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Kirenol upregulates nuclear Annexin-1 which interacts with NF-κB to attenuate synovial inflammation of collagen-induced arthritis in rats

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ABSTRACT

Ethnopharmacological relevance: Kirenol is a diterpenoid compound purified from the Chinese Herba Siegesbeckiae. Siegesbeckiae has been employed for the treatment of arthritis for centuries, its safety and efficacy are documented through a long history of human use.
Aim of the study: To investigate the effects on collagen-induced arthritis (CIA) and anti-inflammatory mechanism of Kirenol.
Materials and methods: Kirenol was administrated intragastrically in rats after the onset of CIA. Pathological changes were evaluated by paw swelling and histopathology. Concentration of IL-1β in synovial fluid and adrenal corticotropin (ACTH) in plasma were determined by Elisa. Western blot was performed to detect the expression of Annexin-1 and glucocorticoid receptor alpha (GRα) in synovium. NF-κB DNA binding activity was assessed by electrophoretic mobility shift assays (EMSA).
Results: Kirenol (1, 2, and 4 mg/kg) and Prednisolone depressed paw swelling and reduced IL-1β of synovial fluid in the CIA rats ($p < 0.05$ or $p < 0.01$). Kirenol and Prednisolone upregulated nuclear Annexin-1 and inhibited NF-κB activity in synovium of CIA. The inhibitory effect of Kirenol and Prednisolone on NF-κB activity was enhanced by anti-Annexin-1 Ab. Prednisolone, but not Kirenol, downregulated plasma ACTH and GRα expression significantly ($p < 0.01$).
Conclusion: Kirenol and Prednisolone can upregulate nuclear Annexin-1 which interacts with NF-κB to inhibit NF-κB activity, reduce cytokines expression and thereby attenuate inflammation of CIA joints. Kirenol does not lead to ACTH or GR downregulation, which is in contrast to classic glucocorticoid Prednisolone. Kirenol shares with GCs similar anti-inflammatory mechanism but bypass the considerable limitation of GCs treatment.

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1. Introduction

Rheumatoid arthritis (RA) is a systemic and chronic inflammatory autoimmune disease characterized by symmetric inflammation of synovial joints leading to progressive erosion of cartilage and bone (Smith and Tak, 2002; Doan and Massarotti, 2005). Cytokines have emerged as crucial players in mediating synovial inflammation in the rheumatoid joint. Among the cytokines, tumor necrosis factor-alpha (TNF-α) and interleukin-1 (IL-1) are considered to be of great importance in the pathogenesis of the disease (Gay et al., 1993; Odeh, 1998). The nuclear factor kappa B (NF-κB) plays a pivotal role in transcriptional activation of TNF-α and IL-1β and has been regarded as an important therapeutic target (Lee and Burckart, 1998; Tak and Firestein, 2001; Roman-Blas and Jimenez, 2006; Sethi et al., 2008).

Annexin-1 is an important mediator of the anti-inflammatory actions of glucocorticoids (GCs) (Perretti and Gavins, 2003), belonging to the annexin family of calcium-dependent phospholipid binding proteins (Lim and Pervaiz, 2007). Annexin-1 and its N-terminal fragment scan affect many components of the inflammatory reaction and play regulatory roles in synthesis of arachidonic acid, leukocyte migration and apoptosis of inflammatory cells (Flower and Rothwell, 1994; Damazo et al., 2005; D'Acquisto et al., 2007). Disruption of the Annexin-1 gene exacerbates arthritis severity and proinflammatory cytokine expression in antigen-induced arthritis of mice. The absence of Annexin-1 is also associated with insensitivity to the anti-inflammatory effects of dexamethasone (Hannon et al., 2003; Yang et al., 2004). Recent studies have shown that Annexin-1 binds to NF-κB and inhibits its transcriptional activity by preventing NF-κB binding to DNA (Zhang et al., 2010).

Abbreviations: AA, adjuvant arthritis; ACTH, adrenal corticotropin; CIA, collagen-induced arthritis; EMSA, electrophoretic mobility shift assays; GCs, glucocorticoids; GRα, glucocorticoid receptor alpha; RA, rheumatoid arthritis.

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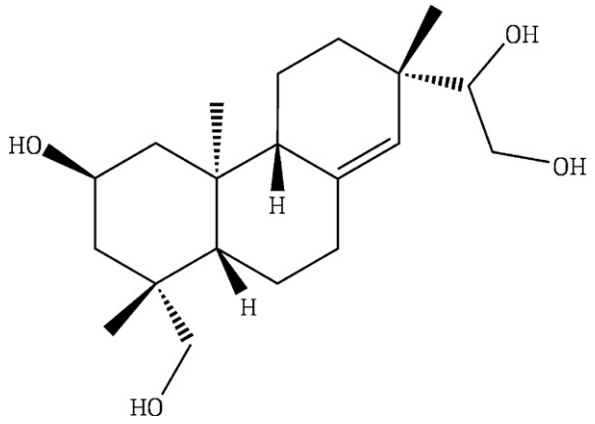

Fig. 1. Chemical structure of Kirenol.

Glucocorticoids have been used to treat rheumatoid arthritis for decades (Hench et al., 1950). GC-activated GC receptor α (GRα) is the most important physiologic inhibitor of inflammation. GRα inhibits inflammation through three mechanisms: direct and indirect genomic effects and nongenomic mechanisms (Rhen and Cidlowski, 2005). The anti-inflammatory mechanisms of GCs involve with inhibition of NF-κB (Meduri et al., 2005; Gossye et al., 2009) and upregulation of Annexin-1 (John et al., 2002; Hannon et al., 2003). Though glucocorticoids are the most effective anti-inflammatory treatment available for many inflammatory and immune diseases (Barnes and Adcock, 2009), side effects including hypothalamic-pituitary–adrenal (HPA) suppression and development of resistance limit their widespread and long-term use (Kirwan and Power, 2007). An international effort is in progress to discover more effective and safe agents.

Kirenol is a compound derived from *Herba Siegesbeckia*, which has been used for the treatment of arthritis for centuries (Fig. 1). Our previous work have demonstrated that Kirenol can attenuate the pathologic response (Qian et al., 2000; Xin et al., 2005) and suppress the levels of IL-1β and TNF-α in serum of adjuvant arthritis (AA) rats (Bi et al., 2007). The present study was designed to clarify the anti-inflammatory mechanism of Kirenol and the relation with that of GCs.

2. Materials and methods

2.1. Experimental animals

Wistar rats (male, 120 ± 10 g, Grade II, Certificate No. 0008) were obtained from the Department of Laboratory Animal Science of Peking University (Beijing, China). All rats were fed on a standard chow pellet diet, had free access to water, housed under standard laboratory conditions and were maintained on a 12-h light 12-h dark cycle. All experiments were approved by Biomedical Ethics Committee of Peking University, Animal Welfare and Ethics Branch (Approval No. LA2011-020).

2.2. Materials and reagents

Type II collagen (CII) and incomplete Freund's adjuvant were purchased from Chondrex (WA, USA). Nuclear and cytoplasmic extraction reagents and anti-glucocorticoid receptor alpha were from Pierce (IL, USA). ACTH Elisa kit was obtained from Phenix (CA, USA); IL-1β Elisa kit from Shanghai ExCell Biology (Shanghai, China) and chemiluminescent EMSA kit from Beyotime Institute of Biotechnology (Shanghai, China). Anti-Annexin-1 antibody and other antibodies were all bought from Cell Signaling Technology (MA, USA).

2.3. Drug

Kirenol (purity more than 99%) was provided by the State Key Laboratory of Natural and Biomimetic Drugs of Peking University (Beijing, China). Prednisolone was purchased from Sigma–Aldrich (St. Louis, MO). Both Kirenol and Prednisolone were suspended in distilled water before use.

2.4. Induction of collagen-induced arthritis (CIA)

Type II collagen was dissolved at 2.0 mg/ml in 0.05 M acetic acid overnight at 4 °C with constant, but gentle stirring, then the collagen was emulsified in the adjuvant at a 1:1 ratio. Each mouse was injected approximately 1 cm from the base of the tail with 0.2 ml of the emulsion subcutaneously. A booster injection was given subcutaneously at the tail avoiding the primary injecting site on d7 post-immunization. The day of the first immunization was defined as d0.

2.5. Treatment of CIA

After the onset of arthritis, animals were randomly divided into six groups of 10 animals each. Rats with CIA were given intragastrically Kirenol (1, 2, and 4 mg/kg) and Prednisolone (2 mg/kg) once per day from d10 to d35 after immunization. Normal and CIA model rats were given an equal volume of distilled water at the same time.

2.6. Arthritis assessment

Each paw was marked with a standard line 0.5 cm above ankle joint. Paw volume was measured bilaterally three times below the standard line and the average was taken as the current volume. Paw swelling (Δml) was calculated by current volume subtracting the paw volume of d0. The arthritic severity in each paw was graded from 0 to 4: grade 0, paws with no swelling and focal redness; grade 1, paws with swelling of finger joints; grade 2, paws with mild swelling of ankle or wrist joints; grade 3, paws with severe inflammation of the entire paw; and grade 4, paws with deformity or ankylosis. Each paw was graded and the four scores were totaled so that the possible maximum score per rat was 16 (Alonzi et al., 1998).

2.7. Synovial membrane and fluid

The rats were anesthetized and the joint capsules were exposed. Intra-articular (i.a.) injected with 50 μl of saline (Singh et al., 1997), pumped the syringe repeatedly and collected the fluid. Joint cavity was opened and the synovial membrane was excised together with patella, patellar ligament and joint capsule after detachment of patellar ligament from the upper part of the tuberosity of the tibia. Synovial membrane, together with infrapatellar fat pad, were separated from the patellar ligament and joint capsule (Hyc et al., 2007).

2.8. Histological examination

The ankle joints were dissected and fixed in 10% buffered formalin for 7 days. Fixed tissues were decalcified for 4 weeks in 15% EDTA, dehydrated, and embedded in paraffin. Sagittal sections (5 μm) of each ankle joint were stained with hematoxylin and eosin (HE). The slides were evaluated histologically by two independent observers who were not aware of the treatment, and the gradation of arthritis was scored from 0 to 4 according to the

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