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Original article

FGF21 exerts comparable pharmacological efficacy with Adalimumab in ameliorating collagen-induced rheumatoid arthritis by regulating systematic inflammatory response

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ABSTRACT

Previous studies have reported that Fibroblast growth factor 21 (FGF21) can regulate inflammation and may play an important role in inflammatory and immune-mediated diseases, such as autoimmune diseases. Adalimumab is one of the clinically effective anti-rheumatoid arthritis (RA) drugs. The aim of this study was to compare the therapeutic efficacy of FGF21 and Adalimumab on collagen-induced arthritis (CIA) model mice. Mice with CIA were subcutaneously treated with FGF21 or Adalimumab at dose of 1 mg kg^{-1} d^{-1}, respectively. Our results showed that FGF21 significantly alleviated the severity of arthritis by reducing cellular immune responses and exerted the similar anti-inflammatory effects with Adalimumab in decreasing the mRNA and protein expression levels of IL-2, IL-6 and IL-17. However, the expression levels of IL-1β, RANKL and IL-10 in the mice treated with FGF21 were decreased 2.2-fold, 2.5-fold and increased 4.3-fold compared with Adalimumab, respectively. However, the levels of TNF-α in the mice treated with Adalimumab were lower than those in the mice treated with FGF21. Western blotting results demonstrated that FGF21 displayed equivalent effects with Adalimumab by inhibiting NF-κB/IκBα signaling pathway. However, FGF21 could also regulate systematic inflammatory response and the mechanism maybe related to other signal pathway. In summary, FGF21 exerts comparable pharmacological efficacy with Adalimumab by regulating systematic inflammatory response, providing that FGF21 may be a promising therapeutic agent for RA patients.

1. Introduction

RA is known as a common autoimmune disease, which causes joint inflammation and progressive cartilage and bone erosion [1]. Studies have reported that the pathophysiology of this disease is initiated by the immune system recognizes self-joint antigens as non-self, leading to trigger many distinct inflammatory factor mechanisms [2,3]. The inflammatory cytokines and soluble mediators are secreted by immune cells and synovial tissues [4]. The degree of inflammation is determined by the balance between pro-inflammatory and anti-inflammatory cytokines [5,6]. Pro-inflammatory cytokines like tumor necrosis factor-alpha (TNF-α), are important pathogenic factors and mainly expressed in joint [7]. Anti-inflammatory cytokines as IL-10 are treatment factors in relieving the destruction in RA [8].

Clinically, there are many drugs and treatments used for RA. TNF-α inhibitors are the most effective drugs in improving clinical, functional and radiographic outcomes [9,10]. Adalimumab and Etanercept are the worldwide best-selling TNF-α inhibitor drugs [11,12]. However, treatments with TNF-α inhibitors also have its drawbacks, including dose-dependent adverse effects, increased risk of infections and skin cancer and costly, *etc* [13–15]. Thus, patients with RA are in urgent need of efficiency, safety and economic new drugs [16–18].

Previous studies demonstrated that several adipokines played a significant role in RA patients [19–24] and may reflected disease

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activity. They also modulated inflammation and joint destruction in RA [25]. FGF21 was a promising anti-diabetic drug and associated with metabolic disorders in regulating glucose and lipid metabolism, while not causes adverse effects [26–30]. Recently, scientists found that circulating FGF21 was elevated in RA patients [31]. It was also reported that FGF21 could regulate inflammation and protect inflammation-induced toxicity [32–34]. Yu et al. demonstrated that FGF21 can ameliorate arthritis through improving nuclear factor-kappa B (NF-κB) pathway [1]. Considering above results, our studies were designed to compare the pharmacological efficacy of FGF21 with Adalimumab in treatment of RA.

2. Materials and methods

2.1. Preparation of FGF21 and Adalimumab

FGF21 was produced in *E. coli* Rosetta (DE3) and purified by Ni Sepharose 4FF and Capto Q ion exchange chromatography [35]. SDS-PAGE was used to identify the purity and molecular weight of FGF21. Glucose uptake assay was used to detect the *in vitro* activity of the FGF21 protein. Adalimumab was purchased from Vetter Pharma-Fertigung GmbHCo.KG.

2.2. Ethics statement

Male C57BL/6 mice weighing 25–30 g, 6–8 weeks old, were purchased from the Experimental Animal Center of Changchun YiSi Company. They were housed at the animal facility of Pharmaceutical Biotechnology Laboratory of Northeast Agricultural University. All mice were bred and maintained under constant conditions (SPF) at temperature about 25 °C, humidity 40%–50%, with 12 h light and 12 h dark cycles. 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.

2.3. Induction of CIA and treatment with two proteins

Chick Type II collagen (CII, Sigma) was dissolved in 0.1 M acetic acid overnight at 4 °C to a concentration of 2 mg/mL, and then emulsified with equal volume of complete Freund's adjuvant (Sigma). The C57BL/6 mice were immunized by intradermal injection with 100 μL emulsion at the base of the tail. Mice were boosted with the same dose 15 days later. Observations were made on the joints of mice. After the second immunization, redness or erythema on the paw of the mice were identified to be arthritic. Eighty percentages of C57BL/6 mice become arthritic in this experiment. The CIA mice were randomly divided into three groups (n = 6): FGF21 (1 mg kg^{-1} d^{-1}), Adalimumab (1 mg kg^{-1} d^{-1}) and CIA control group (0.9% saline). Six male C57BL/6J mice of the same age were taken as the normal control group (0.9% saline). For treatment, the mice were received daily subcutaneous injection and sacrificed on the fifty-fourth day. Mice were considered as arthritic if there were significant changes in redness or erythema on any digits or in other parts of the paws. Joint swelling was observed and scored every two days at 8:30 A.M. before administration until the end of the experiment, using the following scale based on the previous study [36]: 0 representing no swelling; 1 representing little joint swelling or redness; 2 representing toe joints and toe swelling, 3 representing severe redness and swelling of the entire paw including digits, 4 representing maximally inflamed limb with involvement of multiple joints. The arthritis score (between 0 and 16) of all four paws of each animal showed the overall disease severity and progression. Scoring was carried out by 3 independent observers.

2.4. Histopathologic analysis

At the end of this study, the mice were sacrificed by cervical dislocation. Hind paws from each group were resected and fixed for 48 h in 10% buffered formalin and decalcified in 15% EDTA, then trimmed and embedded in paraffin, sectioned and stained with hematoxylin and eosin (HE). According to the severity of arthritis from synovial proliferation, joint inflammation and cartilage destruction, the severity of the joints in the tissue slice was scored on an established scoring system of 0–5 (0 = normal, 1 = minimal, 2 = mild, 3 = moderate, 4 = marked, 5 = severe) as shown in previous study [37]. Scoring was performed by 3 independent observers in a blind manner.

2.5. Quantification of the mRNA expression levels of cytokines by real-time PCR

Total RNAs from the spleens were isolated with TRIzol (Invitrogen), and converted to cDNA using oligo(dT)18. According to the instruction of the Thermal Cycler DiceTM real-time PCR kit (TaKaRa Code: TP800), the cDNA was used for real-time quantitative PCR (ABI 7500, USA) to detect the gene expression levels of IL-1β, IL-2, IL-6, IL-10, IL-17, TNF-α, IFN-γ and RANKL. The gene expression level of β-actin in spleen was used as a reference. Real-time PCR primers were as shown in Table 1.

2.6. Measurement of the levels of serum inflammatory cytokines

At the end of the experiment, blood samples were taken from each mouse. The levels of IL-1β, IL-6, IL-10, IL-17 and TNF-α in serum were measured by using commercially available ELISA kits (R&D Systems, USA). According to the operation procedures of the ELISA kits, the levels of inflammatory cytokines in serum were calculated from standard curves.

2.7. Western blotting

After treatments, nuclear and cytoplasmic proteins were isolated from the spleens by extraction kit (Beyotime Institute of Biotechnology, China). Equal amount of proteins were separated by SDS-PAGE and trans-blotted to nitrocellulose membrane (GE Healthcare). A standard immunodetection protocol was employed

Table 1
Forward and reverse primers for quantitative real-time PCR analysis.

Target	Sequence
β-actin forward	ACA TCT GCT GGA AGG TGG AC
β-actin reverse	GGT ACC ACC ATG TAC CCA GG
IL-1β forward	CCA TGG CAC ATT CTG TTC AAA
IL-1β reverse	GCC CAT CAG AGG CAA GGA
IL-2 forward	GCA CCC ACT TCA AGC TCC A
IL-2 reverse	AAA TTT GAA GGT GAG CATCCTG
IL-6 forward	GAA CTC CTT CTC CAC AAG CGC CTT
IL-6 reverse	CAA AAG ACC AGT GAT GAT TTT CAC CAG G
IL-10 forward	TGC CTT CAG CCA GGT GAA GAC TTT C
IL-10 reverse	CTT GAT TTC TGG GCC ATG CTT CTC TG
IL-17 forward	TCA GCG TGT CCA AAC ACT GAG
IL-17 reverse	CGC CAA GGG AGT TAA AGA CTT
TNF-α forward	GGA AAC CCA GAG GCA TTG AC
TNF-α reverse	TCA GGA TCT GGC CCT TGA AC
IFN-γ forward	TGC TGA TGG GAG GAG ATGTCT
IFN-γ reverse	TGC TGT CTG GCC TGC TGT TA
RANKL forward	TGT ACT TTC GAG CGC AGA TG
RANKL reverse	CCA CAA TGT GTT GCA GTT CC