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Quercetin ameliorates kidney injury and fibrosis by modulating M1/M2 macrophage polarization

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ABSTRACT

Interstitial inflammation is the main pathological feature in kidneys following injury, and the polarization of macrophages is involved in the process of inflammatory injury. Previous studies have shown that quercetin has a renal anti-inflammatory activity, but the potential molecular mechanism remains unknown. In obstructive kidneys, administration of quercetin inhibited tubulointerstitial injury and reduced the synthesis and release of inflammatory factors. Further study revealed that quercetin inhibited the infiltration of CD68+ macrophages in renal interstitium. Moreover, the decrease in levels of iNOS and IL-12, as well as the proportion of F4/80+/CD11b+/CD86+ macrophages, indicated quercetin-mediated inhibition of M1 macrophage polarization in the injured kidneys. In cultured macrophages, lipopolysaccharide-induced inflammatory polarization was suppressed by quercetin treatment, resulting in the reduction of the release of inflammatory factors. Notably, quercetin-induced inhibitory effects on inflammatory macrophage polarization were associated with down-regulated activities of NF-κB p65 and IRF5, and thus led to the inactivation of upstream signaling TLR4/Myd88. Interestingly, quercetin also inhibited the polarization of F4/80+/CD11b+/CD206+ M2 macrophages, and reduced excessive accumulation of extracellular matrix and interstitial fibrosis by antagonizing the TGF-β1/Smad2/3 signaling. Thus, quercetin ameliorates kidney injury via modulating macrophage polarization, and may have therapeutic potential for patients with kidney injury.

1. Introduction

Interstitial macrophages in kidney tissues are important inflammatory cells in the process of kidney injury, but its role is controversial [1,2]. Macrophages induce the production of reactive oxygen species (ROS), promote the synthesis of nitric oxide (NO), and release various inflammatory factors, resulting in glomerular intrinsic cells and basement membrane damage, and then cause proteinuria, glomerulonephritis and interstitial fibrosis [3]. Therefore, the excessive accumulation of macrophages is an important factor inducing the occurrence of kidney injury, and the removal of renal macrophages can effectively reduce the course of kidney injury [4]. However, macrophages also secrete growth factors, promote tissue repair and remodeling, to maintain the integrity of the organ, suggesting that macrophages have a protective effect of kidney injury [5]. This difference in biological effects of macrophages in kidney injury may be related to its plasticity and heterogeneity [6].

In different microenvironments, macrophages are polarized into two distinct functional phenotypes: classically activated inflammatory (M1) or alternatively (M2) activated [7,8]. In response to activation of toll-like receptors (TLRs) and cytokines such as interferon-γ (IFN-γ), M1 macrophages secrete interleukin 12 (IL-12) and inducible nitric oxide synthase (iNOS), which has the effects of proinflammation, chemotaxis and inducing matrix degradation [9]. In contrast, M2 macrophages express arginase-1 (Arg-1) and IL-10, which play an anti-inflammation, repair tissue and angiogenesis [10]. In the early stage of kidney injury, M1 macrophage polarization and subsequent release of inflammatory factors may be a key factor for the induction of inflammatory injury [11,12]. Thus, the inhibition of M1 macrophage polarization may be an effective strategy for the prevention and treatment of kidney injury.

Abbreviations: Arg-1, arginase-1; BUN, blood urea nitrogen; ELISA, enzyme-linked immunoabsorbent assay; IFN-γ, interferon-γ; IL-12, interleukin 12; iNOS, inducible nitric oxide synthase; IRF, interferon regulatory factor; LPS, lipopolysaccharide; NF-κB, nuclear transcription factor-κB; NO, nitric oxide; ROS, reactive oxygen species; SCr, serum creatinine; TGF-β1, transforming growth factor β1; TLRs, toll-like receptors

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Screening highly effective pharmaceutical agents to inhibit the polarization of M1 macrophages provides a large opportunity for the development of anti-inflammatory drugs.

Quercetin is a natural flavonoid compound, which mostly in the form of glycosides exists in the plant flowers, leaves and fruit. Many Chinese herbal medicines such as locusta, red dates, buckwheat, etc. also contain this ingredient. Quercetin has anti-tumor, anti-inflammation, anti-oxidation, anti-platelet aggregation and free radical scavenging, and other biological activities [13,14]. In skeletal muscle inflammation, quercetin reduces the release of inflammatory factors and inhibits macrophage infiltration by down-regulating the activity of nuclear transcription factor-κB (NF-κB) [15]. In kidneys, quercetin decreases oxidative stress and exerts DNA-protective effects in alloxan-induced diabetic mice [16]. Additionally, quercetin also inhibits fibroblast activation and kidney fibrosis by the suppression of mammalian target of rapamycin and β-catenin signaling [17]. Taken into account of all these above, we hypothesize that quercetin may have an anti-inflammatory effect via antagonizing M1 macrophage polarization.

In the present study, we investigated the protective effects of quercetin on kidney injury in mice with unilateral ureteral obstruction (UUO) in vivo, and cultured macrophages (RAW264.7) stimulated with the TLR4 ligand lipopolysaccharide (LPS) or IL-4 in vitro. Furthermore, the infiltration of inflammatory cells, the polarization of macrophages, and the activities of NF-κB and interferon regulatory factor (IRF) were also evaluated. Our findings suggest that quercetin is able to inhibit M1 macrophage polarization via the NF-κB and IRF5 signaling, and thereby ameliorates kidney injury. Interestingly, quercetin also inhibits M2 macrophage polarization and reduces excessive accumulation of extracellular matrix by the TGF-β/Smad pathway. As a result, interstitial fibrosis in the kidneys is reduced by quercetin treatment. Thus, quercetin has therapeutic potential for patients with kidney injury.

2. Materials and methods

2.1. Animal models

Thirty-six ICR/JCL mice weighing 18–20 g and 6–8 weeks old were purchased from the Experimental Animal Center of Wenzhou Medical University (Wenzhou, China). Mice were housed in a temperature-, humidity- and light-controlled environment, and fed a standard chow and water. Mice were fasted in the day prior to experiments being conducted. Weight-matched mice were randomly assigned to three groups: (1) a sham operation group (n = 12); (2) a vehicle group (n = 12, obstructive mice treated with normal saline); and (3) quercetin group (n = 12, obstructive mice treated with quercetin). Obstruction surgery was performed as previously described [18], and after 2 h postoperatively, UUO mice received daily intragastric administration consecutively for 3 days with either normal saline or quercetin (20 mg/kg/d, lot No. 20120330, Yuanye Biotechnology, Shanghai, China) according to previous report [19]. The kidneys were excised on days 3 after surgery, and serum samples were collected to examine the levels of SCr and BUN using an AU5800 automatic biochemistry analyzers (Beckman Coulter Inc., Kraemer Boulevard Brea, CA, USA). The Scr levels were measured via the sarcosine oxidase method, and the BUN method employs a urease/glutamate dehydrogenase coupled enzymatic technique. All mice were sacrificed by cervical dislocation and were anesthetized by 0.2% pentobarbital natrium (Sigma-Aldrich; Merck Millipore, Darmstadt, Germany).

The animal study protocols (wydw2016-0030) were approved by the Institutional Animal Care and Use Committee of Wenzhou Medical University, China.

2.2. Histopathological examination

Kidney specimens fixed in formalin and embedded in paraffin were cut into 4-μm sections and stained with Hematoxylin and eosin (HE, Yuanye) and Masson's trichrome (Yuanye). Slides were examined and pictures taken using a DM4000 B LED microscope system (Leica Microsystems, Wetzlar, Germany) and a DFC 420 C 5M digital microscope camera (Leica Microsystems). Tubulointerstitial damage and the degree of interstitial collagen deposition were graded as described previously [20].

2.3. Immunohistochemical staining

Immunohistochemical analysis was performed with 4-μm-thick kidney sections that had been dewaxed with xylene and hydrated using sequential ethanol (100, 95, 85, and 75%) and distilled water. Endogenous peroxidase was blocked with 3% hydrogen peroxide. Antigen retrieval was performed by heating sections in 0.1% sodium citrate buffer (pH 6.0). Anti-CD68 (1:100, Biogot Technology, Shanghai, China) and anti-MCP-1/CCL2 (1:200, Santa Cruz Biotechnology, CA, USA) antibodies were used by immunochemical streptavidin-perosidase method. All samples were semi-quantitatively or quantitatively assessed by two independent investigators in a blinded manner.

2.4. Cell culture and treatment

The mouse macrophage line (RAW264.7) was purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). RAW264.7 cells were maintained in Dulbecco's Modified Eagle Medium (DMEM, Invitrogen, CA, USA) supplemented with 5% fetal bovine serum (FBS, Invitrogen), 100 U/ml penicillin and 100 μg/ml streptomycin (Invitrogen). The RAW264.7 cells were seeded in the complete medium containing 5% FBS at approximately 70% confluence in six-well culture plates. After 24 h, the complete medium was replaced with serum-free medium for 24 h before treatment with 10 ng/ml recombinant LPS (Lot No. 5164948, Lianke Biotechnology, Hangzhou, China), 20 ng/ml IL-4 (Lot No. 081449, PeproTech, RockyHill, NJ, USA) or quercetin (10, 100 μmol/ml).

2.5. Enzyme-linked immunoabsorbent assay (ELISA)

Mice kidney tissues (100 mg) were homogenized, centrifuged and the supernatant was collected. In addition, macrophages culture supernatant was also collected. Avidin-biotin complex-ELISA was used according to the manufacturer's protocol to determine the levels of IL-1β, IL-6, IFN-γ, TNF-α, iNOS, IL-12, Arg-1, and TGF-β1. ELISA kits were purchased from Xitang Biotechnology (Shanghai, China). All experiments were repeated at least three times.

2.6. Immunocytochemical staining

Macrophages were cultured with or without treatment in the six-well plates containing glass slides and were then washed with PBS and fixed with 4% paraformaldehyde (Sigma-Aldrich, St. Louis, MO, USA) at 4 °C for 30 min. After permeabilization with 0.1% Triton X-100 for 10 min, the specimens were washed with PBS and then blocked with 10% FBS to eliminate the nonspecific fluorescence. Immunofluorescence staining was performed using NF-κB p65 (1:200, Cell Signaling Technology, CST, Beverly, MA, USA), IRF4 (1:200, Bioworld Technology, Nanjing, China), IRF5 (1:200, Proteintech, Wuhan, China), iNOS (1:200, Proteintech), CD163 (1:200, Proteintech), and Smad2/3 (1:200, Bioworld) as the primary antibodies, and the cell preparations were incubated with DyLight 488/594 labeled secondary antibodies (Beyotime Biotechnology, Jiangsu, China). Immunocytochemical samples were semiquantitatively or quantitatively assessed by two independent investigators in a blinded manner.