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Original article

Therapeutic effect of quercetin in collagen-induced arthritis

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ABSTRACT

Quercetin, a bioactive flavonoid with anti-inflammatory, immunosuppressive, and protective properties, is a potential agent for the treatment of rheumatoid arthritis (RA). Collagen-induced arthritis (CIA) is the most commonly used animal model for studying the pathogenesis of RA. This study analysed the therapeutic role of quercetin in collagen-induced arthritis in C57BL/6 mice. The animals were allocated into five groups that were subjected to the following treatments: negative (untreated) control, positive control (arthritis-induced), arthritis + methotrexate, arthritis + quercetin, and arthritis + methotrexate + quercetin. Assessments of weight, oedema, joint damage, and cytokine production were used to determine the therapeutic effect of quercetin. This study demonstrated for the first time the anti-inflammatory and protective effects of quercetin *in vivo* in CIA. The results also showed that the concurrent administration of quercetin and methotrexate did not offer greater protection than the administration of a single agent. The use of quercetin as a monotherapeutic agent resulted in the lowest degree of joint inflammation and the highest protection. The reduced severity of the disease in animals treated with quercetin was associated with decreased levels of TNF-α, IL-1β, IL-17, and MCP-1. In conclusion, this study determined that quercetin, which was non-toxic, produced better results than methotrexate for the protection of joints from arthritic inflammation in mice. Quercetin may be an alternative treatment for RA because it modulates the main pathogenic pathways of RA.

1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory and degenerative joint disease that can affect all ages, sexes, and races [1,2]. RA is characterized by swelling, pain, and stiffness in joints, but it can also damage the heart, eyes, lungs, kidneys, and skin [3–7]. Patients with RA experience progressive disability and an increased risk of death [8]. Genetic, hormonal, and environmental factors have been related to RA [9–12].

The development of new treatments for RA has relied on animal models. The collagen-induced arthritis (CIA) model of RA closely replicates the pathogenesis of the disease. The current therapeutic products for RA include: 1) conventional synthetic disease-modifying antirheumatic drugs (DMARDs) such as methotrexate (Mtx), sulfasalazine, leflunomide, and hydroxychloroquine; 2) biological DMARDs such as TNF inhibitors, anti-B cell, anti-T cell, and anti-IL6 R antibodies; and 3) targeted synthetic DMARDs such as Janus kinase inhibitors [13]. Methotrexate is the most widely used treatment for RA because of its immunosuppressive activity, anti-inflammatory effects, and low cost. However, owing to its toxicity, methotrexate is not an ideal therapeutic agent. The adverse effects of methotrexate on the neuronal, gastrointestinal, reproductive, respiratory, urinary, integumentary, cardiovascular, and immune systems have been reported [14]. Therefore, there is a need to develop improved and affordable therapeutic agents for RA.

Quercetin (Que) is a natural flavonoid found in vegetables, fruits, grains, and nuts [15,16]. This flavonoid does not cause toxicity or have secondary effects [17–21]. Plants containing quercetin, such as *Bridelia ferruginea, Betula pendula*, and *Poligonum hydropiper* L., have been used in traditional medicine for the treatment of arthritic pain [22–24]. Several effects of

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quercetin have been described, including antioxidant, antiproliferative, anti-inflammatory, antihistamine, antiviral, anticarcinogenic, psychostimulatory, immunosuppressive, and protective properties. [16]. *In vitro*, *ex vivo*, and *in vivo* studies have indicated that quercetin disturbs different pathways of inflammation. For example, quercetin has been reported to a) inhibit dendritic cell (DC) and T cell activation, maturation, and migration; b) reduce the production of nitric oxide (NO), rheumatoid factor (RF), C-reactive protein (CRP), cytokines, chemokines, and prostaglandins, including TNF-α, IL-1β, IL-1α, IFN-γ, MCP-1, IL-6, IL-10, IL-12 p70, MIP-1a, MIP-1b, RANTES, and PGE-2; c) reduce the expression of costimulatory molecules such as CD40, CD80, and CD86; d) prevent angiogenesis; e) inhibit transcription factors such as NF-κB; f) reduce enzymes such as MMPs, COX, hyaluronidases, and collagenases; g) promote the apoptosis of pathogenic cells; and h) inhibit oxidative metabolism [21,25–33].

In summary, despite the demonstrated efficacy of methotrexate in the treatment of RA, it is not specific to the disease and may endanger the life of the patients; therefore, alternative therapies are required to treat RA. In the present study, the anti-inflammatory and joint-protective properties of quercetin were studied using a C57BL/6 collagen-induced arthritis mouse model.

2. Materials and methods

2.1. Reagents

Chicken type II collagen, Complete Freund's Adjuvant (CFA), Incomplete Freund's Adjuvant (IFA), methotrexate and quercetin were purchased from Sigma-Aldrich (St Louis, MO, USA). Phosphate buffer saline (PBS) was obtained from Thermo Fisher (Newstead, QLD, Australia). The cytokine kits were purchased from BD Biosciences (North Ryde, NSW, Australia).

2.2. Induction and evaluation of collagen-induced arthritis

The mouse CIA model was established by immunizing 4 mg/ml chicken type II collagen emulsified 1:1 with CFA. The mice were injected 0.1 mL of emulsion intradermally into the back and the tail base on day 0. A booster injection of an emulsion of chicken type II collagen and IFA was administered into the tail on day 21. Every 5 days, collagen-induced arthritis was evaluated by visual scoring of the paws, including the adjoining area. In addition, swelling in the ankles was measured using digital callipers. Each paw was scored from 0 to 4 as follows: 0 = normal; 1 = erythema and mild swelling; 2 = erythema and swelling extending to ankle joints and one or two toes; 3 = erythema and swelling extending to metatarsal joints and more than two toes; and 4 = ankylosing deformity with joint swelling. The scores from each paw were added to obtain a cumulative score between 0 and 16.

2.3. Treatment groups

Ten- to twelve-week-old C57BL/6 mice were selected for this study. All studies were approved by the Institutional Animal Ethics Committee. Mice were allocated into five groups: 1) negative control (Control), 2) positive control (Arthritis), 3) Arthritis + Mtx, 4) Arthritis + Que, and 5) Arthritis + Mtx + Que.

The negative control group did not receive any treatment. Arthritis was induced in the following groups: Arthritis, Arthritis + Mtx, Arthritis + Que, and Arthritis + Mtx + Que. In addition, mice from these groups received daily treatments between day 21 and day 70. The Arthritis group received subcutaneous injections of 100 μL PBS. The Arthritis + Mtx group was treated intraperitoneally (i.p.) with 0.5 mg/kg methotrexate. The Arthritis + Que group was treated daily by oral gavage with 30 mg/kg quercetin. The Arthritis + Mtx + Que group was treated with both methotrexate (0.5 mg/kg i.p.) and quercetin (30 mg/kg oral gavage, daily).

2.4. Cytokine assay

Mice were sacrificed by exsanguination under deep anaesthesia 24 h after final treatment (day 71) and blood was collected by cardiac puncture. The plasma was separated from the blood by centrifugation (1000*g* for 15 min). Plasma was stored at −80 °C until it was tested to detect pro- and anti-inflammatory cytokines. Quantitative measurement of cytokines was performed with a Quantibody: Multiplex array (BD Pharmigen). Samples were assayed following the protocol of BD Pharmigen in a CyAn ADP (Beckman Counter) flow cytometer.

2.5. Radiographic examination

At the end of the experiment (day 70), one mouse was chosen from each group randomly and anesthetized. X-ray examination was carried out with a multi soft x-ray film apparatus.

2.6. Histopathological analysis

Left hind knees were dissected out and cleaned, and the samples were fixed, decalcified, and embedded in paraffin to be sectioned. Sections of 5-μm thickness were stained with haematoxylin and eosin for histopathological examination. Histopathological scoring (Table 1) was performed by a pathologist blinded to the identity of the sample group.

As the histopathological scores differed among the groups, the medians of each group – including synovitis, pannus formation, cell infiltration, cartilage and bone damage – were obtained to illustrate the arthritic distribution. Further analysis of the minimum, maximum, and total scores of synovitis, including pannus formation, cell infiltration, cartilage damage, and bone damage, was performed using column analysis. The means were rounded to the nearest integer and represented as (−) normal, (+) minimum, (++) moderate, and (+++) maximum damage in the knee.

2.7. Statistical analysis

GraphPad Prism Version 7.0a was used for data analysis. Data were analysed by two-way ANOVA with Dunnett's multiple comparisons test, one-way ANOVA with Kruskal-Wallis test, and Dunn's multiple comparisons test, column statistics, and the

Table 1
Histopathological score.

Score	Characteristics
0	No synovitis and no pannus formation, no cell infiltration, no damage to the cartilage, no damage to the bone
1	Mild synovitis with limited pannus formation, low cellular density of infiltrating cells, small isolated lesions in the cartilage and small isolated lesions in the bone
2	Moderate synovitis with moderate pannus formation, moderate cellular density of infiltrating cells, moderate lesions in the cartilage and moderate lesions in the bone
3	Severe synovitis with severe pannus formation, high cellular density of infiltrating cells, extensive damage to the cartilage and extensive damage to the bone.