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Fibroblast growth factor 21 (FGF21) ameliorates collagen-induced arthritis through modulating oxidative stress and suppressing nuclear factor-kappa B pathway

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ABSTRACT

It has been demonstrated that circulating FGF21 levels are elevated in the serum and synovial fluid of patients with rheumatoid arthritis (RA). The aim of this study is to investigate efficacy of FGF21 for treatment of RA and the molecular mechanisms of the therapeutic effect on collagen-induced arthritis (CIA). Mice with CIA were subcutaneously administered with FGF21 (5, 2 or 1 $mg \cdot kg^{-1} \cdot d^{-1}$), IL-1β antibody (5 $mg \cdot kg^{-1} \cdot d^{-1}$), IL-17A antibody (5 $mg \cdot kg^{-1} \cdot d^{-1}$) and dexamethasone (DEX) (1 $mg \cdot kg^{-1} \cdot d^{-1}$), respectively. The effects of treatment were determined by arthritis severity score, histological damage and cytokine production. The activation of NF-κB was analyzed by Western blotting. We also detected the levels of oxidative stress parameters. Our results showed that FGF21 had beneficial effects on clinical symptom and histological lesion of CIA mice. Similar to antibody and DEX, FGF21 treatment alleviated the severity of arthritis by reducing humoral and cellular immune responses and down-regulating the expression of pro-inflammatory cytokines. FGF21 treatment also reduced the expression of TNF-α, IL-1β, IL-6, IFN-γ and MMP-3 and increased level of IL-10 in the spleen tissue or the plasma of CIA mice in a dose-dependent manner. Furthermore, FGF21 inhibited IκBα degradation and NF-κB p65 nuclear translocation and induced significant changes of oxidative stress parameters (MDA, SOD, CAT, GSH-PX and GSH) in the plasma. FGF21 exerts therapeutic efficacy for RA through antioxidant reaction and inhibiting NF-κB inflammatory pathway. This study provides evidence that FGF21 may be a promising therapeutic agent for RA patients.

1. Introduction

Rheumatoid arthritis (RA) is a common and systemic autoimmune disease that leads to joint inflammation and progressive cartilage and bone erosion, in which immune cells (lymphocytes and macrophages) and synovial tissues produce a series of complex inflammatory cytokines and other soluble mediators in a pathogenic inflammatory cascade [1]. In RA, the balance between pro-inflammatory and anti-inflammatory cytokines determines the degree and extent of inflammation [15,16]. Pro-inflammatory cytokines like tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) are highly expressed in the rheumatoid joint and play key roles in the pathogenesis of RA [17]. Anti-inflammatory cytokine, such as IL-10, is an immunoregulatory cytokine that can reduce the levels of Th17, which suppresses the destruction of cartilage and bone pathology in RA [2].

Reactive oxygen species (ROS), produced in the course of cellular oxidative phosphorylation by activating phagocytic cells during oxidative bursts, exceed the physiological buffering capacity and result in oxidative stress [3]. In humans, oxidative stress has attracted attention as a potentially causative factor for several diseases with an inflammatory component, such as cardiovascular disease, and diabetes [8–12]. Recent studies indicated that ROS plays an important role in the development of RA, and oxygen-free radicals have been implicated as mediators of

Abbreviations: RA, rheumatoid arthritis; FGF21, fibroblast growth factor 21; CIA, collagen-induced arthritis; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor; IL-6, interleukin-6; IFN-γ, interferon-γ; MMP-3, matrix metalloproteinase-3; IL-10, interleukin-10; DEX, dexamethasone; ROS, reactive oxygen species; MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase; GSH-PX, glutathione peroxidase; GSH, glutathione; NF-κB, nuclear factor-kappa B.

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joint tissue and cartilage damage in RA [6,7]. An excessive production of ROS can serve as important intracellular signaling molecules that amplify the synovial inflammatory–proliferative response. On the other hand, nuclear factor-kappa B (NF-κB), a redox-sensitive transcription factor found in human RA synovium, has been referred to play against apoptosis followed by pannus formation [4], which has been shown to be activated by pro-inflammatory cytokines or ROS [13,14]. Therefore, as one of the central downstream targets regarding the pathobiology of RA, inhibition of NF-κB may be a potential therapeutic way for RA.

Fibroblast growth factor 21 (FGF21), as a hormone secreted predominantly in the liver, pancreas and adipose tissue, is identified as a critical regulator of long-term energy balance, glucose and lipid metabolism. Administration of recombinant FGF21 decreases blood glucose in both diabetic mice and rhesus monkeys [21–24]. However, there is no report about the relationship between FGF21 and autoimmune diseases. Recently, H. Hulejová et al. found that circulating FGF21 levels were elevated in the serum and synovial fluid of patients with RA [25], suggesting that FGF21 may play an important role in RA. The aim of the present study is to investigate the bio-function of FGF21 in RA.

2. Materials and methods

2.1. FGF21 and antibody preparation

The humanized anti-IL-1β and IL-17A antibodies were expressed and purified from Chinese hamster ovary (CHO) cells as described [29]. Recombinant human FGF21 (hFGF21) was expressed from *Escherichia coli* and purified with AKTA purifier (GE, USA). The purified hFGF21 (endotoxin removal) has been demonstrated a high activity in glucose uptake assay in vitro (data not shown).

2.2. Animals

Ethics statement. All experiments were carried out in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and were approved by Harbin Veterinary Research Institute Animal Care and Use Committee.

Male DBA/1 mice 6–8 weeks old with a mean weight of 25–30 g were purchased from Changchun Yisi Animal Research Institute (Changchun, China). They were housed at the animal facility of Pharmaceutical Biotechnology Laboratory of Northeast Agricultural University. Animals were provided water ad libitum and fed with standard rodent chow.

2.3. Induction of CIA and administration of FGF21 and antibodies

Mice were immunized as that in previous report [30]. Each mouse was immunized with 100 μL of a 1:1 (v/v) emulsion 0.1 mol/L acetic acid containing 2 mg/mL of chicken type II collagen (SIGMA) and Freund's complete adjuvant (SIGMA) into the right hind paw. Mice were immunized a second time 7 days later.

The treatment was started on day 14 after the second immunization with chicken type II collagen. For the first experiment, mice were randomly divided into 5 CIA groups and 1 healthy control group (n = 9 mice per group). The CIA groups were injected with one of the following treatments everyday: saline (0.9%), IL-1β antibody (5 mg/kg subcutaneously), IL-17A antibody (5 mg/kg subcutaneously), dexamethasone (DEX) (1 mg/kg) and FGF21 (5 mg/kg subcutaneously). For the second experiment, mice were randomly divided into 4 CIA groups and 1 normal control group (n = 9 mice per group). The CIA mice received daily subcutaneous injection of FGF21 (5, 2, 1 mg/kg) or saline, and normal control were injected with saline (0.9%). The mice were sacrificed at day 47.

2.4. Evaluation of arthritis

According to the previous report [31], we measured the thickness ankle of each mouse (maximal lateral) with a caliper in order to determine the baseline in both left and right ankles. Each ankle was measured 3 times and averaged.

The response of each mouse was monitored every two days for the first 14 days, then every three days during the remaining 33 days (end of the experiment). Likewise, a reading of the response consisted of 3 measurements with a caliper per ankle. Each group of mouse ankle measurements were averaged and recorded as one data point to produce a graphical representation of the data.

The severity of the arthritis in each limb was scored on an established scoring system of 0–4: 0 = normal, 1 = mild but definite redness and swelling of the ankle or wrist, or apparent redness and swelling limited to individual digits, 2 = moderate redness and swelling of the ankle and wrist, 3 = severe redness and swelling of the entire paw including digits and 4 = maximally inflamed limb with involvement of multiple joints. Scores for the four limbs were summed and each animal given the resulting score (between 0 and 16). Assessment of the arthritis score was carried out by three observers independently.

2.5. Determination of oxidative stress parameters

Sera were collected at the day of sacrifice (day 48) by retro-orbital blood drawing. All the sera samples were centrifuged and were stored at −80 °C for later use.

The end product of lipid peroxidation (MDA), antioxidant enzyme, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-PX), and glutathione (GSH) were determined in the plasma of all test groups according to the manufacturer's protocols. The normal mice served as healthy controls.

To determine the activity of MDA we used a commercial kit (Beyotime Institute of Biotechnology, Suzhou, China) to quantify the generation of MDA according to the manufacturer's protocol. The MDA levels were determined at 532 nm using tetra methoxypropane as standard. The results were expressed as the contents umol/L.

The assay for total SOD in plasma was based on its ability to inhibit the oxidation of oxyamine by the xanthine–xanthine oxidase system according to the manufacturer's protocol (Nanjing Jiancheng Bioengineering Institute). One unit (U) of SOD activity was defined as the amount that reduced the absorbance at 550 nm by 50%, and enzymatic activity was expressed as U/mL.

CAT activity was determined by measuring the intensity of a yellow complex formed by molybdate and H_2O_2 at 405 nm according to the manufacturer's protocol (Nanjing Jiancheng Bioengineering Institute), after ammonium molybdate was added to terminate the H_2O_2 degradation reaction catalyzed by CAT. The enzymatic activity was expressed as U/mL.

GSH-PX activity assay was based on the method of Paglia and Valentine (1967). tert-Butylhydroperoxide was used as substrate. The assay measures the enzymatic reduction of H_2O_2 by GSH-PX through consumption of reduced glutathione (GSH) that is restored from oxidized glutathione GSSG in a coupled enzymatic reaction by GR. GR reduces GSSG to GSH using NADPH as a reducing agent. The decrease in absorbance at 340 nm due to NADPH consumption was measured in a Molecular Devices M2 plate reader (Molecular Devices, Menlo. Park, CA). GSH-PX activity was computed using the molar extinction coefficient of 6.22 mM^{-1} cm^{-1}. One unit of GSH-PX was defined as the amount of enzyme that catalyzed the oxidation of 1.0 mol of NADPH to NADP+ per minute at 25 °C.

GSH content was determined using a thiol-specific reagent, dithionitrobenzoic acid (DTNB) according to the manufacturer's protocol (Nanjing Jiancheng Bioengineering Institute), and the adduct was