Biological Evidence for the Benefit of Green Tea and EGCG in Arthritis

Salahuddin Ahmed*

Department of Pharmacology, College of Pharmacy, University of Toledo, OH, USA

Abstract: The use of complimentary and alternative medicine (CAM) approaches is becoming increasingly popular among patients with rheumatoid arthritis (RA) and osteoarthritis (OA). Arthritis is a leading cause of work-related disabilities affecting approximately 1.0% of the United States population. The lack of adequate response combined with increased risk of adverse events to conventional therapy in RA or OA patients has prompted interest in evaluating CAM options for arthritis. In this regard, the last decade has shown a growing popularity of green tea (GT) in the management of arthritis. Researchers are studying GT and its constituents to provide scientific rationale for its benefit in arthritis. This review summarizes the disease pathogenesis and novel therapeutic targets for the treatment of arthritis. I also tried to address the current treatment options that are available as well as their limitations. Finally, this article reviews the emerging role of GT and its polyphenol, epigallocatechin-3-gallate (EGCG), in arthritis. Although the recent findings provide scientific evidence of the efficacy of GT or EGCG in several in vitro and in vivo models of arthritis, further preclinical studies to validate its safety profile and additional phase-clinical trials in RA patients are warranted to authenticate its beneficial effect in arthritis and possibly other rheumatic conditions.

Keywords: Green tea, EGCG, rheumatoid arthritis, osteoarthritis, alternative medicine.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by robust infiltration of leukocytes into the synovium, resulting in hyperplasia of the synovial lining, formation of lymph follicles, and development of the mature pannus [1]. This results in progressive cartilage destruction and, finally, erosion of the underlying bone. The severe morbidity and structural damage of joints caused by chronic inflammation require early and effective treatment [2, 3]. For patients, RA causes chronic joint pain and stiffness with eventual deformity and progressive difficulties with activities of daily living [4].

RA affects ~ 1% of the adult population, with more women being afflicted than men [5, 6]. Recent reports suggest that patients with RA not only have a higher chronic disease burden but may also have increased morbidity and mortality from cardiovascular disease compared with persons without RA [7]. The high prevalence of the disease, expensive long-term treatments, disability, and loss of productivity has made RA a significant health challenge. An estimated 43 million Americans were diagnosed with RA in late 1990s, with the number expected to increase to an estimated 60 million by the year 2020 as the population ages [5]. RA pathogenesis is regulated by proinflammatory cytokines such as interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-α (TNF-α) that activate a broad array of intracellular signal transduction mechanisms [1, 8, 9]. In RA, migration of leukocytes into the synovial tissue (ST) occurs. These leukocytes and other cells in the ST, particularly RA synovial fibroblasts, produce several mediators of inflammation, including chemokines, chemotactic cytokines that recruit leukocytes to the inflamed joint and also play a role in angiogenesis [10].

Treatment for RA has gradually improved, but is still far from ideal. Many of the currently available therapeutic options for RA were introduced empirically, have significant toxicities and lack efficacy over time [5]. Recently, biologically-based antagonists of cytokines (TNF-α, and IL-1β) have been introduced into clinical practice [5]. While it has been shown that blocking TNF-α results in reduced joint inflammation in RA, still around 25% of patients fail to respond to TNF-α inhibitors [11]. It has also been shown that mice lacking the TNF-α gene can still develop severe joint inflammation and destruction [12]. IL-6 is a pleiotropic cytokine that plays pathological role in arthritis with a wide range of biological activities, including immunoregulation, mediation of acute-phase responses, and effects on bone metabolism [13-15]. Despite its important physiological roles, dysregulated overproduction of IL-6, resulting in the elevated levels of IL-6 in serum and synovial fluid of RA patients, has been shown to correlate with clinical and laboratory indices of disease activity [15]. Animal studies have shown that IL-6 deficiency suppressed the development of arthritis in SKG mice and resulted in DBA1/J mice becoming resistant to collagen-induced arthritis [16, 17]. In humans, a humanized monoclonal antibody against the IL-6 receptor α (IL-6Rα), tocilizumab, was shown to be effective in the clinical trials for RA [18]. This has intensified the research in developing more effective therapies for RA, besides presently available treatment options, which may also be of therapeutic value in other chronic inflammatory conditions.

Novel therapies in particular biological agents, have resulted in major breakthroughs in the treatment of RA [19]. However, these new and promising biological agents show no evidence of sustained benefits after the termination of therapy. The majority of therapeutic agents available target

*Address correspondence to this author at the Department of Pharmacology, 2232 Wolfe Hall, College of Pharmacy, 2801 W. Bancroft Street, Toledo, OH 43606, USA; Tel: 419-530-1913; E-mail: Salah.Ahmed@utoledo.edu
the inflammatory cells in the joint, including macrophages, T and B lymphocytes, vascular endothelium, and the activated synovial fibroblasts. RA synovial fibroblasts are important mediator cells in joint destruction since they are hyperactive in the diseased condition and show invasive growth into the adjacent tissue [20]. Together, these limitations indicate an opportunity to test alternative approaches that may be explored further in an effort to develop successful preventive and/or therapeutic strategies for the treatment of RA.

Similar to RA, osteoarthritis (OA) of the knee and hip is a chronic joint disorder in which the risk increases precipitously with age. The severity of OA differs from patient to patient, but very common clinical symptoms include pain, reduced range of motion, inflammation and deformity [21]. The high prevalence of OA with its associated loss of joint function results in expensive and long-term conventional therapies that pose a significant socioeconomic burden. Although OA is not considered as inflammatory as RA, symptoms of local inflammation and synovitis are present in many patients with OA and are also seen in animal models of OA [22]. The presence of elevated levels of cytokines such as IL-1 and TNF-α has been demonstrated in OA synovial fluid, and it has been shown that these proinflammatory cytokines can stimulate the expression of inflammatory mediators and matrix degrading metalloproteinases (MMPs) in an arthritic joint [23].

TREATMENT OF ARTHRITIS

Traditionally, anti-inflammatory strategies have been broadly divided between two approaches: acting outside of the cell and inside the cell, respectively [24]. The first approach targets receptors and cytokines using antibodies or cytokine traps (e.g., anti-TNF-α or soluble receptor antagonist of IL-1 (IL-1Ra) or IL-6 (sIL-6R) therapies) to block ligand/cell-surface receptor interactions. In 1999, Enbrel® (Etanercept) and Remicade® (Infliximab), anti-TNF-α therapies [25], signaled a new concept for RA treatment. This was followed by the development of biological therapy against IL-6 in the form of humanized monoclonal antibody against IL-6Ra, Actemra® (tocilizumab) [13]. The second approach uses small-molecule inhibitors such as cyclosporine, cyclooxygenase-2 (COX-2) inhibitors, and steroids that easily enter the cell. Such intracellular interference with signaling pathways has generated very effective and wide-ranging anti-inflammatory therapeutics, with the caveat that they also work as general immunosuppressant [26]. Along these lines, the majority of cytokine or chemokine networks could be intervened by both approaches. Current treatment options for RA include fast-acting non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin and the disease modifying corticosteroids (e.g. prednisolone), and slow-acting disease-modifying anti-rheumatic drugs (DMARDs). These include methotrexate, gold salts, hydroxychloroquine, sulfasalazine, and azathioprine. Although DMARD use decreases markers of inflammation such as swollen joint counts, their long-term use is sometimes unacceptable due to their limited effectiveness, poor tolerability, significant toxicity, and overall inability to slow the irreversible joint destruction and progression of RA. Pharmacological management of OA includes analgesics and NSAIDs. However, their use only provides symptomatic relief from pain and does not affect the progress of OA in some patients.

In addition, with the recent understanding in the chemokinobiology, the novel approach of targeting chemokines and chemokine receptors was boosted in 1998 when potent small-molecule antagonists for CXCR2 (by SmithKline Beecham, now GlaxoSmithKline) and CCR1 (by Berlex Biosciences) were developed [27, 28]. This spurred a rigorous search for potent small-molecule antagonists which may act specifically on a wide variety of therapeutically relevant chemokine receptors (CCR1, 2, 3, 5 and 10, and CXCR2, 3, and 4) [29].

EMERGING ROLE OF ANTI-INFLAMMATORY BOTANICALS IN TREATING ARTHRITIS

Despite early detection of RA, the present treatment modalities are limited to the symptomatic relief from pain with no effect on disease progression, and often compounds have toxic effects. A number of safety concerns have been raised since the introduction of anti-TNF-α therapies which included complications such as sepsis, drug-induced systemic lupus erythematosus (SLE), demyelination, tuberculosis, and lymphomas [30, 31]. The long-term risks and benefits of these drugs are not yet known and the extent of these side effects need to be determined and carefully monitored. These treatments have additional disadvantages, including the need for repeated injections and their relatively high cost compared to small organic chemical drugs. Consequently, there has been a growing interest in the use of alternative treatments and herbal therapies, as shown by a recently shifting trend in which 60-90% of dissatisfied arthritis patients are likely to seek the option of complementary and alternative medicine (CAM), mostly phytotherapy. Phytotherapy is a broad category of medications that includes drugs used in traditional systems of medicine, folkloric and ethnomedicinal products, as well as drugs discovered from plants having no documented therapeutic use [33]. Despite the fact that modern organic chemistry provides us numerous synthetic compounds for medicinal use, many important drugs are still extracted from medicinal plants [34]. Aspirin, quinine, quercetin, oltipraz, and tamoxifen are a few examples of what traditional medicine has given us in the past. Careful documentation of traditional knowledge, together with extensive modern scientific/pharmacological experimentation, is necessary to achieve a true validation of the efficacy of any botanical with purported medicinal value.

In this regard, identification of common dietary substances capable of affording protection or modulating the onset and severity of arthritis may have important human health implications. Recently, some studies have reported positive therapeutic effects of naturally occurring and synthetic compounds on the animal models of arthritis and in vitro conditions. Inhibition of collagen-induced arthritis (CIA) has been reported in taxol, an active principle from Taxus baccata treated mice [35]. In another study, the use of an extract of cat’s claw (Uncaria tomentosa) from the part of the vine that is rich in pentacyclic alkaloids (roots) showed a reduction in the number of painful joints in
patients with RA, when compared to placebo, and was found relatively safe and beneficial to the tender joints [36]. Studies have shown that curcumin, a dietary constituent and an anti-inflammatory agent, blocks mediators of inflammation in the joints by inhibiting the activation of nuclear factor kappa B (NF-κB) and activation protein-1 (AP-1) [37]. In a double-blind crossover clinical trial of 18 patients with RA given curcumin (1200 mg/day) for 2 weeks followed by 300mg/day of phenylbutazone for another 2 weeks, respondents showed a significant improvement in morning stiffness, walking time and reduction in joint swelling [38]. In another in vitro study, an extract of Zingiber officinale (ginger) showed inhibition of PGE2, TNF-α, and COX-2 expression in human synovial fibroblasts by regulating NF-κB activation and IκB-α degradation [39]. Ginger extract administered daily for 4 weeks, either orally or intraperitoneally, caused significant reduction in PGE2 levels in rats [40]. In a study reported by Srivastava and Mustafa, ginger was effective in relieving pain and swelling in the joints of seven RA patients [41].

A prospective, double-blind, placebo-controlled study of Tripterygium wilfordii Hook F (TwHF) ethanol/ethyl acetate extracts was performed in RA patients. Patients achieved an ACR-20 response with 50% of patients showing a significant decrease in the number of tender and swollen joints within the first 4 weeks [42]. In another phase-I study, eight of nine patients treated with TwHF extract (> 360 mg/day) showed improvements in both clinical manifestations and laboratory findings [43]. In animal studies, triptolide, an active constituent of TwHF, inhibited the arthritis in animal models [44, 45] and markedly suppressed the production of proMMP-1 and -3 in cultured synovial fibroblasts and mouse macrophages [46].

GREEN TEA (CAMELLIA SINENSIS) IN ARTHRITIS

Tea (Camellia sinensis), especially green tea, is one of the most commonly consumed beverages in the world with no reported side effects [47]. Green tea is sold as fresh or dried unfermented leaves. Dried leaves are composed mainly of polyphenols known as catechins (30-36% of dry weight). The main catechins found in green tea are epicatechin (EC), epigallocatechin (EGC), epigallocatechin 3-gallate (EGCG) and epicatechin 3-gallate (ECG) [48]. The established pharmacological properties of green tea are attributed to its high content of EGCG, which constitute up to 63% of total catechins in green tea (Fig. 1) [48]. EGCG has been shown to possess about 25-100 times more antioxidant potential than Vitamins C and E [49]. A cup of green tea typically provides 60-125mg of catechins including EGCG, and 20-40mg of L-theanine [50].

BENEFITS OF GREEN TEA IN RA

Extensive studies in the past two decades have verified the antioxidant, anti-inflammatory, and cancer chemopreventive properties of a polyphenolic mixture derived from green tea (GT) in many animal tumor bioassay systems [51]. The potential disease-modifying effect of green tea on arthritis came to light when it was shown that consumption of GT in drinking water ameliorated CIA in mice [52]. The reduced CIA incidence and severity was reflected in a marked inhibition of the inflammatory mediators COX-2, interferon-γ (IFN-γ), and TNF-α in arthritic joint of GT-fed mice. Additionally, total immunoglobulins (IgG) and type II collagen-specific IgG levels were found to be lower in serum and arthritic joints of GT-fed mice. Overall, this study suggested that polyphenols present in GT extract may be

**Fig. (1).** Mechanisms of arthritis suppression by green tea-EGCG.
useful in preventing the onset and disease severity of RA. Since then, we and others have extensively evaluated the efficacy of EGCG in animal models of RA and also in the synovial fibroblasts isolated from human joints to provide the exact mechanism through which antioxidants present in GT inhibit or suppress RA. Our study showed that EGCG pretreatment significantly inhibited both the constitutive and IL-1β-induced chemokine (MCP-1/CCL2, RANTES/CCL5, Gro-a/CXCL1, and epithelial neutrophil-activating peptide 78, ENA-78/CXCL5) production and MMP-2 activation by RA synovial fibroblasts. Interestingly, we found that EGCG distinctly inhibited IL-1β-induced protein kinase C (PKC)δ and NF-kB pathways to elicit its response [53]. Furthermore, we also found that EGCG significantly inhibited MMP-2 activity induced by RANTES/CCL5, Gro-a/CXCL1, and ENA-78/CXCL5, suggesting a novel mechanism of MMP-2 regulation by EGCG in RA synovial fibroblasts in vitro [53].

Another recent study using human osteoblastic cells evaluated the effect of EGCG on oncostatin M (OSM)-induced MCP-1/CCL2 synthesis [54]. The results of the study showed that EGCG at 10 μg/ml concentration inhibited OSM-induced MCP-1/CCL2 synthesis in human osteoblastic cells and MG-63 cells [54]. More experiments in the study suggested that EGCG attenuated AP-1-MCP-1/CCL2 promoter interaction by reducing c-Fos synthesis. Further in vivo testing of EGCG in rat CIA model showed that intraperitoneal administration of EGCG (20 mg/kg) resulted in amelioration of CIA, macrophage infiltration, and the amount of MCP-1/CCL2 synthesizing osteoblasts [54]. In a recent study, Yun et al. showed EGCG treatment resulted in dose-dependent inhibition of TNF-α-induced production of MMP-1 and MMP-3 at the protein and mRNA levels in RA synovial fibroblast [55]. EGCG treatment also inhibited TNF-α-induced phosphorylation of ERK1/2, p38, JNK. DNA binding activity assay revealed that EGCG inhibits binding of AP-1 proteins to its response elements in treated synovial fibroblast, suggesting its role in regulating inflammation and bone destruction in RA patients [55].

Recent pharmacological studies using EGCG to inhibit or suppress arthritis have focused more on bone resorption observed in RA [56-59]. In 2006 Haijees et al. showed that GT polyphenols may be effective in inhibiting uncontrolled growth of osteocarcoma using SaOs-2 cells [60]. The study also found that GT extract at the dose of 20-60 μg/ml caused a time- and dose-dependent inhibition in the proliferation rate of SaOs-2 cells [60]. It was also shown that GT polyphenols triggered caspase-3 dependent apoptosis in these cells by regulating the constitutively active NF-κBp65 to induce BAX/BAX fragmentation and apoptosis [60]. A recent study by Momobu et al. showed that EGCG treatment reduced the bone resorption as determined by tartrate-resistant acid phosphatase (TRAP) staining [57]. The study also showed that EGCG at 20μM concentration was effective in inhibiting the generation of TRAP-positive multinucleated cells, bone resorption activity, and osteoblast-specific gene expression of nuclear factor of activated T cells (NF-ATC1), but not of NF-κB, c-Fos, and c-Jun [57]. However, the in vivo effect of osteoclast differentiation in CIA mice was not clear, as EGCC (20 mg/kg) inhibited inflammation in experimental arthritis [57]. We further studied the mechanism through which EGCG inhibits inflammation and tissue destruction in RA. Our novel finding showed that EGCG inhibits IL-6 synthesis both in human RA synovial fibroblast cultures and rat adjuvant-induced arthritis (AIA) model [61], thus providing a missing link to the reduction in inflammation observed in earlier studies. Our study also showed that EGCG enhances the synthesis of soluble gp130 protein (sgp130), an endogenous inhibitor of IL-6 signaling and transsignaling, by inducing alternative splicing in gp130 gene, resulting in enhanced sgp130 synthesis [61]. Intra-peritoneal administration of EGCG (100 mg/kg) during the onset of arthritis in rats showed a specific inhibition of IL-6 levels in the serum and joints of EGCG-treated animals by 28% and 40%, respectively. The study also revealed that EGCG inhibited IL-6/sIL-6R-induced MMP-2 activity in human RA synovial fibroblasts, which correlated to the inhibition of MMP-2 activity in the joints of EGCG treated animals when compared to the activity level in arthritic rats.

Overexpression of the antiapoptotic Bcl-2 proteins in RA synovial fibroblasts, in particular myeloid cell leukemia-1 (Mcl-1) protein, is a major cause of their resistance to apoptosis [1, 62]. We recently undertook a study to evaluate the efficacy of EGCG in down-regulating Mcl-1 expression and its mechanism of RA synovial fibroblast sensitization to TNF-α-induced apoptosis. This study showed that in RA synovial fibroblasts, EGCG (5-50 μM) inhibited constitutive and TNF-α-induced Mcl-1 protein expression in a concentration- and time-dependent manner [63]. Importantly, EGCG specifically abrogated Mcl-1 expression in RA synovial fibroblasts and affected Mcl-1 expression to a lesser extent in OA or normal synovial fibroblasts or endothelial cells. Inhibition of Mcl-1 by EGCG caused activation of caspase 3 in RA synovial fibroblasts, which was mediated via down-regulation of the TNF-α-induced Akt and NF-κB pathways [63]. Caspase 3 activation by EGCG also suppressed RA synovial fibroblast growth, and this effect was mimicked by Akt and NF-κB inhibitors. Interestingly, Mcl-1 degradation by EGCG sensitized RA synovial fibroblasts to TNF-α-induced cleavage of poly ADP-ribose polymerase (PARP) protein and apoptotic cell death. Our finding suggests that EGCG may induce apoptosis and further sensitize RA synovial fibroblasts to TNF-α-induced apoptosis, and hence may be of therapeutic value in regulating the invasive growth of synovial fibroblasts in RA.

**BENEFIT OF GREEN TEA IN OA**

Soon after a discovery that GT polyphenols may inhibit arthritis in murine model, the majority of studies were focused on its beneficial effect on progressive cartilage degradation, a hallmark of OA that is observed in RA as well [64-66]. Proinflammatory cytokines such as IL-1β, TNF-α, and IL-6 have been shown to modulate extracellular matrix (ECM) turnover, accelerate the degradation of cartilage, and induce apoptosis in chondrocytes [64-66]. Besides promoting imbalance between excessive cartilage destruction and cartilage repair processes, IL-1β has been a potent inducer of reactive oxygen species (ROS), including nitric oxide (NO) and inflammatory mediators such as PGE2, via enhanced expression of enzymes inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), respectively [67, 68]. Several studies have shown that most of the effects of GT extract are mimicked by its primary constituent polyphenol EGCG [51]. Consequently, we further studied

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this compound and found that in human chondrocytes derived from OA cartilage, EGCG inhibited the IL-1β-induced iNOS and COX-2 expression and activity, which resulted in the reduction of NO and PGE2 synthesis [68, 69]. Further evaluation of the signaling pathways through which EGCG inhibits iNOS expression both at transcriptional and translational levels revealed that EGCG inhibits IL-1β-induced phosphorylation and consequent degradation of IkBα, an endogenous inhibitor of transcription factor NF-κB, resulting in the suppression of NF-κB nuclear translocation [69]. The reduced translocation of NF-κB to the nucleus resulted in decreased DNA binding activity of NF-κB to suppress iNOS gene expression. In an another study, Singh et al. showed that EGCG selectively inhibited the IL-1β-induced phosphorylation of c-Jun-N-terminal kinase (JNK) p46 isoform, resulting in lower levels of phospho-c-Jun and DNA binding activity of AP-1, a transcription factor implicated in the inflammatory response, in human OA chondrocytes [70].

MMPs play a crucial role in tissue remodeling as well as in the destruction of cartilage and bone in an arthritic joint due to their ability to degrade a wide variety of extracellular matrix components [71]. Among the various MMPs, MMP-1 and MMP-13 are of particular importance because they are found elevated in joint disorders and can more efficiently cleave type II collagen, the major component of the cartilage matrix [72-74]. In chondrocytes, JNK pathway and the transcription factor AP-1 were shown to be necessary for MMP activation, and their pivotal role in the pathogenesis of arthritis is also evident from studies showing that the inhibitors of JNK kinase protect animals from developing experimentally-induced arthritis [75, 76]. EGCG has been shown to inhibit the activities of MMP-2 and MMP-9 [53, 77]. In a recent study, we evaluated the potential of EGCG to protect human cartilage explants from IL-1β-induced release of cartilage matrix proteoglycans and the induction and expression of MMP-1 and MMP-13 in human chondrocytes [78]. Results from the study showed that pretreatment of cultured human OA chondrocytes with EGCG significantly inhibited the expression and activities of MMP-1 and MMP-13 at a physiologically achievable dose [78]. It has also been shown that EGCG was similarly effective in inhibiting IL-1β-induced MMP-1, -3, and -13 in human tendon fibroblasts [79]. Also, it was recently shown that catechins from green tea inhibited the degradation of human cartilage proteoglycan and type II collagen, and selectively inhibited the aggrecanases ADAMTS-1, -4, and -5 [80, 81]. These findings defined a novel role of green tea constituents in the inflammatory response, in human OA chondrocytes [82].

CONCLUSIONS

Current treatment modalities for joint diseases are able to provide symptomatic relief from pain and discomfort, but there are few pharmaceutical-based treatments that have proven to regain the functionality or slow down the progression of tissue invasion and joint destruction observed in arthritis. Although biological therapies have revolutionized the treatment of RA, their high costs for long-term treatment have limited their clinical appeal and still a majority of patients in the United States and other parts of the world live with pain and disabilities associated with arthritis or undergo joint replacement surgery. This limitation, along with the risk of developing side effects with these therapies, has given patients an option to try nutraceuticals or dietary supplements that are generally regarded safe and have not shown significant adverse effects over long-term consumption [83]. This has also encouraged scientists and researchers to evaluate and prove evidence of the beneficial effects of such agents to validate their efficacy and safety in patients with joint disorders. Since the area CAM research is in budding stages, it will require further maturing to provide some extensive scientific evidence at the cellular and molecular levels to explain the mechanisms of action of such CAM options. We and others have extensively studied GT and EGCG in recent years for their efficacy in RA and OA, with the help of in vitro and in vivo models of inflammation and joint destruction. However, further pre-clinical studies and additional clinical trials are needed to elucidate the dose and mechanisms through which GT or EGCG may afford protection to the progressively degrading tissue in human arthritic joints. Furthermore, the interaction of GT or EGCG with the conventional medicines for RA or OA is of major concern to the medical community for verifying its applicability in treating the inflammatory symptoms with concomitant reduction in the disease pathogenesis.

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