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Mechanisms of the anti-inflammatory actions of the angiotensin type 1 receptor antagonist losartan in experimental models of arthritis

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ABSTRACT

Angiotensin (Ang) II and its AT_1 receptors have been implicated in the pathogenesis of rheumatoid arthritis. Activation of the counter-regulatory Ang-(1–7)–Mas receptor axis may contribute to some of the effects of AT_1 receptor blockers (ARBs). In this study, we have used losartan, an ARB, to investigate the role of and the mechanisms by which AT_1 receptors participated in two experimental models of arthritis: antigen-induced arthritis (AIA) in mice and adjuvant-induced arthritis (AdIA) in rats. Treatment with losartan decreased neutrophil recruitment, hypernociception and the production of TNF-α, IL-1β and chemokine (C–X–C motif) ligand 1 in mice subjected to AIA. Histopathological analysis showed significant reduction of tissue injury and inflammation and decreased proteoglycan loss. In addition to decreasing cytokine production, losartan directly reduced leukocyte rolling and adhesion. Anti-inflammatory effects of losartan were not associated to Mas receptor activation and/or Ang-(1–7) production. Anti-inflammatory effects were reproduced in rats subjected to AdIA. This study shows that ARBs have potent anti-inflammatory effects in animal models of arthritis. Mechanistically, reduction of leukocyte accumulation and of joint damage was associated with local inhibition of cytokine production and direct inhibition of leukocyte–endothelium interactions. The anti-inflammatory actions of losartan were accompanied by functional improvement of the joint, as seen by reduced joint hypernociception. These findings support the use of ARBs for the treatment of human arthritis and provide potential mechanisms for the anti-inflammatory actions of these compounds.

1. Introduction

Clinical trials using angiotensin converting enzyme (ACE) inhibitors and angiotensin type 1 (AT_1) receptor blockers (ARBs) have shown that the actions of the renin–angiotensin system (RAS) extend far beyond blood pressure control and electrolyte balance [9,17,28,31]. Angiotensin II (Ang II) exerts several cytokine-like actions *via* the AT_1 receptor by stimulating multiple signaling pathways, several growth factor receptors, reactive oxygen species (ROS) and other pro-inflammatory responses [37]. Animal models and clinical data have also helped to establish that inhibition of Ang II action in non-classical target sites, such as immune cells, explains some of the unanticipated therapeutic effects of ACE inhibitors and AT_1 blockade [9,17,28,31]. Ang II is also implicated in the up-regulation of pro-inflammatory cytokines, such as tumor necrosis factor α (TNF-α), interleukin-1β (IL-1β) and IL-6 [1,23], in the interactions between leukocytes and endothelium, in neutrophil accumulation and in the release of chemokines [25,27].

Rheumatoid arthritis (RA) is a chronic inflammatory disease that mainly targets the synovial membrane, cartilage and bone. It affects 1% of the population and is associated with significant morbidity and increased mortality [13]. Cytokines are directly implicated in many of the immune processes that are associated with the pathogenesis of RA, mostly TNF-α, IL-1β and IL-6 [3,4]. In recent years, the

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targeted blockade of these cytokines has been a major therapeutic advance in the management of RA. Nevertheless, cytokine-based therapy must be administered *via* a systemic route, current treatments are costly, and many patients fail to respond to blockade of either TNF-α [26] or IL-1β [20]. Moreover, minor adverse events, and susceptibility to serious infection is a commonly reported risk [24]. Thus, new therapeutic options for the treatment of arthritis are clearly needed. Since Ang II participates in important steps of the inflammatory response, a reasonable approach to treat arthritis might include the inhibition of local Ang II formation or the blockade of the AT_1 receptor. Moreover, angiotensin receptors, AT_1 and AT_2, are present in some articular structures such as synovia and chondrocytes [21,50]. In this regard, some studies have shown that treatment with ACE inhibitors produces beneficial effects in arthritis models [9]. The implication of the latter findings is that Ang II production and action are important in the cascade of events leading to inflammation in the joint. Indeed, some studies have shown that ARBs decrease joint edema and TNF production in a model of collagen-induced arthritis and in humans [31,39].

Recently, many studies evidenced the counter-regulatory role of Angiotensin-(1–7) [Ang-(1–7)] and its receptor, the G-protein coupled receptor Mas [40]. In this regard, Silveira et al. [42] have shown that the receptor Mas oral agonist, AVE0991, exerts anti-inflammatory actions in animal models of arthritis. In addition, there are many data supporting a role for AT_1 receptor blockade in potentiating the effects of Ang-(1–7) [11,19]. Ferrario et al. [11] showed that the treatment with losartan produced an increase in Ang-(1–7) levels, which, in turn, probably contributed to beneficial renal effects elicited by AT_1 receptor blockade. Iyer et al. [19] have previously reported that chronic treatment with losartan also increased urinary levels of Ang-(1–7) in hypertensive patients. These authors and others suggested that the elevation of Ang-(1–7) levels might contribute to the beneficial effects of ARBs [11,18,19]. On the other hand, the role of ARBs and their interaction with the anti-inflammatory actions of Ang-(1–7) in experimental arthritis have never been previously investigated in any detail.

Therefore, in the present study, we have used losartan, an AT_1 receptor blocker, to investigate the role of ARBs and the mechanisms by which AT_1 receptors participated in two experimental models of arthritis: antigen-induced arthritis (AIA) in mice and adjuvant-induced arthritis (AdIA) in rats. Furthermore, we have also evaluated the interaction between AT_1 blockade and Ang-(1–7)–receptor Mas axis.

2. Methods

2.1. Animals

Eight- to 10-week old male C57Bl/6 (20–25 g) and female Holtzman rats (140–170 g) were obtained from the animal facility of our Institution. Animals were maintained under temperature-controlled condition with an artificial 12-h light–dark cycle, and were allowed standard chow and water *ad libitum*. The study was approved by the local Ethics Committee of Federal University of Minas Gerais.

2.2. Experimental approach

2.2.1. Antigen-induced arthritis (AIA) in mice

To evaluate the inflammatory *milieu* of arthritis (leukocytes migration, cytokines levels, histological changes and pain) and the effect of AT_1 blockade upon these parameters, plasma levels of Ang II and Ang-(1–7) levels and systolic blood pressure, we used wild-type male mice on C57Bl/6 background ($Mas^{+/+}$). In order to induce arthritis, animals were immunized with an intradermal injection of 100 μg of methylated bovine albumin (mBSA, Sigma, St. Louis, MO), emulsified in 500 μg of Freund's complete adjuvant (CFA, Sigma) at the base of the tail (day 0). Two weeks after immunization, antigen challenge was produced by intra-articular injection of 10 μg of mBSA diluted in 10 μl of sterile saline into the left knee joint. As sham group, a subset of $Mas^{+/+}$ mice received intra-articular injection of sterile saline (10 μl) into the same site.

As an attempt to evaluate if Ang-(1–7) might contribute to the anti-inflammatory response to losartan administration in antigen induced arthritis (AIA), the same experimental protocol was performed in mice with genetic deletion of receptor Mas ($Mas^{-/-}$). For this subset of experiments, $Mas^{+/+}$ animals were used as controls.

Mice were treated with an intraperitoneal injection (200 μl) of losartan (1, 3.0 and/or 10.0 mg/kg), or vehicle (NaCl 0.9%) 60 min before and 6 h after antigen challenge [5]. Neutrophil recruitment in the joint and periarticular tissues, production of TNF-α, IL-1β and chemokine (C–X–C motif) ligand 1 (CXCL1), histopathological index and loss of proteoglycans, hypernociception and leukocyte–endothelial cell interactions were assessed at 24 h, peaked of inflammatory responses [5]. All surgical procedures were performed under ketamin and xylazine anesthesia (150 mg/kg and 10 mg/kg, respectively) following by euthanasia.

2.2.2. Rat adjuvant induced arthritis (AdIA)

Female Holtzman rats were immunized with a single subcutaneous injection of 0.2 ml mineral oil–water emulsion (10:1, v/v) containing 400 μg of dried *Micobacterium butyricum* into the dorsal root of the tail at day 0, as previously described [2]. Control animals were injected subcutaneously with a single dose of 0.2 ml mineral–water emulsion (10:1) without *M. butyricum* in the same site. Rats were treated daily from day 10 to 16 with oral administration of losartan (10 mg/kg, 0.5 ml) or vehicle (0.9% NaCl). Periarticular edema was evaluated daily and tissue neutrophil infiltration, production of IL-1β and CXCL1, histopathological index and loss of proteoglycans were evaluated at day 16.

2.3. Measurements

2.3.1. Tissue neutrophil accumulation

The extent of neutrophil accumulation in the periarticular tissues was measured by assaying myeloperoxidase (MPO) activity, as described elsewhere [5]. Briefly, periarticular tissues (near knee) were removed and frozen at −20 °C. Upon thawing and processing, the tissue was assayed for MPO activity by measuring the change in optical density (OD) at 450 nm using 3,3′-5,5′-tetramethylbenzidine (Sigma) in PBS (pH 5.4) as the color reagent. The number of neutrophils in each sample was calculated with reference to a standard curve of the number of neutrophils obtained from the peritoneal cavity of 5% casein-treated animals processed in the same way. The results in synovial tissue were expressed as relative number of neutrophils (relative units – R.U.).

2.3.2. Intraarticular neutrophil accumulation

The total number of intraarticular leukocytes was evaluated in the knee cavity washed with PBS (2 × 5 μl), 24 h after antigen challenge. Total leukocytes were counted in a Neubauer chamber after staining with Turk's solution and differential leukocyte counts obtained after staining with May–Grunwald–Giemsa using standard morphologic criteria.

2.3.3. Tissue cytokines

TNF-α, IL-1β and CXCL1 concentrations were measured in periarticular tissues (around the knee or paw) by specific enzyme-linked immunoassay (ELISA) kits according to the procedures supplied by the manufacturer (R&D Systems, Minneapolis, MN, USA). Periarticular tissues were obtained from dissection of the