

Pharmacological Aspects of Natural Quercetin in Rheumatoid Arthritis

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Abstract: Rheumatoid arthritis (RA) is a chronic autoimmune disease that can lead to severe joint damage, disability and mortality. Quercetin (QUE) is a natural flavonoid that is ubiquitous in fruits and vegetables. This article reviews the effect of QUE on articular and extra-articular manifestations of RA in vitro and in vivo. In general, for articular manifestations, QUE inhibited synovial membrane inflammation by reducing inflammatory cytokines and mediators, decreasing oxidative stress, inhibiting proliferation, migration and invasion, and promoting apoptosis of fibroblast-like synoviocytes (FLS), regulated autoimmune response through modulating Th17/Treg imbalance and Th17 cells differentiation, reducing autoantibodies levels and regulating ectonucleoside triphosphate diphosphohydrolase (E-NTPDase)/ectoadenosine deaminase (E-ADA) activities, reduced bony damage via lowering matrix metalloproteinase (MMP)-1, MMP-3, receptor activator of nuclear factor kappa B ligand (RANKL) expression and osteoclasts formation. For extra-articular manifestations, QUE could reverse the neurodegenerative processes of the enteric nervous system (ENS) and exhibited cytoprotective, genoprotective and hepatoprotective effects. In addition, we also summarize some contradictory experimental results and explore the possibility for these differences to form a sound basis for the clinical application of QUE for RA.

Keywords: rheumatoid arthritis, quercetin, pharmacological

Introduction

Rheumatoid arthritis (RA), a systemic inflammatory autoimmune disease, is more common in women than men with a worldwide prevalence of 0.5%–1.0%.¹ RA is featured with progressive cartilage and bone destruction by invasive hyperplastic synovial membrane, leading to an increased risk of disability and mortality.² Though current therapeutic options have, to some extent, improved the prognosis of RA, the pharmacological treatment seems to be continuously inadequate in preventing the progression of RA; therefore, new anti-arthritis therapies are essential.^{3,4} Currently, there is growing interest in the pharmacological potential of natural products.

Quercetin (QUE) is a type of flavonoid, which is ubiquitous in fruits and vegetables, such as onion, apples, beans and various berries.⁵ A growing body of evidence has shown the anti-hypertensive,⁶ anti-inflammatory,⁷ anti-angiogenic,⁸ anticancer,^{9–11} hepatoprotective,¹² anti-diabetic,¹³ anti-aging,¹⁴ and neuroprotective¹⁵ potential of QUE. In addition, QUE is also proved to be effective in management of RA in pre-clinical or clinical studies. In this review, we discuss the effect of QUE on articular and extra-articular manifestations in RA.

Pharmacological Mechanism of QUE in Pre-Clinical Studies (Figure 1)

The zymosan-induced arthritis, collagen-induced arthritis (CIA) and adjuvant-induced arthritis (AA) models are commonly used animal models of RA, which is helpful for understanding the complex pathogenic mechanisms involving inflammation, autoimmunity, and cartilage and bone destruction in RA.^{16,17}

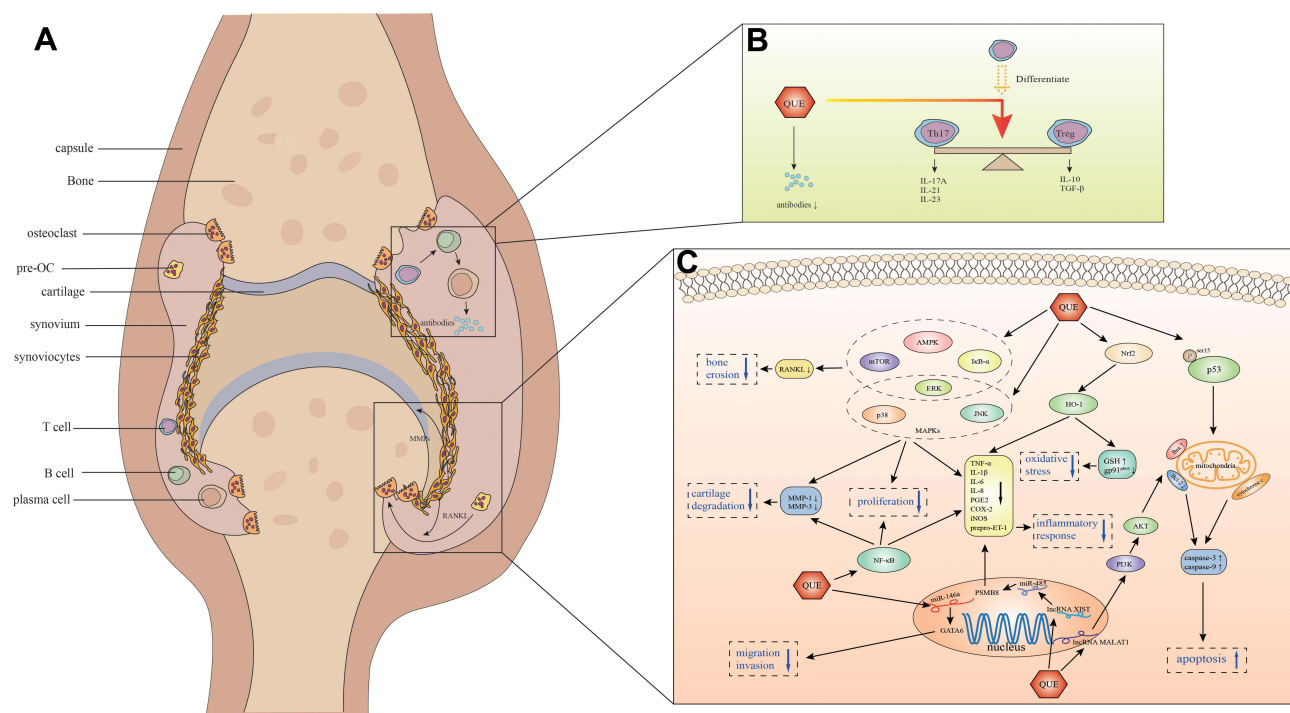


Figure 1 The anti-articular effect of QUE on RA in pre-clinical studies. **(A):** Rheumatoid arthritis joint. **(B): The immune-regulatory effect of QUE on RA:** QUE regulated Th17/Treg cell balance, decreased Th17 cells-related cytokines (IL-17A, IL-21 and IL-23), increased Treg cells-related cytokines (IL-10 and TGF-β), and reduced the autoantibodies levels. **(C): The anti-inflammatory and bone protective mechanisms of QUE on RA: Anti-inflammatory effect:** QUE decreased pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, IL-8, PGE2, COX-2, iNOS, and prepro-ET-1) by regulating MAPKs (ERK, p38, JNK), NF-κB and Nrf2/HO-1 signaling pathway as well as lncRNA XIST/miR-485/PSMB8 axis; QUE decreased gp91^{phox} expression, increased GSH levels through regulating Nrf2/HO-1 signaling pathway, indicating the participation of HO-1 in the anti-inflammatory effect of QUE; QUE inhibited FLS proliferation via inhibiting the activation of intracellular MAPKs (ERK, p38, JNK) and NF-κB signaling pathways; QUE inhibited the migration and invasion of FLS, which might be related to the up-regulatory effect on miR-146a and the down-regulatory effect on the downstream target GATA6; Besides, QUE upregulated lncRNA MALAT1, inducing the inhibitory effect on the activation of PI3K/AKT signaling pathway, leading to caspase-3, caspase-9, and Bax elevated, Bcl-2 diminished, the Bcl-2/Bax ratio decreased, ultimately to promote RAFLS apoptosis; QUE could also enhance p53 phosphorylation at ser15, leading to caspase-3 and caspase-9 activated, Bcl-2 expression diminished, Bax expression elevated, the Bcl-2/Bax ratio decreased, caused a loss in mitochondrial membrane potential, and enhanced the subsequent release of cytochrome c from mitochondria in FLS, ultimately to promote RAFLS apoptosis. **Bone-protective effect:** QUE suppressed RANKL expression of FLS by regulating mTOR, ERK, IκB-α and AMPK signaling pathway. QUE inhibited MMP-1 and MMP-3 expression of FLS via suppressing the activation of MAPKs (ERK, p38, JNK) and NF-κB signaling pathway.

Anti-Inflammatory Effect

Effect of QUE on Clinical Parameters and Inflammatory Parameters

Synovial inflammation is a hallmark of RA, and joint swelling reflects synovial membrane inflammation, characterized by leukocyte infiltration into the normal synovial compartment. Besides, the inflammatory milieu of the synovial compartment is correlated with the complex cytokine and chemokine network.^{1,18} Previous studies have proved that QUE could decrease paw edema in arthritis model.^{19,20}

In zymosan-induced arthritis mice, QUE significantly reduced mechanical hyperalgesia and joint edema, inhibited the recruitment of total leukocytes, neutrophil and mononuclear cells, decreased the tumor necrosis factor (TNF)-α and interleukin (IL)-1β production, and inhibited zymosan-induced prepro-endothelin-1 (prepro-ET-1) and cyclooxygenase-2 (COX-2) mRNA expression in the knee joint of mice. These effects of QUE might be related to the inhibitory effect on nuclear factor (NF)-κB activation.²¹

Similarly, QUE remarkably mitigated the paw edema and arthritis index scores, down-regulated the total pathological score (including inflammatory cells infiltration, synovium congestion and hyperplasia, and cartilage and bone erosion), decreased TNF-α, IL-1β, IL-6 and prostaglandin E2 (PGE2), inhibited nucleotide-binding oligomerization domain-like receptor family pyrin domain containing-3 (NLRP3) inflammasome (NLRP3, Caspase-1 and IL-1β) activation with no remarkable effect to pro-IL-1β and pro-Caspase-1 in CIA models.^{22–25} Further mechanistical studies suggested that QUE could improve impaired mitochondrial biogenesis and function via regulating silent information regulator 1 (SIRT1)/peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α)/nuclear respiratory factor 1 (NRF1)/mitochondrial

transcription factor A (TFAM) signaling pathway, and QUE could inhibit inflammation by regulating high-mobility group box 1 (HMGB1)/Toll-like receptor 4 (TLR4)/p38/extracellular signal-regulated kinases (ERK)-1/2/NF- κ B p65 signaling pathway.²⁴ But, interestingly, combined administration of QUE and methotrexate (MTX) exhibited no greater protection than administration of QUE alone for CIA mice.²⁶

As well, in AA model, oral or intra-cutaneous QUE significantly decreased arthritis index score^{27,28} and paw thickness, increased paw thermal latency, reduced infiltration of inflammatory cells and decreased p-P65 level in histological analysis of joint tissue.^{28–32} What's more, intra-cutaneous QUE simultaneously with adjuvant induced and prior to the appearance of clinical signs also resulted in reduction of clinical scores, suggesting the preventive property of QUE on RA.²⁷ However, another study indicated that, in AA rats treated with QUE group, the changes in arthritis score observed were not obvious compared to the AA group, which were, partly, in contradiction with the related experimental results observed in zymosan-induced arthritis, CIA and AA model.³³ This might be partially associated with the difference in experimental models and administration dosage and ways. ELISIA analysis of the levels of inflammatory cytokines in AA model serum indicated that QUE decreased proinflammatory cytokines, including interferon (IFN)- γ , TNF and IL-6, increased anti-proinflammatory cytokines, including IL-4 and IL-10³⁰. QUE also ameliorated nitric oxide (NO), decreased macrophage-derived inflammatory cytokines, including TNF- α , IL-1 β , IL-6, monocyte chemotactic protein-1 (MCP-1),^{27,28,32,33} and myeloperoxidase (MPO) activity.²⁹ Adenosine deaminase (ADA), another inflammatory biomarker for RA.³⁴ QUE could inhibit ADA enzyme activity and gene expression in sera and joints.²⁸ Furthermore, the regulatory effect on miRNA-26b, miRNA-20a and glycogen synthase kinase-3 β (GSK-3 β)/NF- κ B/NLRP3 signaling pathway, possibly, made contributions to the anti-inflammatory effect of the atorvastatin and QUE combination therapy on AA model.³² In addition, suppression of lipoxygenase (LOX) production ameliorates inflammation of RA.³⁵ Meanwhile, QUE decreased the activity of 12/15-LOX in liver and lung of AA model, which might be correlated to the inhibitory effect of QUE on the activation of NF- κ B in joint, lung and liver, and the activation of extracellular signal-regulated kinases (ERK) in joint and lung.³³

In vitro, an early study showed that QUE inhibited TNF- α -induced stimulation of IL-8 and MCP-1, partly, by inhibiting the activation of NF- κ B in human RA fibroblast-like synoviocytes (RAFLS).³⁶ Furthermore, a recent study found that QUE could suppress TNF- α -induced production of IL-1 β , IL-6 and IL-8 by targeting long non-coding RNA (lncRNA) (si)-X-inactive specific transcript (XIST), sponging microRNA (miR)-485, which, subsequently, targeting proteasome subunit β type-8 (PSMB8).³⁷ Besides, IL-1 β stimulated the expression of COX-2 and PGE2, but for COX-1 in RAFLS. QUE inhibited the effects of IL-1 β on COX-2 and PGE2, which were due partly to the inhibitory effect on activation of intracellular mitogen-activated protein kinase (MAPK) signaling pathways, including ERK, p38, c-Jun N-terminal kinase (JNK), and NF- κ B signaling pathways.³⁸

In short, QUE attenuated clinical parameters, decreased pro-inflammatory cytokines and increased anti-inflammatory cytokines, which might be related to the regulatory effect of QUE on numerous signaling pathways, including GSK-3 β , NF- κ B, MAPKs (ERK, p-38, JNK), SIRT1/PGC-1 α /NRF1/TFAM, and HMGB1/TLR4/p38/ERK1/2/NF- κ B p65 signaling pathways, as well as lncRNA XIST/miR-485/PSMB8 axis.

Inhibition of Oxidative Stress

In rheumatoid joint, neutrophil can release various potentially harmful peptides and enzymes, and toxic oxygen metabolites to drive inflammation.³⁹ Reactive oxygen species (ROS) can activate signaling pathways involved in the inflammation of RA,⁴⁰ which is related to the levels of reduced glutathione (GSH), glutathione S-transferase (GST), glutathione reductase (GSR) and glutathione peroxidase (GSH-Px).⁴¹

A recent study indicated that QUE could inhibit neutrophil infiltration and activation in joint of RA model and in LPS-mediated air pouch model. QUE could attenuate neutrophil invasion and increase apoptosis of activated neutrophil. In addition, QUE inhibited the formation of neutrophil extracellular traps (NETs) by suppressing autophagy.³⁰

QUE could counteract the oxidative stress associated with AA-induced in the joint tissues and plasma of rats.^{28,33} Also, QUE reduced ROS level and increased catalase activity in serum in complete Freund's adjuvant (CFA)-induced arthritis.⁴² As well, in zymosan-induced arthritis mice, QUE decreased gp91^{phox} (a subunit of NADPH oxidase) mRNA expression, increased GSH levels, nuclear factor erythroid 2 related factor 2 (Nrf2) and heme oxygenase (HO-1) mRNA expression,

indicating these antioxidant molecular effects of QUE might be associated with Nrf2/HO-1 signaling pathway.²¹ As coincided with in zymosan-induced arthritis mice, QUE increased HO-1 at protein level in synovium and FLS (concentration- and time-dependent) of CIA rats.²² Furthermore, in HO-1 siRNA transfected CIA-FLS, QUE or cobalt protoporphyrin IX (CoPP, the inducer of HO-1) failed to downregulate inflammatory cytokines and mediators (TNF- α , IL-1 β , IL-6, PGE2, iNOS and COX-2), providing sound evidences indicating the participation of HO-1 in the anti-inflammation effects of QUE.²² Interestingly, in AA rats, HO-1 protein level increased in joint, while decreased in lung, QUE treated further increased HO-1 expression in joint, and restored HO-1 to control level in lung compared to AA rats.³³

Briefly, QUE regulated neutrophil activities and exhibited antioxidant molecular effects to inhibit oxidative stress through Nrf2/HO-1 signaling pathway, to attenuate synovial inflammation.

Regulation of Behavior of FLS

The process that hyperplastic synovial membrane, acting as cytokine-producing tissue, facilitates structural damage is based on sustainable activation and aggressive behavior of FLS, including proliferation, migration, invasiveness and resistance to apoptosis.^{18,43}

QUE inhibited IL-1 β -induced proliferation of RAFLS by inhibiting the activation of intracellular MAPKs (ERK, p38, JNK) and NF- κ B signaling pathways.³⁸ QUE also induced RAFLS apoptosis.³⁸ Moreover, QUE promoted RAFLS apoptosis by upregulating the lncRNA metastasis associated with lung adenocarcinoma transcript 1 (MALAT1), and MALAT1 induced RAFLS apoptosis via inhibiting the activation of the phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) signaling pathway. Besides, in the RAFLS transfected with si-MALAT1, the expression of caspase-3, caspase-9 and Bax decreased, while the expression of Bcl-2 increased at protein level.⁴⁴ Similarly, another study indicated that QUE induced RAFLS apoptosis, activated caspase-3 and caspase-9, diminished Bcl-2 expression, elevated Bax expression, decreased the Bcl-2/Bax ratio, caused a loss in mitochondrial membrane potential, and enhanced the subsequent release of cytochrome c from mitochondria in concentration-dependent manner in RAFLS, which indicated that quercetin-induced RAFLS apoptosis through mitochondrial pathway.⁴⁵ It has been suggested that protein 53 (p53), a tumor suppressor, plays a vital role in regulating cellular behaviors in rheumatoid synovium.^{46,47} And, the phosphorylation of p53 at ser15 reflects its functional response to the cellular stress and leads to cell apoptosis.⁴⁸ QUE treatment could enhance p53 phosphorylation at ser15 in a concentration-dependent manner with no effect on the expression of total p53. Besides, further experimental results indicated that RAFLS treated with pifithrin- α (PFT- α , an inhibitor of p53) or siRNA targeting to p53 significantly abrogated quercetin-induced apoptosis, indicating that p53 activation was contributed to quercetin-induced apoptosis in RAFLS.⁴⁵

QUE could inhibit the migration and invasion of RAFLS in vitro, which was proved to be related to the up-regulatory effect on miR-146a and the down-regulatory effect on the downstream target GATA transcription factor 6 (GATA6) of QUE. miR-146a inhibitor transfection could enhance the migration and invasion of RAFLS. Moreover, miR-146a inhibitor transfection could inverse the inhibitory effect of QUE on RAFLS migration and invasion, abolish the suppression of QUE on GATA6 and F-actin, indicating that QUE inhibited the migration and invasion of RAFLS might be related to the regulatory effect on miR-146a/GATA6 axis.⁴⁹

In brief, QUE attenuated hyperplastic synovial membrane by regulating FLS behavior, including inducing apoptosis, inhibiting proliferation, migration and invasion.

Immune-Regulatory Effect

RA is an autoimmune disease. It has been identified that T cells and B cells play a crucial role in pathogenesis and pathology of RA.^{50,51} Moreover, it has proved that the presence of autoantibodies is related to more severe clinical symptoms and joint damage in RA patients.¹

In CIA model, the relative weight of spleen increased when compared with healthy controlled group, QUE could decrease spleen index.²⁴ Besides, QUE decreased the proportion of CD4⁺IL-17A⁺T cells, increased the percentage of CD4⁺CD25⁺Foxp3⁺T cells.^{22,25} Besides, the further studies proved that QUE could significantly increase Foxp3 and decrease ROR γ t of Th17 cells (IL-17-producing CD4⁺T cells) and Treg cells (Foxp3⁺ regulatory T cells) could inhibit CD4⁺T cells polarized into Th17 cells and could decrease the proportion of CXCR3⁺IL-17A⁺T cells and IFN- γ IL-17A⁺CD4⁺T cells,

indicating the anti-arthritis effects of QUE might be partially due to the modulation of Th17/Treg cells balance and Th17 cells differentiation.^{22,25} Moreover, QUE activated peroxisome proliferator activator receptor γ (PPAR γ) to promote the suppressor of cytokine signaling 3 (SOCS3) gene transcription then to inhibit signal transducer and activator of transcription 3 (STAT3) activation, QUE also redistributed the corepressor retinoid and thyroid-hormone receptors (SMRT) from PPAR γ to STAT3 to inhibit the STAT3 transcriptional activity, namely, QUE target PPAR γ to inhibit STAT3 by dual mechanisms, ultimately inhibiting Th17 differentiation.²⁵ But that, a little paradoxically, some experimental results were different from these. In Peripheral blood mononuclear cells (PBMCs) cultured with Th17-differentiation conditions, QUE reduced IL-17 cytokine production in culture medium, suppressed the percentage of IL-17-expressing CD4⁺ T cells, but exhibited no effect to the percentage of CD25Foxp3-expressing CD4⁺ regulatory T cells.⁵² Different cell sources might contribute to these contradictory experimental results. QUE could also regulate the serum levels of Th17/Treg-related cytokines, namely, decreased Th17 cells-related cytokines (IL-17A, IL-21 and IL-23), increased Treg cells-related cytokines (IL-10 and TGF- β).^{22,25} In addition, QUE decreased the elevated levels of IFN- γ , IL-4 and CXCR3.^{25,29} Besides, QUE could reduce the serum autoantibodies levels, including anti-CII IgG, anti-CII IgG1 and anti-CII IgG2a in CIA models.^{22,24} A recent study indicated that QUE could diminish the sera levels of anti-cyclic citrullinated peptide antibody (anti-CCP) and rheumatoid factor (RF) in AA rats model.²⁸

Ectonucleoside triphosphate diphosphohydrolase (E-NTPDase) and ectoadenosine deaminase (E-ADA) in lymphocytes are involved in the pathogenesis of RA.^{53,54} QUE could reverse the increase of E-NTPDase activity and the decrease of E-ADA activity in lymphocytes. Besides, though there were no significant changes in the serum levels of ATP, ADP and AMP in QUE group compared with AA group, QUE lowered the increased serum adenosine levels of AA rats.²⁹

Collectively, QUE exhibited the immune-regulatory effect by restoring T cell homeostasis, regulating Th17 cells differentiation, regulating the levels of Th17/Treg-related cytokines, reducing autoantibodies production, and regulating E-NTPDase/E-ADA activities.

Bone-Protective Effect

In RA, proinflammatory cytokines, receptor activator of nuclear factor kappa B ligand (RANKL) and antibodies directed against citrullinated proteins secreted by synovitis mediate articular bone erosion, including stimulating the differentiation of bone-resorbing osteoclasts.⁵⁵ And, matrix metalloproteinases (MMPs) made contributions to the cartilage destruction.⁵⁶

QUE could inhibit IL-1 β induced the expression of MMP-1, MMP-3 at mRNA and protein level in RAFLS, except for tissue inhibitor of metalloproteinase (TIMP)-1, which might be related to inhibiting the activation of intracellular MAPKs (ERK, p38, JNK) and NF- κ B pathways.³⁸

QUE also suppressed IL-17 induced RANKL expression of RAFLS at mRNA and protein level, inhibited RANKL- and IL-17- produced TRAP positive osteoclasts formation, and decreased the expression of the osteoclast markers, including TRAP, cathepsin K, NF-ATc1, DC-STAMP, ATP6vOd2, and OC-STAMP. In addition, QUE exhibited similar inhibitory effect on osteoclastogenesis (TRAP positive osteoclasts formation and increased expression of the osteoclast markers) in culture system of monocytes with IL-17-prestimulated RAFLS, and in osteoclast precursors (pre-OC) cultured with Th17 cells and M-CSF. Moreover, QUE suppressed the phosphorylation of mTOR, ERK and I κ B- α , while enhanced the phosphorylation of AMPK in IL-17 stimulated RAFLS, implying that these molecular effects of quercetin for RA might be mediated by aforementioned signaling pathways.⁵²

In brief, QUE exerted the bone-protective effect by decreasing MMPs, RANKL production, and osteoclasts formation by regulating MAPKs (ERK, p38, JNK), NF- κ B, mTOR, ERK, I κ B- α and AMPK.

Anti-Extra-Arthritic Effect

RA is a multi-systemic disease and some patients may develop extra-articular manifestations at the onset or in the progression of RA.⁵⁷

In CFA-induced arthritis model, QUE reduced the increased aspartate aminotransferase (AST), except for alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in serum, decreased production of thiobarbituric acid-reactive substances (TBARS), and DNA damage, suggesting the hepatoprotective, genoprotective and cytoprotective effect of

QUE in arthritic model.⁴² Another study indicated that the levels of ALT and AST of methotrexate (MTX) treatment group were higher than control group, while QUE co-administered with MTX could reverse the hepatotoxicity.³¹

Besides, in CFA-induced arthritis rats, the density of the enteric neurons and the enteric glial cells (EGC) in the myenteric and submucosal plexuses with neurodegeneration in the jejunum were remarkably decreased, glial fibrillary acidic protein (GFAP) and glial cell derived neurotrophic factor (GDNF) expression reduced, the mucosa and intestinal wall atrophied, and intestinal inflammation presented, and QUE substantially reversed most of these effects except the intestinal atrophy of the jejunum.⁵⁸

The antioxidant, anti-inflammatory and/or neuroprotective mechanisms might make contributions to the hepatoprotective, genoprotective and cytoprotective effect, and to the improvement of RA-induced arthritic neuropathy of QUE.

Potential Effect of QUE in Clinical Studies

Based on the effectiveness of QUE on RA in preclinical studies, some clinical studies have been performed to illuminate the protective effect on RA though related studies are few.

In a randomized double-blind clinical trial, 51 women with RA aged 19–70 years were assigned into quercetin (500 mg/day) or placebo groups for 8 weeks, finally, there were no significant differences in markers of oxidative stress including total antioxidant capacity (TAC), oxidized low-density lipoprotein (ox-LDL), malondialdehyde (MDA), and high sensitivity C-reactive protein (hs-CRP), and blood pressure between quercetin and placebo groups.⁵⁹

Meanwhile, subsequently, in another double-blind, placebo-controlled, randomized clinical trial, 50 women with RA were allocated to two groups: a quercetin group and a placebo group, receiving quercetin (500 mg/day) or a placebo, respectively, for 8 weeks. QUE groups presented a reduced early morning stiffness (EMS), morning pain, and after-activity pain, and a decreased plasma high-sensitivity TNF- α (hs-TNF- α) level, Disease Activity Score 28 (DAS-28) and health assessment questionnaire (HAQ) scores.⁶⁰

The grouping, dosage and administration method of QUE, gender of subject in the two clinical trials above were same. A study suggested that QUE exhibited no effect on oxidative or inflammatory status in RA patients, but another study indicated that QUE could significantly improve clinical symptoms and decrease inflammatory factors of RA patients. Subjects with different levels of disease activity and different evaluation indexes were the possible reasons for these differences in the two studies.

Conclusion and Future Directions

RA is a chronic inflammatory joint disease, the main pathological process includes synovial membrane inflammation, autoimmune response, cartilage and bone damage, and oxidative stress. RA patients with insufficient treatment can have various extra-articular manifestations.^{1,2,61}

In this review, the anti-RA effect of QUE is summarized on anti-articular and anti-extra-articular (Table 1) in pre-clinical studies. Firstly, QUE decreased synovial inflammation through reducing joint clinical parameters and inflammatory cytokines, inhibiting oxidative stress, and regulating behavior of FLS. Secondly, QUE exhibited immune-regulatory effect by regulating Th17/Treg balance and Th17 cells differentiation, reducing autoantibodies levels, and regulating E-NTPDase/E-ADA activities. Thirdly, QUE exerted bone-protective effect by suppressing MMPs, RANKL expression, and inhibiting TRAP positive osteoclasts formation. Fourthly, for extra-articular manifestations, QUE exhibited cytoprotective, genoprotective and hepatoprotective effect in arthritic model, and QUE could reverse the neurodegenerative processes of enteric nervous system (ENS) in arthritic rats. Furthermore, some experimental results are paradoxical which might be due to the different experimental models, administration methods or dosages, and most of the pre-clinical evidences are derived from anti-inflammatory effect. Therefore, more pre-clinical studies, especially research on immune-regulatory, bone-protective and anti-extra-articular effect, are urgently needed in future.

For clinical studies, the conclusion of the current two clinical studies seemed to be confusing and the existing evidence did not show the corresponding excellent therapeutic potential in pre-clinical studies. The major gap about the efficacy of QUE on RA between pre-clinical and clinical might be attributed to the limited clinical evidence. Furthermore, clinical trials of RA patients with different disease activity, larger sample size, gender difference,

Table 1 Potential Mechanism of QUE in Pre-Clinical Studies

Therapeutic Effect		Experimental Model	Dosage Information	Molecular Mechanism	Signaling Pathway
Anti-inflammatory effect	Effect on clinical parameters and inflammatory cytokines	Zymosan-induced arthritis mice	0, 10, 30, 100 mg/kg s.c. 30 min before zymosan injection ²¹	Reduced mechanical hyperalgesia, joint edema, and recruitment of total leukocytes, neutrophil, mononuclear cells in joint, decreased TNF- α , IL-1 β , prepro-ET-1, COX-2 ²¹	NF- κ B ²¹
		CIA model	150 mg kg ⁻¹ 0.5 mL ⁻¹ three times a week orally administrated for 17 or 28 days; ²³ 0, 50, 100mg/kg/d orally administrated for 5 weeks; ²⁴ 150mg/kg daily orally administrated for 14 days ²² or 21 days; ²⁵ 30mg/kg daily orally administrated for 49 days ²⁶	Mitigated paw edema, inflammatory cells infiltration, synovium hyperplasia, cartilage and bone erosion, decreased TNF- α , IL-1 β , IL-6, PGE2, and NLRP3 inflammasome (NLRP3, Caspase-1 and IL-1 β) ²²⁻²⁶	SIRT1/PGC-1 α /NRF1/TFAM and HMGB1/TLR4/p38/ERK1/2/NF- κ B p65 ²⁴
	AA model	150 mg/rat (30 mg every 2 days, orally administrated for 10 days) or 25, 50 mg/rat (5 or 10 mg every 2 days, intra-cutaneous injection following the appearance of first arthritic symptoms); ²⁷ 25 mg/kg/d orally administrated for 14 days; ³¹ 0, 5, 25 50 mg/kg/d orally administrated for 45 days; ²⁹ 30mg/kg/d injected intra-peritoneally 31 days; ³⁰ 100mg/kg injected intra-peritoneally three times a week for 3 weeks; ²⁸ 0, 50, 100, 150 mg/kg/d orally administrated for 28 days; ³² 150mg/kg/d orally administrated for 28 days ³³	Reduced arthritis scores, paw thickness and inflammatory infiltration, increase paw thermal latency; ²⁷⁻³² Increased IL-4 and IL-10, decreased TNF- α , NO, IFN- γ , IL-6, IL-1 β , MCP-1, MPO, ^{27-30,32,33} decreased I2/15-LOX in liver and lung. ³³ Decreased ADA enzyme activity and gene expression in sera and joints ²⁸	GSK-3 β , NF- κ B and ERK ^{30,32,33}	
Inhibition of oxidative stress	Zymosan-induced arthritis mice	Human RAFLS	50 nmol/L; ³⁷ 0, 20, 100, 200 μ M ³⁸	Decreased IL-1 β stimulated COX-2 and PGE2, ³⁸ decreased TNF- α -induced IL-1 β , IL-6, IL-8 and MCP-1 ^{36,37}	MAPKs (ERK, p-38, JNK) ³⁸ and NF- κ B, ^{36,38} lncRNA XIST/miR-485/PSMB8 axis ³⁷ Nrf2/HO-1 ²¹
		CIA model	0, 10, 30, 100 mg/kg s.c. 30 min before zymosan injection ²¹	Increased GSH, decreased gp91 phox ²¹	
	AA model	150mg/kg daily orally administrated for 14 days ²²	Increased HO-1 in synovium and FLS, failed to downregulate inflammatory cytokines and mediators (TNF- α , IL-1 β , IL-6, PGE2, iNOS and COX-2) in HO-1 siRNA transfection CIA-FLS ²²	-	
Regulation of behavior of FLS	Human RAFLS	AA model	30mg/kg/d injected intra-peritoneally 31 days; ³⁰ 0, 5, 25, 50 mg/kg/d orally administrated for 45 days; ⁴² 150mg/kg/d orally administrated for 28 days ³³	Decreased neutrophil infiltration, activation, and invasion, increased neutrophil apoptosis, decreased NETs formation by inhibiting autophagy. ³⁰ Reduced ROS level and increased catalase activity in serum. ⁴² Further increased HO-1 expression in joint, and restored HO-1 to control level in lung ³³	NF- κ B and ERK ³³
		Human RAFLS	0, 10, 20, 30 μ M; ⁴⁹ 0, 10, 50, 100, 150, 200, 300 μ M; ⁴⁴ 0, 100, 200, 300 μ M, ⁴⁵ 0, 20, 100, 200 μ M ³⁸	Increased apoptosis and decreased IL-1 β -induced proliferation. ³⁸ Increased apoptosis via upregulating lncRNA MALAT1. ⁴⁴ Upregulated caspase-3, caspase-9, Bax, downregulated Bcl-2, ^{44,45} caused a loss in mitochondrial membrane potential, and enhanced the subsequent release of cytochrome c from mitochondria. ⁴⁵ Inhibited migration and invasion ⁴⁹	MAPKs (ERK, p38, JNK), NF- κ B ³⁸ and PI3K/AKT, ⁴⁴ p53 activation, ⁴⁵ miRNA-146a/GATA6 axis. ⁴⁹

(Continued)

Table I (Continued).

Therapeutic Effect		Experimental Model	Dosage Information	Molecular Mechanism	Signaling Pathway
Immune-regulatory effect	-	CIA model	0, 50, 100mg/kg/d orally administrated for 5 weeks; ²⁴ 150mg/kg daily orally administrated for 14 days ²² or 21 days ²⁵	Decreased IL-17A, IL-21, IL-23, increased IL-10, TGF- β , decreased CD4 ⁺ IL-17A ⁺ T cell proportion, increased CD4 ⁺ CD25 ⁺ Foxp3 ⁺ T cells proportion; upregulated Foxp3 and downregulated ROR γ t of Th17 cells and Treg cells, ^{22,24,25} decreased serum autoantibodies levels (anti-CII IgG, anti-CII IgG1 and anti-CII IgG2a) ^{22,24}	PPAR γ ²⁵
		AA model	100mg/kg injected intra-peritoneally three times a week for 3 weeks; ²⁸ 0, 5, 25 50 mg/kg/d orally administrated for 45 days ²⁹	Decreased anti-CCP, RF; ²⁸ Reversed the increase of E-NTPDase activity and the decrease of E-ADA activity in lymphocytes, lowered the increased serum adenosine levels, decreased the elevated levels of IFN- γ and IL-4 ²⁹	-
		Human PBMC cultured with Th17-differentiation conditions Mouse naïve CD4 ⁺ T cells	0, 1, 5, 25 μ M ⁵² 0, 1, 3, 10 μ M ²⁵	Reduced IL17 production in the culture medium, suppressed the percentage of IL-17-expressing CD4 ⁺ T cells, but exhibited no effect to the percentage of CD25Foxp3-expressing CD4 ⁺ regulatory T cells ⁵² Decreased CXCR3, inhibited CD4 ⁺ T cells polarized into Th17 cells, and decrease the proportion of CXCR3 ⁺ IL-17A ⁺ T cells and IFN- γ ⁺ IL-17A ⁺ CD4 ⁺ T cells ²⁵	mTOR, ERK, I κ B- α and AMPK ⁵²
Bone-protective effect	-	Human RAFLS	0, 1, 5, 25 μ M; ⁵² 100 μ M ³⁸	Suppressed IL-17 produced RANKL expression at mRNA and protein level, inhibited RANKL- and IL-17- produced TRAP positive osteoclasts formation, and decreased the expression of the osteoclast markers, including TRAP, cathepsin K, NF-ATc1, DC-STAMP, ATP6vOd2, and OC-STAMP; decreased TRAP positive osteoclasts formation and the osteoclast markers in culture system of monocytes with IL-17-prestimulated RAFLS, and in osteoclast precursors (pre-OC) with Th17 cells and M-CSF. ⁵² Inhibit IL-1 β stimulated MMP-1, MMP-3 at mRNA and protein level ³⁸	mTOR, ERK, I κ B- α , AMPK, ⁵² MAPKs (ERK, p38, JNK) and NF- κ B ³⁸
Anti-extra-articular effect	Hepatoprotective Cytoprotective Genoprotective Neuroprotective	AA model	0, 5, 25, 50 mg/kg/d orally administrated for 45 days ⁴²	Decreased the increased serum AST except for ALT, ALP, decreased TBARS, DNA damage ⁴²	-
		AA model	50 mg/kg/d orally administrated for 60 days ⁵⁸	Reversed the density of the enteric neurons and the enteric glial cells (EGC) in the myenteric and submucosal plexuses, the expression of GFAP and GDNF expression, reduced intestinal inflammation ⁵⁸	-

exploration of administration methods or dosages, pharmacokinetics, etc. will also be needed. Only sufficient evidence of pre-clinical and clinical could form a sound basis for the clinical application of QUE for RA.

Ethics

There are no ethical issues involved in the article.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This study was supported by the Natural Science Foundation of HUNAN province (2021JJ30934); Hunan Provincial Health Committee 225 Talent Project (2019-196); Educational Fund of Hunan Provincial Finance Department (2021-22-2050205).

Disclosure

The authors report no conflicts of interest in this work.

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