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ORIGINAL ARTICLE

Therapeutic effects of spironolactone on a collagen-induced arthritis model of rheumatoid arthritis

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KEYWORDS

Spironolactone;
Methotrexate;
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Inflammation;
Inflammatory cytokines;
Oxidative stress

Abstract *Background:* Rheumatoid arthritis (RA) is an autoimmune disease characterized by increased inflammation of synovial joint. The collagen-induced arthritis (CIA) is a widely used animal model for RA. Spironolactone possesses potent anti-inflammatory and immune modifying properties that might make it an excellent medical intervention for rheumatic diseases.

Aim of the work: The present study was conducted to evaluate the therapeutic effect of spironolactone (SPIR) in collagen-induced arthritis model in mice.

Materials and methods: DBA/1mice were divided into eight groups and CIA mice treated with SPIR (20, 40 and 80 mg/kg/day), methotrexate (MTX) and vehicle was administered beginning on day 21 (arthritis onset) until day 42. The effects of treatment in the mice were assessed by clinical, oxidative markers, inflammatory cytokines; tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) as well as histological changes in ankle joints.

Results: Mice immunized with collagen II with complete and incomplete Freund's adjuvant developed inflammatory arthritis. Spironolactone (40 and 80 mg/kg/day) was effective in bringing significant changes on all the parameters (paw swelling, arthritis score, oxidative markers) studied. Oral administration of SPIR significantly reduced the level of TNF-α and IL-6. The protective effect of SPIR against RA was also evident from the ankle joint histopathology and its effect was found comparable to that of MTX.

Conclusion: Amelioration of paw swelling, antioxidant properties, inflammatory mediators TNF-α and IL-6 and histopathological changes indicates that SPIR can be considered with MTX among the treatment armamentarium of arthritis. Spironolactone may be considered for use as a novel therapeutic treatment against human arthritis.

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune and chronic inflammatory disorder which results in inflammation of multiple joints with subsequent destruction of joint cartilage and

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erosive destruction of bone with evolving treatment strategies [1]. RA is a potentially devastating condition which lacks good treatment options. Although a number of synthetic disease-modifying antirheumatic drugs (DMARDs) are used for the clinical treatment of RA, an effective drug with few side effects and of low cost is still lacking [2–4]. Now a days, much attention has been focused on biologic agents as anti-tumor necrosis factor-α (anti-TNF-α), interleukin-6 (IL-6) and IL-1 that inhibit the activity of proinflammatory cytokines, which are believed to play a primary role in joint destruction [5]. Biologic DMARD therapy seems to be even more effective than synthetic DMARDs in RA, ankylosing spondylitis (AS) and psoriatic arthritis (PsA) [5,6]. Despite their efficacy, biologic DMARDs have major limitations as parenteral administration, risk of infection, neutralizing antibodies and the cost [3,7]. Due to the chronic nature of disease and side effects associated with these drugs, patients with RA rely on other option like use of complementary and alternative safe, economical and acceptable oral therapeutic agents for treating arthritis to achieve remission and to prevent collateral damage.

Spironolactone was first known to possess anti-inflammatory potential as early as 1961 [8]. However, that observation seemed to have gone largely unnoticed until the last few years. However, in a Danish study population spironolactone significantly reduced the proinflammatory cytokines as well as decreased gene transcription for many regulators of inflammation and also demonstrated reduction in interferon system in incubated human whole blood in arthritis patients [9]. In 2006, Miura et al. reported the potential anti-inflammatory effect of spironolactone for reduction in inflammation-related cardiovascular risk factors [10]. In another study, the immunomodulatory and anti-inflammatory properties of spironolactone have been shown in autism [11]. Several researchers have shown that spironolactone has the potential to improve endothelial dysfunction in RA, AS and polycystic ovary syndrome [12–14] and to attenuate alleviated oxidative stress [15,16]. This is relevant because oxidative stress has been well described in RA [17–19]. Despite its widespread use in the treatment of different disorders, there is no dearth of scientific evidence regarding its anti-inflammatory, immunomodulatory and antioxidant activity in experimental animal model of arthritis.

In view of above research, the present study was carried out to evaluate the immunomodulatory and anti-inflammatory effect of spironolactone on type II collagen-induced arthritis (CIA) in DBA/1 mice. Collagen-induced arthritis model is the most extensively studied autoimmune model of RA. It is induced by immunization with an emulsion of complete Freund's adjuvant (CFA) and type II collagen (CII) [20].

2. Materials and methods

2.1. Animals

DBA/1 mice (7–10 weeks of age) were purchased from Central Drug Research Institute (CDRI), Lucknow, India. The animals were housed in a controlled environment and provided with standard rodent chow and distilled water. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) and animal care was done in compliance with Committee for the purpose of control and Supervision of Experimental on Animals (CPCSEA) regulations on the protection of animals used for experimental and other scientific purposes (Reg. No- 107/99/CPCSEA).

2.2. Reagents and drugs

Bovine type II collagen (CII), CFA and Freund's incomplete adjuvant (FIA) were purchased from Sigma Aldrich Co., St. Louis, MO, USA. Spironolactone (SPIR) and methotrexate (MTX) were obtained from R.P.G life Sciences, Mumbai, India and Cadila Pharmaceutical Ltd., Mumbai, India, respectively. All the reagents used in the present study were of analytical grade.

2.3. Collagen-induced arthritis (CIA)

Bovine type II collagen was dissolved overnight at −4 °C in 0.01*M* acetic acid at a concentration of 2 mg/ml. For the induction of CIA, mice were immunized with 100 μg of bovine CII in CFA containing 4 mg/ml heat-killed mycobacterium tuberculosis by 50 μl volume intradermal injection at the base of the tail of DBA/1 mice on day 0. On day 21st, a booster injection of CII in FIA was administered at the base of the tail, but proximal to the primary injection site [20].

2.4. Experimental protocol

In total, forty-eight DBA/1 mice were randomly divided into eight groups each comprising six animals. Group I and group II served as normal control and arthritic control/positive control. Groups III–V arthritic mice were treated orally with SPIR 20, 40 and 80 mg/kg/day, respectively given for 21 days after the administration of booster injection of CII in FIA. Group VI arthritic mice treated with MTX (5 mg/kg/week). Groups VII and VIII arthritic mice were treated with carboxymethyl cellulose (CMC) (SPIR groups) and phosphate buffered saline (PBS) (MTX group) respectively and served as a vehicle control. The animals of different groups were sacrificed by cervical decapitation. The blood samples from all groups were collected from the orbital vein at the end of experiment for oxidative markers and inflammatory mediators. The levels of oxidative stress markers including glutathione (GSH), superoxide-dismutase (SOD), thiobarbituric acid reactivity (TBARS) and nitric oxide were determined.

2.5. Evaluation of arthritis

To evaluate the incidence and severity of arthritis, mice were examined 3 times/week. The severity of arthritis was scored on a 4-point scale, in which 0 = normal appearance, 1 = erythema and/or mild swelling, 2 = erythema and/or moderate swelling, and 3 = erythema and moderate swelling extending from the ankle to metatarsal joints, and 4 = erythema and severe swelling encompass the ankle, foot and digits, or ankylosis of the limb. An arthritis score for each mice was calculated by the sum of the four paws. The joint diameter was measured by