Archives of Biochemistry and Biophysics 583 (2015) 150–157

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

Quercetin reduced inflammation and increased antioxidant defense in rat adjuvant arthritis

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ARTICLE INFO

Article history:
Received 14 July 2015
Received in revised form
14 August 2015
Accepted 17 August 2015
Available online 20 August 2015

Keywords:
Quercetin
Adjuvant arthritis
NF-κB
ERK pathway
Inflammation
Oxidative stress

ABSTRACT

Novel therapies for rheumatoid arthritis also include the use of naturally occurring compounds possessing antioxidant properties. In the present work, the effects of oral administration of quercetin were investigated in a rat model of adjuvant arthritis. Arthritis was induced by a single intradermal injection of heat-inactivated *Mycobacterium butyricum* in incomplete Freund's adjuvant. The experimental groups were treated with an oral daily dose of 150 mg/kg b.w. of quercetin for 28 days. Results indicated that quercetin was able to ameliorate all markers of inflammation and oxidative stress measured. Quercetin lowered levels of interleukin-1β, C-reactive protein, and monocyte chemotactic protein-1 and restored plasma antioxidant capacity. In addition, quercetin inhibited the enzymatic activity of pro-inflammatory 12/15-lipoxygenase in lung and liver and increased the expression of heme oxygenase-1 in joint and lung of arthritic rats. Finally, quercetin inhibited the 2-fold increase of NF-κB activity observed in lung, liver and joint after induction of arthritis.

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease affecting approximately 1% of the whole world population. Patients with RA have a reduced life quality (joints and bones degeneration, muscle weakness, persistent pain) and require long-life therapy. A common effect of long-term therapy is the development of resistance to treatment and also an increased occurrence of adverse effects. Due to these reasons, a continuous need for new agents in the therapy of RA is envisaged. Primary and dominant processes in the etiopathogenesis of RA are immunological mechanisms, closely related to redox imbalance in the organism, which may potentiate chronic inflammatory processes [1]. Our studies [2–4] are in agreement with findings of other authors who referred to the important role of oxidative stress in the pathogenesis of RA [5–7].

In the last decade, the potential involvement of flavonoids with antioxidant properties in RA has been evaluated [8–10]. In this context, the limited side effects of quercetin (QUE) and its well-known pharmacological activities suggested a potential application as an adjuvant natural drug for the treatment of RA [10]. QUE (3,30,40,5,7-pentahydroxyflavone) is the major dietary flavonol found in fruits, vegetables and beverages, such as tea and red wine [11]. Several epidemiological and experimental studies support the antioxidant, anti-inflammatory, anti-angiogenic, anti-proliferative and pro-apoptotic effects of this molecule [12–14]. In Western populations, the estimated daily intake of total flavonols is in the range of 20–50 mg/day, of which about 15–20 mg correspond to QUE glycosides [15].

The existence of a functional link between the intake of QUE and other flavonoids and RA is supported by circumstantial evidence deriving from pre-clinical studies on primary cells and animal models, as well as clinical studies. Early in the 1997, it was reported that QUE suppressed the increase in the mRNA for interleukin 8 (IL-8) and monocyte chemotactic protein-1 (MCP-1) in cultured human synovial cells stimulated by tumor necrosis factor-alpha (TNF-α) in

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http://dx.doi.org/10.1016/j.abb.2015.08.008
0003-9861/

a dose dependent manner. TNF-α is present in synovial fluid and induces the expression of pro-inflammatory cytokines in synovial cells of patients with RA. The suppression was dose dependent and probably induced by the inhibition of TNF-α mediated nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation [16]. A decade later, the anti-RA capacity of QUE was confirmed in synoviocytes isolated from rabbit where the molecule inhibited proliferation of 30–40% at very low micromolar concentrations (<10 μM). It must be considered that proliferation of synoviocytes in RA contributes to the establishment of the so-called "pannus formation", a lesion accompanied by restriction of joint movement and the generation of pro-inflammatory cytokines [17]. In human rheumatoid synovial fibroblasts activated by interleukin 1 beta (IL-1β), QUE inhibited proliferation and induced apoptosis starting from 20 μM concentration. The mode of action was double: i. inhibition of both the expression of IL-1β-induced mRNA and protein of matrix metalloproteases MMP-1, MMP-3, and COX-2 and PGE2 production; ii. inhibition of extracellular signal-regulated kinases (ERK) signal pathways and NF-κB activation both mediated by IL-1β [18].

The anti-RA effect of QUE was confirmed in several animal models of experimentally induced arthritis. In a rat model of gouty arthritis, QUE treatment (100–400 mg/kg) ameliorated edema by decreasing histological signs of acute inflammation and attenuating several markers of inflammation [19]. QUE was more effective than hesperidin, but less than rutin (all tested at a dose of 80 mg/kg and administered intraperitoneally) in inhibiting acute and chronic inflammation in rats where experimental arthritis was induced following the method of adjuvant-carrageenan-induced inflammation [20]. Considering that rutin differs from QUE for the presence of rutinose in position 3, it is possible to hypothesize that the 2-fold higher efficacy of rutin than QUE in arthrogram scores can be attributed to pharmacokinetic factors [20]. In a subsequent work, the same group, comparing the effects of different flavonoids on different rats and mice models, confirmed that rutin was the only effective against chronic-like arthritis, principally in adjuvant arthritis (AA), but QUE resulted the most active in reducing the paw edema induced by carrageenan [21]. In AA induced in female Lewis rats by subcutaneous injection of inactivated *Mycobacterium butyricum*, oral administration of QUE (5 × 160 mg/kg) clearly decreased clinical signs of arthritis. Importantly, the dosage was selected to be comparable to that administered to patients affected by prostatitis who received QUE as dietary supplement 1 g/day [22]. When the molecule was given by intracutaneous injection in AA-induced rats at lower doses (5 × 60 mg/kg), the anti-arthritic effects were similar, while injection of relatively low doses (5 × 30 mg/kg) prior to AA induction significantly reduced arthritis signs, suggesting multiple approaches (different doses and modes of administrations) to exploit the clinical potentiality of QUE as an anti-arthritic agent. Finally, analysis of cumulated arthritic scores clearly indicated that high oral doses were most efficient in reducing arthritic signs, followed by lower intracutaneous therapeutic or preventive QUE doses [22].

More recently, the study of the effects of QUE in RA was extended to human subjects with contradictory results compared to pre-clinical studies. In a randomized controlled trial aimed to investigate the efficacy of antioxidant supplementation in RA patients, QUE was administered together with vitamin C (166 mg + 133 mg/capsule, respectively) for 4 weeks in 26 subjects. No changes in the levels of serum pro-inflammatory cytokines and C-reactive protein (CRP) in RA patients after supplementation were observed [23]. In a more recent work, 51 women affected by RA were supplemented with 500 mg/day of QUE for 8 weeks. As in the previous study, measurements of several markers of inflammation, such as plasmatic total antioxidant capacity, malondialdehyde, oxidized low density lipoprotein (Ox-LDL) and high sensitivity CRP did not show any significant difference between QUE and placebo groups [24]. On the opposite, in an *ex vivo* study, where neutrophils were isolated from RA patients versus healthy subjects and stimulated by *in vitro* prepared immune complex before treatment with 4 different flavonols (galangin, kaempferol, QUE, and myricetin), QUE was the most effective in reducing superoxide anion production with an IC_{50} of 1.71 μM [25]. It is worthwhile to note that the applied concentrations were in the physiological range of those measured *in vivo* after supplementation with QUE or other flavonols [15,26]. Matsuno et al. [27] performed a study with osteoarthritic patients and RA patients, in which QUE was administered in form of glucosamine-chondrotin-QUE glucoside combination. The patients were treated for 3 months with oral doses of QUE glucoside (45 mg/day). Significant improvement in pain symptoms, daily activities (walking and climbing up and down stairs) and changes in the synovial fluid properties were observed in osteoarthritic patients. No beneficial effects were observed in RA subjects [27].

Therefore, in our study we re-investigated the effect of QUE orally administered in a dose of 150 mg/kg in AA with the aim to prove its anti-arthritic potential, as well as to study its mechanisms of action. We focused on the key two processes in arthritis: inflammation and oxidative stress. Both processes were evaluated in plasma and in selected tissues as joint, liver and lung homogenates.

2. Materials and methods

2.1. Animals, experimental design and treatments

Male Lewis rats weighing 160–180 g were obtained from the Breeding Farm Dobra Voda (Slovakia). The rats had free access to standard pelleted diet and tap water. The experimental protocol was approved by the Ethics Committee of the Institute of the Experimental Pharmacology and Toxicology, by the Slovak State Veterinary and Food Administration in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, and by Slovak legislation. AA was induced by a single intradermal injection of heat-inactivated *M. butyricum* in incomplete Freund's adjuvant (Difco Laboratories, Detroit, MI, USA). The injection was performed near the tail base. The experiment included healthy animals (CO), healthy animals treated with QUE (CO-Q) in an oral daily dose of 150 mg/kg b.w. (body weight) during 28 days, arthritic animals not treated (AA), arthritic animals treated with QUE (AA-Q) in an oral daily dose of 150 mg/kg b.w. during 28 days. In each group 10 animals were used. After the animals have been sacrificed under deep ketamin/xylasine anesthesia, blood for plasma preparation and tissues for homogenate preparation (joint, lung and liver) were taken at the end of the experiment (day 28). Tissue were immediately frozen and stored at −80 °C until analysis. Blood samples were centrifuged at 2400 × g for 15 min at 4 °C and stored at −80 °C until analyses.

2.2. Change of body weight

Change of body weight (CBW; g) was measured on days 1, 14 and 28. CBW was calculated as the difference of the body mass measured on days 14 and 28 and the body weight measured at the beginning of the experiment (day 1).

2.3. Arthritic score

The arthritic score was measured as the total score of hind paw volume (ml, max. points 8) plus paw diameter of forelimb (mm,