Biomedicine & Pharmacotherapy 102 (2018) 175–184

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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

Quercetin attenuates zymosan-induced arthritis in mice

Carla F.S. Guazelli[a,1], Larissa Staurengo-Ferrari[a,1], Ana C. Zarpelon[a,1], Felipe A. Pinho-Ribeiro[a], Kenji W. Ruiz-Miyazawa[a], Fabiana T.M.C. Vicentini[b], Josiane A. Vignoli[c], Doumit Camilios-Neto[c], Sandra R. Georgetti[d], Marcela M. Baracat[d], Rubia Casagrande[d,2,*], Waldiceu A. Verri Jr[a,2,*]

[a] Departamento de Ciências Patológicas – Centro de Ciências Biológicas, Universidade Estadual de Londrina, Londrina 86051-990, Brazil
[b] Farmacore Biotecnologia LTDA, Rua Edson Souto, 728, Lagoinha, 14095-250 Ribeirão Preto, Brazil
[c] Departamento de Bioquímica e Biotecnologia - Centro de Ciências Exatas, Universidade Estadual de Londrina 86057-970, Brazil
[d] Departamento de Ciências Farmacêuticas – Centro de Ciências de Saúde, Universidade Estadual de Londrina, Londrina 86038-350, Brazil

ARTICLE INFO

Keywords:
Quercetin
Rheumatoid arthritis
Oxidative stress
Inflammation
NF-κB
Nrf2/heme Oxygenase (HO-1) pathway

ABSTRACT

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by articular lesions, recruitment of inflammatory cells and increased levels of pro-inflammatory cytokine. The intra-articular administration of zymosan is an experimental model that promotes inflammatory parameters resembling RA. Therefore, this model was used to investigate the efficacy of quercetin as a treatment of articular inflammation. Treatment with quercetin dose-dependently reduced zymosan-induced hyperalgesia, articular edema and the recruitment of neutrophils to the knee joint cavity. Histological analysis confirmed that quercetin inhibited zymosan-induced arthritis. The treatment with quercetin also inhibited zymosan-induced depletion of reduced glutathione (GSH) levels, TNFα and IL-1β production, and $gp91^{phox}$, prepro-endothelin-1 (preproET-1), and cyclooxygenase-2 mRNA expression. These molecular effects of quercetin were related to the inhibition of the nuclear factor kappa-B and induction of Nuclear factor erythroid 2- related factor (Nrf2)/home oxygenase (HO-1) pathway. Thus, quercetin exerted anti-inflammatory, analgesic and antioxidant effects in experimental arthritis, suggesting quercetin is a possible candidate for arthritis treatment.

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune disease that affects the joints inducing pain, stiffness, synovitis, cartilage destruction and loss of function [1]. The proliferation of resident fibroblast-like and macrophage-like synoviocytes, angiogenesis and the infiltration of macrophages, lymphocytes, and neutrophils into the synovial space characterize RA [2]. Regarding the limitations of experimental models of articular inflammation, they have been essential to unveil the underlying physiopathological mechanisms and development of novel treatments for RA [3].

The intra-articular (ia) administration of zymosan in mice promotes the emergence of inflammatory parameters akin to RA, and for this reason, it is considered a suitable animal model for studying novel therapeutic approaches for this disease [4,5]. Zymosan-induced articular hyperalgesia is a result of TLR2 activation that signals through the adaptor molecule MyD88 [5]. This signaling pathway triggers the production of inflammatory cytokines including TNFα, CXCL1 and IL-1β [5]. Arachidonic acid lipid products, complement system and endothelin-1 (ET-1) [6–8] are also involved in zymosan inflammation. ET-1 is a mediator of innate and adaptive inflammation [9,10], which recruits neutrophils by inducing the production of TNF-α and CXCL1 in adaptive immune responses [9]. Endogenous endothelin is an inflammatory mediator that also participates in zymosan-induced knee joint inflammation by acting through distinct cell surface specific G protein-coupled receptors and modulating edema formation, neutrophil recruitment, and production of TNF-α and chemokines [8]. In collagen-induced arthritis, the endothelin receptor antagonist bosentan, reduces articular inflammation [11]. Thus, targeting ET-1 could be an important therapeutic approach to treat articular diseases.

Abbreviations: ARE, antioxidant response element; COX-2, cycloxigenase-2; DTNB, dithiobisnitrobenzoic acid; ET-1, endothelin-1; GSH, glutathione; GR, glutathione reductase; GSH-Px, glutathione peroxidase; HO-1, heme oxygenase-1; ia, intra-articular; Maf, members of the basic leucine zipper family of transcription factors; NADPH, nicotinamide adenine dinucleotide phosphate; NF-κB, nuclear factor kappa-B; Nrf2, nuclear factor erythroid 2-related factor; phox, phagocyte oxidase; preproET-1, prepro-endothelin-1; PKC, protein kinase C; PG, prostaglandin; RA, rheumatoid arthritis; ROS, reactive oxygen species; VEGF, vascular endothelial growth factor

* Corresponding author.
E-mail addresses: rubiacasa@uel.br (R. Casagrande), waverri@uel.br (W.A. Verri).
[1] These authors contributed equally.
[2] These authors share senior authorship.

https://doi.org/10.1016/j.biopha.2018.03.057
Received 22 October 2017; Received in revised form 9 March 2018; Accepted 11 March 2018

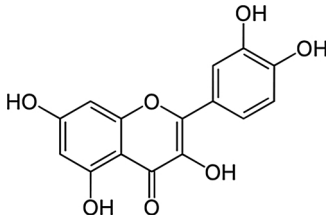

Fig. 1. Chemical structure of quercetin.

Neutrophils are key cells in articular inflammation. Neutrophils release proteolytic enzymes and have their respiratory burst activated generating superoxide anion, hydrogen peroxide, and hydroxyl radical [12]. ROS can also function as second messengers to activate NF-κB, which induces the expression of inflammatory genes [13]. Additionally, neutrophils perpetuate the inflammation by orchestrating the recruitment of lymphocytes and macrophages [3]. Oxidative stress participates in the pathogenesis of RA, which is characterized by modulation of reduced glutathione (GSH), glutathione reductase (GR) and glutathione peroxidase (GSH-Px) levels [14,15]. This information supports the notion that antioxidant defense mechanisms are impaired in RA, and therefore, replenishing the system with antioxidants would reduce inflammation in RA.

Quercetin (Fig. 1) is the most common flavonoid in nature and presents prominent antioxidant properties including oxygen radicals scavenging, reduction of lipid peroxidation and metal ion chelation [16,17]. Evidence shows that quercetin inhibits pro-inflammatory cytokines (e.g. TNF-α) expression by suppressing NF-κB signaling [18–20], reduces paw edema [21,22] and hyperalgesia, prevents the decrease in GSH levels caused by inflammatory agents [22,23], and diminishes neutrophil recruitment by inhibiting cellular signaling responsible for actin polymerization [24]. Furthermore, quercetin effectively inhibits the proliferation of synoviocytes and angiogenesis in an inflammatory process associated with arthritis indicating its potential as anti-rheumatic drug [25]. Taking into account the above-mentioned evidence, quercetin effects were investigated in zymosan-induced experimental arthritis in mice focusing on molecular events regulated by NF-κB and Nrf2 transcription factors.

2. Materials and methods

2.1. Experimental animals

The experiments were performed on male Swiss mice weighing between 20 and 25 g. Animals were housed in standard clear plastic cages with water and food ad libitum in temperature-controlled rooms (22–25 °C) and a light/dark cycle of 12:12 h. All behavioral testing was performed between 9:00 am and 5:00 p.m. Mice were acclimatized in the testing room at least 1 h before the experiments and were used only once. Mice were anesthetized with isoflurane 5% (Abbot, Abbott Park, IL, USA) by inhalation overdose, and terminally euthanized by cervical dislocation followed by decapitation at the end of experiments described in the following sections. Animal care and handling procedures were in accordance with the guidelines of the International Association for Study of Pain (IASP) and other Laws that stablish directions on experimental research in animals. This study was approved by the Animal Ethics Committee of the Universidade Estadual de Londrina (CEUA-UEL process number: 13,279. 2011.76). A total of 216 mice were used in this study as described in the Materials and Methods section and figure captions. All efforts were made to minimize the number of animals used and their suffering.

2.2. Induction of joint inflammation and pharmacological treatment

Joint inflammation was induced by intra-articular (ia) injection of 10, 30 or 100 μg/cavity of zymosan from *Saccharomyces cerevisiae* (Sigma, St. Louis, MO), in 10 μL of sterile saline into the right knee joint cavity [26]. Control animals received ia injection of an equal volume of sterile saline. In other experimental groups, mice were pre-treated with 10, 30 or 100 mg/Kg of quercetin (95% purity, Acros, Fair Lawn, NJ, USA) or vehicle (saline plus Tween 80) via s.c. 30 min. before zymosan injection.

2.3. Assessment of articular hyperalgesia and joint edema

The articular hyperalgesia of the femur – tibial joint was evaluated with an electronic anesthesiometer [27,28]. This method consists of an electronic pressure-meter, attached to digital force counter fitted with a large polypropylene tip (Insight instruments, Ribeirao Preto, SP, Brazil) [29,30]. Briefly, in a quiet room, mice were placed in acrylic cages with a wire grid floor 15 – 30 min before testing. Stimulations were performed only when animals were quiet and with the four paws on the grid floor. The force applied to induce paw withdrawal was automatically recorded. The absolute values of the mechanical threshold (in grams) were considered the intensity of articular hyperalgesia.

The transverse diameters of the knee joints were measured using an analogic caliper (Digmatic Caliper, Mitutoyo Corporation, Kanagawa, Japan) to determine knee edema. The difference between the diameter measured before (basal) and after induction of articular inflammation are shown in millimeters (mm) [31]. The articular hyperalgesia and joint edema were assessed 1, 3, 5 and 7 h after zymosan injection.

2.4. Leukocyte recruitment

Leukocyte recruitment to the femur-tibial joint was determined 7 h after the inflammatory stimulus injection. The articular cavities were washed 3 times with 3.3 μL of saline with 1 mM EDTA [32]. The total number of leukocytes was determined in a Neubauer chamber diluted in Turk's solution (used to lyse the erythrocytes). Differential cell counts were determined by Roselfeld stained slices using a light microscope and results were expressed as the number of neutrophils and mononuclear cells per cavity.

2.5. Histological analyses

The femur-tibial joints were collected 7 h after ia administration of zymosan or saline, preserved in 10% formaldehyde, decalcified in EDTA and processed for hematoxylin-eosin (HE) staining to assess morphological changes and confirm leukocyte infiltration in response to zymosan. Histopathological score grading was performed according to the inflammatory cell infiltrate. The dimension used was 544 × 582 pixels for analysis (Field).

2.6. GSH assay

Femur-tibial joints were collected 3 h after ia administration of zymosan or saline and frozen at −80 °C until GSH assay. The GSH levels were determined using a spectrophotometric method with modification [33,34]. The samples were homogenized in EDTA 0,02M solution using an ultra-turrax. Homogenates were treated with 50% trichloroacetic acid and centrifuged at 4000 rpm for 15 minutes. Then, 100 μL of sample was mixed with 200 μl of 0.4 M Tris-HCl, pH 8.9, followed by addition of 10 μl of 10 mM dithiobisnitrobenzoic acid (DTNB) in methanol. The absorbance at 412 nm was read after 5 min. The standard curve was prepared with 0.50 μM GSH, and the results were presented as nM GSH/g protein. The total protein content in samples was determined in spectrophotometer by the method described by Lowry et al. [35].