al. 2001). Raised STAT-1 protein expression with concominant increase in its tyrosine-701 and serine-727 phosphorylation suggested that STAT-1 activation in RA synovium plays a major role in RA-associated inflammation (Kasperkovitz et al. 2004; Tedeschi et al. 2002). Therefore, STAT-1 and 3 may represent new molecular targets of anti-inflammatory drugs. Studies have shown that EGCG exert a potent and specific inhibitory effect on IFN-γ-elicited STAT1 activation in a number of human cell types with an estimated EC50 of 2–5 μM without any effect on other STATs, such as STAT3 and 6 (Tedeschi et al. 2002; Menegazzi et al. 2001). Oral administration of EGCG attenuated the magnitude of myocyte apoptosis in the rat heart exposed to I/R injury with concomitant STAT1 inhibition (Townsend et al. 2004). Recently, it was reported that EGCG can suppress oncostatin M induced activation of STAT-3 phosphorylation in human gingival (Hosokawa et al. 2009) and keloid fibroblasts (Park et al. 2008). These studies when taken together clearly identify EGCG as having distinct actions on the nuclear transcription factors activation in a cell-specific manner (Figure 1). This again demonstrates that EGCG or compounds derived from it may be of value in developing new therapeutic approaches for the treatment of OA and RA.

Inhibition of matrix degrading enzymes

Many inflammatory signals are orchestrated within the tissue microenvironment external to cell. A super family of proteases called metzincins (zinc dependent metallopeptidases) includes MMPs, a disintegrin and metalloproteinase (ADAM) and the secreted ADAM with thrombospondin motif (ADAMTS) (Mohammed et al. 2003). Aggrecanase-1 and -2 (ADAMTS-4 and -5) are principal proteases involved in aggrecan degradation and were

found to be increased in OA and RA (Mohammed et al. 2003; Smith 2006). A major component of joint cartilage is collagen, which is susceptible to proteolytic degradation by MMPs. This suggests that MMPs and aggrecanases are promising targets for inhibiting the pathogenesis of OA and RA (Mohammed et al. 2003; Smith 2006). EGCG inhibited the degradation of human cartilage proteoglycan and type II collagen and selectively inhibited the ADAMTS-1,-4 and -5, which are known to cleave aggrecan (Vankemmelbeke et al. 2003). Previously we have shown that EGCG significantly inhibited the expression and activities of MMP-1 and MMP-13 in OA chondrocytes at physiologically achievable doses in vitro (Ahmed et al. 2004). Our recent studies showed that EGCG inhibited advance glycation end products (AGEs) induced expression of MMP-13 in human OA chondrocytes (Rasheed et al. 2009). EGCG was also found to be effective in suppressing the chemokine-induced MMP-2 activity in human colon epithelial cells (Porath et al. 2005) and TNF-α induced production of MMP-1 and MMP-3 in RA synovial fibroblasts (Yun et al. 2008). Studies have also shown that EGCG was an effective inhibitor of IL-1β-induced MMP-1, -3 and -13 expressions in human tendon fibroblasts (Corps et al. 2004). Studies have also documented that EGCG not only decreased the level of MMPs production but also increased the expression of tissue inhibitors of MMP-1 (TIMP-1) in vitro (Lee et al. 2005). Green tea polyphenols have also been reported to inhibit the gelatinolytic activity of MMP-2 and enhances its binding with TIMP-2 (Cheng et al. 2003). Overall, consumption of green tea or EGCG may inhibit the activities of MMPs involved in matrix degradation and this may have a suppressive effect on joint degradation in arthritis.

Modulation of inflammatory cytokines and chemokines expression

A prominent and characteristic feature of arthritis is the persistent production of pro-inflammatory cytokines by the inflamed synovium as well as by chondrocytes in the affected joints (Westacott and Sharif 1996; Malemud et al. 2003; Rasheed and Haqqi 2008). Therefore, the cytokine network constitutes an important target for the development of novel anti-inflammatory drugs (Alkharfy et al. 2000). Consumption of green tea polyphenols has been shown to limit inflammation and maintain joint architecture in an animal model of arthritis (Haqqi et al. 1999). In other studies, EGCG down regulated the LPS-induced inflammatory response in vivo in RAW264.7 macrophages (Yang et al. 1998) and was also shown to influence the migration of CD8+ T cells to sites of inflammation (Kawai et al. 2004). EGCG inhibited T cell proliferation at physiologically relevant concentrations of 2.5 to 10 microM without cytotoxicity or induction of apoptosis (Wu et al. 2009). EGCG supplementation resulted in lower IL-2 receptor expression and higher IL-2 accumulation, suggesting an impeded IL-2/IL-2 receptor signaling (Wu et al. 2009). These results indicate that EGCG supplementation may be beneficial to those with abnormally excessive T cell function such as in autoimmune and inflammatory disorders, but caution should be taken when it is administered at high doses to those with compromised T cell function (Wu et al. 2009). In in vivo studies EGCG was found to inhibit inflammation in mouse models by affecting the functioning of T cells and neutrophils (Aktas et al. 2004; Dona et al. 2003). EGCG (50 mg/L) inhibited the expression and level of IL-1β at wound sites (Shen et al. 2009). EGCG has also been shown to inhibit the TNF-α gene expression and its secretion in different cell types (Fujiki et al. 2003). Recently, EGCG was reported to inhibit the secretion of TNF-α and IL-6 in human mast cells (Shin et al. 2006). The pro-inflammatory cytokine IL-6 has been shown to be over-expressed in RA and is now believed to play a central role in RA joint destruction and the systemic nature of disease (Moller et al. 2006; McInnes and Liew 2005). IL-6 knockout mice were shown to be resistant to collagen induced arthritis and had reduced levels of serum TNF-α (McInnes and Liew 2005). EGCG inhibited IL-1β-induced IL-6 production and trans-signaling in RA synovial fibroblasts by inducing alternative splicing of gp130 mRNA resulting in enhanced sgp130 production (Ahmed et al. 2008). A recent report showed that EGCG significantly inhibited IL-23-induced IL-17 and

TNF-α expression in T-cell lines and this proposed a new nutritional approach for the prevention and treatment of inflammatory bowel diseases (Danesi et al. 2010; Wu et al. 2009). Age related accumulation of AGEs produced by the non-enzymatic glycation of macromolecules could be an important contributing factor for the development of OA (Rasheed et al. 2009). We recently reported that EGCG inhibited AGEs-stimulated gene expression and production of TNF-α in human OA chondrocytes in vitro (Rasheed et al. 2009). Thus consumption of EGCG may prevent pathologies in which AGEs are believed to play a role.

Chemokines are a specialized family of small (8–10 kd), structurally related proteins and well-established regulators of gene transcription, cell proliferation, and leukocyte trafficking to normal and inflamed tissues (Szekanecz et al. 2004). Chemokines such as epithelial neutrophil–activating peptide 78 (ENA-78/CXCL5), RANTES (CCL5), monocyte chemo-attractant protein 1 (MCP-1/CCL2), and growth-regulated oncogene-α (GRO-α/CXCL1) are potent chemotactic agents that have been shown to be constitutively produced by RA synovial fibroblasts and are up-regulated upon stimulation with IL-1β (Szekanecz et al. 2004). Mice with CIA when given EGCG had less severe arthritis, inhibited macrophage infiltration, and the amount of CCL2 synthesizing osteoblasts in arthritic joints (Lin et al. 2008). EGCG has been shown to down-regulate IL-1β induced RANTES, MCP-1, ENA-78, and GRO-α production in human RA synovial fibroblasts (Ahmed et al. 2003, 2006). In addition, it is also reported that green tea blocks the chemokine production but up-regulates chemokine receptor expression in RA-synovial fibroblasts (Marotte et al. 2009). IL-8 is the most powerful chemo-attractant for neutrophils in the target tissue. EGCG is a very effective inhibitor of IL-1β and TNF-α induced IL-8 and macrophage inflammatory protein-3α (MIP-3α) expression in different cell types (Westacott and Sharif 1996; Porath et al. 2005; Netsch et al. 2006). Fractalkine, a chemokine involved in inflammation acts as a chemoattractant as well as an adhesion molecule in endothelial cells activated by pro-inflammatory cytokines (Lee et al. 2009). EGCG decreased TNF-α-induced fractalkine mRNA and protein expression in HUVECs (Lee et al. 2009). Overall these studies suggested that EGCG regulates various Inflammatory and anti-inflammatory cytokines and immune cells in unique ways to reduce inflammation and Table 1 summarizes the reported in vitro and in vivo modulatory effects of EGCG on various catabolic and anabolic mediators related to OA and RA.

Inhibition of inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2) and prostaglandin E_2 (PGE_2) production

One of the hallmark of the inflammatory cytokines IL-1β and TNF-α is to up-regulate the production of nitric oxide (NO), and PGE_2 by stimulating the expression or activities of iNOS, COX-2 and microsomal PGE synthase-1 (mPGES-1) in target cells. High levels of nitrates/nitrites have been found in the synovial fluid and serum of OA and RA patients (Slack et al. 1993; Farrell et al. 1992; Otero and Goldring 2007), which correlated with the increased levels of iNOS expression (McInnes and Liew 2005). Inhibition of iNOS has been shown to exert positive effects in an experimental OA model (Otero and Goldring 2007). EGCG selectively inhibited the production of iNOS in LPS stimulated macrophages (Lin and Lin 1997) and studies from our laboratory have shown that EGCG inhibits NO production in IL-1β stimulated human OA chondrocytes by suppressing iNOS mRNA and protein expression (Singh et al. 2002). Our data also showed that the inhibitory effect of EGCG on the induction and expression of iNOS was in part mediated via inhibition of NF-κ5 in human OA chondrocytes (Singh et al. 2003). Biochemical studies revealed that inhibition of iNOS activity by EGCG was via inhibition of binding of L-arginine and cofactor tetrahydrobiopterins to the active sites of the enzyme (Chan et al. 1997). COX-2 is the rate limiting enzyme in the production of PGE_2 and we have reported that EGCG at 50

μM concentration inhibits the production of PGE_2 via inhibition of COX-2 expression in IL-1β stimulated human OA chondrocytes (Ahmed et al. 2002). On the other hand, Koeberle et al. reported that mPGES-1 is a molecular target of EGCG, and inhibition of mPGES-1 is seemingly the predominant mechanism underlying suppression of cellular PGE-2 biosynthesis by EGCG without any affect on COX-2 up to 30 μM concentrations in vitro (Koeberle et al. 2009). Huang et al. showed down regulation of COX-2 and PGE_2 in IL-1β stimulated synovial fibroblasts (Huang et al. 2009). This inhibitory effect of EGCG on COX-2 expression and activity suggests that EGCG or compound derived from it may be developed for selective and effective inhibition of COX-2 over expression without associated adverse events.

Effects of EGCG on transforming growth factor (TGF)-β expression

The anabolic activity of chondrocytes is sustained by growth factors such as bone morphogenetic proteins (BMP) and TGF-β. TGF-β down regulates the expression of IL-1 receptor (Boumediene et al. 1998) in cultured articular chondrocytes suggesting that TGF-β may be crucial for cartilage homeostasis. This is supported by studies showing that expression of TGF-β receptor II (TGF-βRII) in articular cartilage is dramatically depressed in the rabbit model of OA (Boumediene et al. 1998), and transgenic mice expressing inactive TGF-βRII develop lesions similar to human OA (Serra et al. 1997). OA cartilage break down not only involves IL-1β over-expression but also reduced responsiveness of chondrocytes to TGF-β (Serra et al. 1997). Recently, EGCG was shown to exert a stimulatory effect on TGF-β1, TGF-β2, TGF-βRI and TGF-βRII expression in the presence of IL-1 in mice (Andriamanalijaona et al. 2005). This suggests that EGCG has the potential to prevent the inhibition of TGF-β2 expression and its receptors in diseases such as OA where IL-1β-induced non-responsiveness of chondrocytes to TGF-β may be associated with the absence of cartilage repair (Andriamanalijaona et al. 2005).

Suppression of osteoclast differentiation

Local bone erosion is one of the essential pathological features of RA and osteoclasts seems to play a major role in bone erosion in joints, as evidenced by the lack of bone erosion in experimental arthritis in osteoclast deficient mice (Redlich et al. 2002). Osteoclasts are tartrateresistent acid phosphatase-positive cells in inflamed joints of RA patients (Redlich et al. 2002). Macrophage colony-stimulating factor (M-CSF) and RA synovial fibroblasts produce RANKL which is an essential cytokine for osteoclast development (Teitelbaum et al. 2003). Thus, inhibiting osteoclast development or suppressing M-CSF or RANKL expression in an inflamed joint may be an optimal approach for reducing bone erosion in RA joints (Sato and Takayanagi 2006). EGCG has been shown to be a potent inhibitor of osteoclast development in vitro and in vivo by suppressing the mRNA expression of osteoclast related molecules such as calcitonin receptor, cathepsin H,αv and β3 integrins (Lin et al. 2009; Lee et al. 2010; Morinobu et al. 2008). EGCG selectively down-regulates RANKL induced nuclear factor of activated T cell c1 (NF-ATc1), a transcription factor involved in osteoclast differentiation (Morinobu et al. 2008) suggesting that use of EGCG may be beneficial in suppressing osteoclasts development and activity in arthritic joints.

Antioxidant effects of EGCG in arthritis

Cells of the joint tissue, including chondrocytes, possesses an elaborate antioxidant defense system to cope with the physiological production of ROS and reactive nitrogen species (RNS) and the resulting oxidative stress (Baker et al. 1988). However, excessive and chronic production of ROS and RNS can overwhelm the system and result in oxidative damage to the tissue architecture. Oxygen free radicals in excessive quantities have been identified in synovial fluid of 90% of patients with RA (Kurien et al. 2006). Cytokines such as IL-1β and

TNF-α known to stimulate chondrocytes and synoviocytes to produce high levels of oxygen free radicals (superoxide and hydroxyl radicals) and non-radical species (hydrogen peroxide) (Henrotin et al. 1993). ROS and oxidatively modified proteins and DNA are important mediator in the pathogenesis of arthritic diseases as they modulate intracellular signaling pathways and pro-inflammatory cytokine gene activation and expression (Lo et al. 1996). Several studies have shown that EGCG blunts ROS mediated cytotoxicity in human chondrocytes and other models of oxidative stress (Lo et al.1996; Song et al. 2002; Nie et al. 2002; Bordoni et al. 2002). Investigations from a number of laboratories have demonstrated that green tea polyphenols are efficient free radical and singlet oxygen scavengers (Kurien 1998). Therefore one possible mechanism of the observed chondro-protective effect of EGCG may be the direct ROS scavenging from both intra-cellular and extra-cellular environment. It is now known that the infiltrating leucocytes in arthritic lesions are the major source of NO and H_2O_2 production (Borsiczky et al. 2003) and this can create oxidative stress in the tissue microenvironment. Exposure of different cell types to H_2O_2 activates the MAPKs and H_2O_2-induced activation of MAPKs was inhibited when these cells were pretreated with EGCG indicating that EGCG has the potential to inhibit oxidative stress-mediated phosphorylation and activation of MAPKs in cell types directly relevant to inflammation (Katiyar et al. 2001; Meng et al. 2001). EGCG also considerably increased the gene expressions of catalase, superoxide dismutase, and glutathione peroxidase activities which are essential components of a robust anti-oxidant defense system (Meng et al. 2001). Oxidation of lysine, arginine and proline leads to the formation of carbonyl derivatives that affect the nature and function of the proteins. The presence of carbonylated proteins has become a widely accepted measure of oxidative damage of proteins under conditions of oxidative stress. Elevated blood carbonylated proteins and malondialdehyde with lower levels of blood concentrations of total thiols, glutathione and vitamin C is observed in RA patients and in animal models of arthritis (Kalavacherla et al. 1994; Choi 2007). Shift in the oxidant/antioxidant balance in favor of lipid peroxidation could lead to the tissue damage observed in the disease. A statistically significant increase in the concentration of antioxidants, along with a decrease in the concentration of malondialdehyde was found in RA patients after adjunct treatment with EGCG (Jaswal et al. 2000). Studies have also shown that antioxidant nutrients do protect against cartilage degradation in OA (Tiku et al. 1999; McAlindon et al. 1996). The antioxidant activity of EGCG is also due to its ability to affect the enzyme activity of the antioxidant system (McAlindon et al. 1996). Thus, consumption of a potent anti-oxidant such as EGCG may be helpful in protecting joints from oxidative damage associated with chronic inflammation in arthritis.

Metabolism and Bioavailability of Green Tea Constituents

Methylation, glucuronidation and sulfation of catechins present in the green tea have been observed. In an in vitro study when EGCG was incubated with rat, human or mouse liver microsomes and different human UGT isozymes 6 EGCG glucuronides, namely 4’-O—methyl-EGCG-glucuronide, 4’,4”-di-*O*-methyl-EGCG-glucuronide, EGCG-7-O-glucuronide, EGCG-3’-O-glucuronide, EGCG-3”-O-glucuronide, and EGCG-4”-O-glucuronide, with EGCG-4”-*O*-glucuronide being the major metabolite, were produced in all of the incubations (de Mejia and Ramirez-Mares 2009). Green tea catechins are sparsely absorbed in the small intestine and appear to pass through the biological membranes and are absorbed without deconjugation or hydrolysis. The low absorption of green tea catechins suggests that the majority of catechins, including EGCG, will reach the large intestine where the glycosides will be hydrolyzed into aglycones and various aromatic acids by the microflora (Nakagawa and Miyazawa 1997). Due to their relatively low absorption, rapid metabolism and elimination from the body, consumption of large amounts of flavonols is well tolerated by the humans. At present the safe upper limit for chronic ingestion is about 1 gram of flavonols/day (de Mejia and Ramirez-Mares 2009). The health benefits of tea

consumption in preventing cancer has been intensively investigated (Khan and Mukhtar 2008; Schneider and Segre 2009). However, limited information is available about the protective effect of consumption of green tea or its bioactive components in arthritis.

The bioavailability of EGCG or other catechins is relatively low and this may be due to the short half-life which ranges from 1.87 to 4.58 hours from a 50- to 1600-mg dose (approximately 0.7–23 mg/kg body weight, based on 70 kg body weight) (Ullmann et al. 2003). This might be overcome by repeated administration of EGCG because of its reported low toxicity and high tolerance by human subjects, even when given in doses as high as 1600 mg (vanhet Hof et al. 1999). Drinking one cup of green tea could lead to a level of EGCG of 1 μmol/L in the circulation (vanhet Hof et al. 1999). EGCG at physiological concentrations of 0.1 and 1 μM increased the mRNA expression of the anti-inflammatory cytokine IL-13 by 2.25-and 2.87-fold respectively (Wu et al. 2009). In other studies relevant to arthritis EGCG showed inhibition of T cell proliferation at 2.5 to 10 μM concentration without inducing cytotoxicity or apoptosis (Wu et al. 2009) and a 1600-mg oral dose of EGCG under fasting conditions has been reported to achieve a maximum human plasma level of 7.6 μmol/L (Ullmann et al. 2003). This level is 8 times higher than the highest reported from daily intake of green tea infusion (Ullmann et al. 2003). Further, other factors may be involved in differential response of different human populations to EGCG or other catechins. These may include inter individual variation in the bioavailability of the polyphenols after oral consumption can be substantial due to genetic polymorphism in the enzymes involved in polyphenol metabolism. However, these studies do point out that pharmaceutically prepared formulation of green tea catechins could reach plasma levels equivalent to doses that have been found effective in vitro for the inhibition of inflammatory and joint degrading activities of molecules that are over expressed in arthritis.

Harmful effects of green tea consumption

Tea consumption in general has not displayed any acute or chronic toxic effects, and in fact, it is health promoting. Schwarz et al. (1994) described regular tea drinkers as individuals with a generally healthy lifestyle. However, harmful effects of green tea over consumption could be due to two main factors including (i) caffeine content and (ii) presence of aluminum. Regarding caffeine content, a daylong consumption of green tea improved the cognitive and psychomotor performance of healthy adult in a manner similar to coffee, but green tea contain less caffeine than coffee and is less likely to disrupt sleep quality at night. A cup of green tea contains 40–55 mg caffeine while a cup of coffee has 125–150 mg of caffeine (Willson 1999; McKay and Blumberg 2002). Although, green tea caffeine content is low, the negative effects produced by the overconsumption of caffeine may include nervousness, sleep disorder, vomits, headaches, tachycardia (Bruneton 2001). Studies have also shown that green tea plant has high capacity to accumulate aluminum. Minoia et al. (1994) found concentration of aluminum in green tea (as infusions) accounting for 431–2239 μg/L, which is higher than aluminum concentration of coffee (30.8 μg/L). This knowledge in cases is important of renal failure because aluminum accumulated by the body may results in neurological disorders; therefore drinking green tea of these patients may be counter indicated (Costa et al. 2002). However, black tea contain six fold more aluminum than green tea (Costa et al. 2002) and obviously its consumption too by patients with renal failure should be avoided.

Conclusions

Naturally occurring substances that are derived from diet provide new approaches to the development of therapeutics for chronic diseases like OA and RA. Green tea is regarded as one of the most promising dietary agents for the prevention and treatment of many chronic

diseases. Experimental evidence documenting the health maintenance properties of green tea and its constituents is on the rise. EGCG is the predominant polyphenol in green tea and is regarded as the most active catechin of green tea. Numerous in vitro and in vivo studies have demonstrated that EGCG possesses potent antioxidant and anti-inflammatory activity as it inhibits the activation of MAPKs, AP-1, and NF-κB in different cell types. However, it is becoming clear that the anti-inflammatory effects of EGCG or green tea may be mediated via abrogation of different pathways associated with adjunct inflammatory response. Any single, several, or all the reported biological pathways may yield benefit to reduce the risk of inflammation in OA and RA. Pharmaceutical preparations of EGCG may deliver the in vivo dose equivalent to the concentration used in vitro and may be effective in suppressing inflammation and the catabolic response in arthritic joints. Based on studies reported here use of green tea polyphenols may provide an effective strategy for inhibiting disease progression in OA and RA.

Acknowledgments

This work was supported in part by National Institute of Health/National Centre for Complimentary and Alternative Medicine grant RO1-AT-003267 and R21-AT504615.

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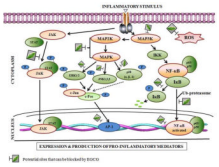

Fig. 1. Effect of EGCG on signal transduction pathways

EGCG regulates inflammation and joint degeneration by modulating MAPKs, AP-1, NF-κB pathway and STAT signaling activated by TNF-α, IL-1β and IFN-γ in various cell types.

Table 1

In vitro and in vivo effects of EGCG on different joint anabolic and catabolic factors.

Cytokines/ mediators	Major activity in joint	Effect of EGCG/Mechanism	Cells /Animal model	Conc. / Dose	Duration	Ref.
Anabolic activity						
TGF-β	Stimulate production of cartilage matrix components	↑ TGF-β1 and TGF-β2 expression in IL-1 induced chondrocytes	Bovine articular chondrocytes	20 or 50 μM, EGC	24 h	Andriamanalijaona et al. 2005
BMP	growth and differentiation factor which induces cartilage or bone formation	↑ cartilage formation in joints using BMP/Fibrous glass membrane implants	Wistar rats	1 or 10μg EGCG	1 to 2 weeks	Takita et al. 2002
Catabolic activity						
IL-1β	Potent inducer of cartilage degradation, bone resorption, MMPs, COX-2, iNOS and PGE_2	↓ expression and level of IL-1β in cutaneous wound	Mouse	50mg/L	72 h	Shen et al. 2009
TNF-α	Similar activity profile to IL-1β but relatively less potent, induces osteoclatic bone resorption more prominent role in RA	↓ PMACI-induced TNF-α expression and production	HMC cells	100 μM	-	Shin et al. 2006
		↓ AGEs-induced TNF-α expression and production	Human OA chondrocytes	25–150 μM	24 h	Rasheed et al 2009
		↓ TNF-α expression and production	PC-9 cells	26 μM	-	Fujiki et al. 2003
			T lymphocytic leukemia cells	10 μg/ml	24 h	Danesi et al. 2010
Modulators of anabolic and catabolic activity						
IL-6	A mediator of OA and RA both, IL-1 induced IL-6 activity is required to inhibit proeoglycan synthesis; involves STAT-3 signaling pathway	↓ IL-1 induced IL-6 production by enhanced production of sgp130	RA-synovial fibroblasts	10–20 μM	24 h	Ahmed et al. 2008
		↓ PMACI-induced IL-6 production	HMC cells	100 μM	-	Shin et al. 2006
		↓ STAT-3 signaling	Keloid fibroblasts	100 μM	24 h	Park et al. 2008
IL-8	Neutrophil chemo-attractant. Stimulates neutrophils to produce superoxide ions	↓ TNF-α-induced IL-8 expression and production	T84 and HT-29 cells	25–50 μM	24 h	Porath et al. 2005
		↓ IL-1β induced IL-8 expression and production	Caco-2 cells	4.02–160 μM	24, 48, 72 h	Netsch et al. 2006
			Synovial fibroblasts	50 μM	12 h	Huang et al. 2009
			HMC cells	100 μM	-	Shin et al. 2007
			Human nasal mucosal Fibroblasts and A549 cells	2, 10, and 50 μg/ml	24 h	Kim et al. 2006
bFGF	Potentiate IL-1 induced protease release by chondrocytes; also secreted by tumor cells	↓ basic fibroblast growth factors via proteosome inhibition	HCT-116 and LoVo cancer cells	50μmol/L	24 h	Sukhthankar et al. 2008
			mice	0.01% in water	2 month	
IFN-γ	Conflicting evidences for presence in OA but have a role in RA; involves	↓IFN-γ induced STAT-1 via interferon regulating factor-1 (IRF-1)	MCF7, HepG2 and HeLa cells	5–20 μM	3 h	Tedeschi et al. 2002

Cytokines/ mediators	Major activity in joint	Effect of EGCG/Mechanism	Cells /Animal model	Conc. / Dose	Duration	Ref.
	STAT signaling induces iNOS and COX-2 expression	↓ STAT-3 activation	MDA MB 231 cells	50 μM	1 h	Menegazzi et al. 2001
			Keloid fibroblasts	100 μM	24 h	Park et al. 2008
Mcl-1	Anti-apoptotic protein; increase expression is observed in RA	↓ TNF-α induced Mcl-1 production	RA-synovial fibroblasts	5–50 μM	72 h	Ahmed et al. 2009
MCP-1, ENA-78 GRO-α, MIP-3α	potent chemotactic agents that have been shown to be constitutively produced by RA synovial fibroblasts and up-regulated upon stimulation with IL-1β, major role in inducing MMPs activity in RA-synovial fibroblasts	↓ IL-1β induced MCP-1, ENA-78, GRO-α and MIP-3α production via specific inhibition of protein kinase Cδ (PKCδ) phosphorylation and NF-κB activation and nuclear translocation.	RA-synovial fibroblasts	10–50 μM	24 h	Ahmed et al. 2006
		↓ PMA-induced MCP-1 expression and production via inhibition of p38 MAPK and NF-κB activity	ECV304 cells	5–30 μM	4 h	Hong et al. 2007
		↓ MCP-1 expression and production	THP-1 cells	100 μM	8 h	Melgarejo et al. 2009
IL-17	Inflammatory cytokine have a role in RA	↓ IL-23 induced IL-17 production	Kit-225 cells	10 μg/mL	24 h	Danesi et al. 2010
MMPs	Principal proteases involved in proteolytic degradation of collagen present in cartilage; induces anti-inflamatory cytokine expression	↓ IL-1β induced MMP-1 and 13 expression and production via NF-κB and AP-1	Human OA chondrocytes	100 μM	24 h	Ahmed et al. 2004
		↓ AGEs- induced MMP-13 expression and production via suppression of p38- and JNK MAPKs and NF-κB activation	Human OA chondrocytes	25–150 μM	24 h	Rasheed et al. 2009
		↓ TNF-α- induced MMP-1 and -3 mRNA and protein production via suppression of p38-, ERK and JNK MAPKs and AP-1 phosphorylation	RA-Synovial fibroblasts	125–500 nM	24 h	Yun et al. 2008
		↓ IL-1β induced MMP-1 and 3 expression and production via JNK/ SAPK phosphoryllation and NF-κB activation	Human tendon-derived fibroblasts	2.5–25 μM	18 h	Corps et al. 2004
		↓ MMP-1 and 3 production, MMP-2 and MMP-9 activity and ↑ TIMP-1 expression	Fibroblasts	0.01–1 μM	24 h	Lee et al. 2005
ADAMTS	Principal proteases involved in aggrecan degradation in cartilage	↓ ADAMTS 1, 4, and 5	Cell free medium	2 nM-2 μM	3 h	Vankemmelbeke et al. 2003
IL-13	Th2 anti-inflammatory cytokine have important immunoregulatory role	↑ IL-13 mRNA expression via JNK-dependent NFATc1 pathway	KU812 cells	0.1–1 μM	11–47 h	Wu et al. 2009
COX-2, PGE-2, iNOS and NO	High levels are associated with OA and RA pathogenesis	↓IL-1β-induced iNOS expression and NO production via suppression of NF-κB activation	Human OA chondrocytes	1–100 μM	12–24 h	Singh et al. 2002
		↓IL-1β-induced iNOS, COX-2 expression NO and PGE2 production	Human OA chondrocytes	20–200 μM 5–10 μM	24 h	Ahmed et al. 2002

Cytokines/ mediators	Major activity in joint	Effect of EGCG/Mechanism	Cells /Animal model	Conc. / Dose	Duration	Ref.
		↓LPS-induced iNOS expression NO production	Macrophages cells	2.5–15 μM	2–24 h	Lin and Lin 1997

BMP, bone morphogenic protein ; TGF-β, transforming gowth factor-β ;bFGF, basic fibroblasts growth factor; IFN-γ, interferon gamma; Mcl-1, myeloid cell leukemia sequence 1;MIP-3α, macrophage inflammatory protein 3 alpha; GRO-α, growth regulated oncogene-alpha; ENA-78, epithelial neutrophil activating protein 78; MCP-1, monocyte chemotactic protein-1 ; NF-κB, Nuclear factor-κB, MAPK, mitogen activated protein kinases;AP-1, activator protein-1; MMP, matrix metalloproteinase; ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs ;NFATc1, Nuclear factor activated T cell-1; IL, interleukin; COX-2, cyclooxygenase-2; PGE2, prosglandin E2; iNOS, inducible nitric oxide synthase; NO, Nitric oxide.