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Evaluation of the effect of losartan and methotrexate combined therapy in adjuvant-induced arthritis in rats

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ABSTRACT

There is increasing body of evidence documenting the involvement of angiotensin II in inflammatory diseases. Moreover the up-regulation of angiotensin II AT_1 receptors in the synovium of rheumatoid arthritis patients has been previously described.

This study aimed at investigating the anti-inflammatory effect of losartan, the selective angiotensin II AT_1 receptor blocker, and comparing the efficacy of methotrexate alone and in combination with losartan in adjuvant arthritis in rats. Twelve days post adjuvant injection, Sprague-Dawley rats were treated with methotrexate (1 mg/kg/week), losartan (20 mg/kg/day) and their combination for 15 days. Severity of arthritis was assessed by hind paw swelling, arthrogram scores. Serum was analyzed for measurement of albumin, C-reactive protein (CRP), nitrite/nitrate concentrations, interleukin 1β (IL-1β), tumor necrosis factor-α (TNF-α), vascular endothelial growth factor (VEGF), aspartate transaminase (AST) and alanine transaminase (ALT). Histopathological examination was done for hind paws and livers. Methotrexate and losartan monotherapies significantly reduced all parameters of inflammation and arthritis with better results in the methotrexate group except for the transaminases where losartan caused more significant reduction in their serum levels. The combined therapy showed better results than methotrexate and losartan alone. Hind paws showed better improvement of inflammatory cell infiltration and bone resorption in the combined therapy group. Disturbances in liver architecture and fibrosis caused by adjuvant arthritis were reverted to normal status in the combined therapy group in contrast to losartan and methotrexate monotherapies. In conclusion, methotrexate and losartan combined therapy provided more effective anti-inflammatory and hepatoprotective effects than either drug alone.

1. Introduction

Rheumatoid arthritis is a systemic autoimmune disease characterized by chronic inflammation of the synovial joints, ultimately leading to a progressive and irreversible joint destruction (Firestein, 2003). Early diagnosis and treatment of rheumatoid arthritis reduce joint destruction, preserve function, and improve survival (Kalpakcioglu and Şenel, 2008). Therefore, critical issues concerning the effect of therapy are to control symptoms and signs of the disease for prolonged periods as well as the capacity to retard the damaging effect of inflammation on articular cartilage and bone (Lipsky et al., 2000). Methotrexate is among the most effective disease modifying anti-rheumatic drugs (DMARDs), because of its efficacy and acceptable safety profile (Tian and Cronstein, 2007). The precise mechanism by which methotrexate, at a low dose, modulates inflammation in rheumatoid arthritis is still unclear, although it is thought that methotrexate prevents de novo pyrimidine and purine syntheses, required for DNA and RNA syntheses, consequently inhibits cellular proliferation of lymphocytes involved in the inflammation process (Wessels et al., 2008). Methotrexate also promotes the release of adenosine with adenosine-mediated suppression of inflammation (Cronstein, 2005), and inhibits the production of inflammatory cytokines (Swierkot and Szechiński, 2006). Unfortunately, methotrexate alone may not fully control disease activity. Increasingly methotrexate is used in combination with other disease modifying anti-rheumatic drugs (Goekoop-Ruiterman et al., 2007). Such combinations are not always effective, and may lose effectiveness with time or may cause adverse effects. Additional therapies, with novel mechanisms of action, are therefore needed and drugs targeting the angiotensin pathway, particularly angiotensin II AT_1 receptors, may be considered one class of them. Angiotensin II is classically known as a cardiovascular mediator,

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with a primary role in the control of blood pressure. There is increasing body of evidence documenting the involvement of angiotensin II in inflammatory diseases (Ruiz-Ortega et al., 2001). Angiotensin II is implicated in the up-regulation of pro-inflammatory cytokines (Lapteva et al., 2002; Arenas et al., 2004) and the production of vascular endothelial growth factor (VEGF) which promotes angiogenesis, increases vascular permeability, and is chemotactic for monocytes (Fuad et al., 2002). Accordingly, angiotensin II may contribute to pathogenesis of rheumatoid arthritis. The presence and up-regulation of angiotensin II AT_1 receptors has been described in synovium samples obtained from rheumatoid arthritis patients (Walsh et al., 1994). Therefore, angiotensin II AT_1 receptors blockade, by a specific inhibitor such as losartan, may present a novel and more effective therapeutic target than angiotensin converting enzyme inhibitors, which lack the specificity for angiotensin conversion, in treatment of rheumatoid arthritis (Burnier and Brunner, 2000).

2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley rats weighing 170–200 g were used. All procedures used in this study complied with regulations of the National Research Council's guide for the care and use of laboratory animals.

2.2. Induction of adjuvant arthritis

To develop a rat model of adjuvant arthritis, rats were injected with 0.1 ml suspension of heat-killed *Mycobacterium butyricum* (Difco Laboratories Co-USA), (12 mg/ml) in incomplete Freund's adjuvant (Sigma Aldrich Co-USA), intradermally at the base of the tail (Rovensky et al., 2008). Chronic inflammation was allowed to progress for 12 days then rats were divided into 5 groups of eight rats each.

2.3. Drugs and experimental groups

Group 1: Adjuvant arthritis rats treated twice weekly with methotrexate (KUP, United Douglas Pharm, USA) prepared in sterile saline at a dose of 1 mg/kg/week intraperitoneally (Morgan et al., 2004).
Group 2: Adjuvant arthritis rats treated daily with losartan (Amriya. Pharm. Ind. Alexandria) dissolved in sterile saline at a dose of 20 mg/kg/day orally (Chua et al., 2008).
Group 3: Adjuvant arthritis rats treated with the combination of losartan orally (20 mg/kg/day) and methotrexate intraperitoneally (1 mg/kg/week).
Group 4: Untreated adjuvant arthritis rats receiving sterile saline orally daily.
Group 5: Non-arthritic healthy control rats.

Drugs were administered for 15 days, from day 12 till day 26 from adjuvant injection.

2.4. Assessment of arthritis progression

2.4.1. Arthrogram scores

The severity of arthritis was scored on a 4-point scale, in which 0=normal, 1=slight edema of the small digital joints, 2=edema of the digital joints and footpad, 3=gross edema of the entire footpad below the ankle or wrist, 4=gross edema of the entire footpad including the ankle joint or wrist joint. The sum of the scores for all 4 limbs was calculated as the arthritic index, with a maximum possible score of 16 per rat (Baggott et al., 1998). Arthrogram scores were evaluated on days 12, 19 and 26.

2.4.2. Hind paw swelling

Hind paw swelling was assessed by caliper measurements of ankle (tibiotarsal) joint width (Bendele, 2001), in both the control and the test groups on day 0, 12, 19 and 26.

2.5. Serum parameters

At the end of the study, on day 27, blood samples were collected from the posterior vena cava through a laparotomy incision. Sera were separated and stored at $-80\,^{\circ}C$ for determination of serum albumin level (Doumas et al., 1997), serum C-reactive protein (CRP) (Otsuji et al., 1982), serum nitrite/nitrate concentration (Guevara et al., 1998), serum aspartate transaminase (AST) and alanine transaminase (ALT) (Bergmeyer et al., 1978). Serum interleukin 1β (IL-1β), tumor necrosis factor-α (TNF-α) and VEGF levels were determined by enzyme-linked immunosorbent assay (ELISA) kit (Invitrogen Corporation, USA) (Rosa and Pinto, 2006). All of the ELISA test kits were used according to the manufacturers' instructions.

2.6. Histopathological examination:

Rat hind paws were removed and processed for histopathological examination to determine the extent of joint inflammation. Livers were fixed, processed and examined to detect any histopathological changes caused by the drugs given alone and in combination.

2.7. Statistical analysis

Data are presented as the mean ± S.D. Mann–Whitney Test was used to analyze two independent populations. If more than two populations were analyzed Kruskal Wallis test was used. Wilcoxon signed ranks test was used to compare between different periods and $P<0.05$ was considered as the significance limit for all comparisons.

3. Results

3.1. Hind paw swelling

Hind paw swelling was followed every other day after adjuvant injection on day 0. The width of the tibiotarsal joints of adjuvant arthritis rats has not changed significantly during the first week but clearly increased afterwards reaching significantly high values by day 12 in all experimental groups, $P<0.01$ (Table 1). Comparison between groups was done according to the percentage of change of hind paw swelling from day 12 value in each group. In the untreated adjuvant arthritis group, the hind paw swelling continued to increase progressively reaching significantly higher values on day 19 and 26, $P<0.05$. Treatment with either methotrexate or losartan alone significantly decreased the progression of hind paw swelling as compared to untreated arthritic rats. The decrease was more evident in the methotrexate group, as joint width values were only 6.24% above control values, compared to 8.93% in rats treated with losartan. The combination therapy caused a more significant reduction in hind paw swelling.