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Ramipril and haloperidol as promising approaches in managing rheumatoid arthritis in rats

Mariam Gamal Fahmy Wahba [a], Basim Anwar Shehata Messiha [b,*], Ali Ahmed Abo-Saif [a]

[a] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Nahda University, Beni-Suef, Egypt
[b] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Beni-Suef University, Beni-Suef, Egypt

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ABSTRACT

Rheumatoid arthritis (RA) is a challenging autoimmune disorder, whose treatments usually cause severe gastrointestinal, renal and other complications. We aimed to evaluate the beneficial anti-arthritic effects of an angiotensin converting enzyme (ACE) inhibitor, ramipril and a dopamine receptor blocker, haloperidol, on Complete Freund's Adjuvant-induced RA in adult female albino rats. Rats were allocated into a normal control group, an arthritis control group, two reference treatment groups receiving dexamethasone (1.5 mg/kg/day) and methotrexate (1 mg/kg/day), and two treatment groups receiving ramipril (0.9 mg/kg/day) and haloperidol (1 mg/kg/day). Serum rheumatoid factor, matrix metalloprotinease-3 (MMP-3) and cartilage oligomeric matrix protein as specific rheumatoid biomarkers, serum immunoglobulin G and antinuclear antibody as immunological biomarkers, serum tumor necrosis factor alpha (TNF-α) and interleukin-10 (IL-10) as immunomodulatory cytokines, serum myeloperoxidase and C-reactive protein as inflammatory biomarkers, as well as malondialdehyde and glutathione reduced (GSH) as oxidative stress biomarkers were assessed. A histopathological study on joints and spleens was performed to support the results of biochemical estimations. Ramipril administration significantly corrected all the measured biomarkers, being restored back to normal levels except for MMP-3, TNF-α and IL-10. Haloperidol administration restored all the measured biomarkers back to normal levels except for TNF-α, IL-10 and GSH. In conclusion, ACE inhibitors represented by ramipril and dopamine receptor blockers represented by haloperidol may represent new promising protective strategies against RA, at least owing to their immunomodulatory, anti-inflammatory and antioxidant potentials.

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1. Introduction

Rheumatoid arthritis (RA), a chronic autoimmune inflammatory disease of joints, affects approximately 1% of population (Vivar and Van Vollenhoven, 2014), with spleen involvement in severe cases (Haleagrahara et al., 2013; Patel and Shah, 2013; Zhang et al., 2014). During the disease course, the synovial membrane becomes autoimmunologically infiltrated with CD^{4+} T-lymphocytes (McInnes and Schett, 2011; Valesini et al., 2015), stimulating B cells to produce rheumatoid factor (RF), an immunoglobulin M (IgM) antiglobulin against immunoglobulin G (IgG), and antinuclear antibody (ANA; Ingegnoli et al., 2013; Biesen et al., 2014; Hügle et al., 2014). Cytokines, particularly interleukins (IL's) and tumor necrosis factor-alpha (TNF-α; Edupuganti et al., 2015), and matrix metalloproteinases (MMPs) that can degrade extracellular matrix protein (Mamehara et al., 2010; Kizaki et al., 2015) are up-regulated. Persistent inflammation results in cartilage destruction and release of cartilage oligomeric matrix protein (COMP; Kawashiri et al., 2010; Andersson et al., 2013). Inflammatory cells are rich in myeloperoxidase (MPO; Stamp et al., 2012), while C-reactive protein (CRP) is an acute-phase inflammatory protein of hepatic origin (Medeiros et al., 2015), representing valuable inflammatory markers. Inflammatory cells release several oxidants causing glutathione (GSH) depletion and malondialdehyde (MDA) production (Yu et al., 2015).

Complete Freund's Adjuvant (CFA) obtained from dried Mycobacteria is a well-documented inducer for RA experimentally (Suke et al., 2013; Huang et al., 2014; Miao et al., 2015). Historically, Pearson (1956) concluded that CFA-induced arthritis is a chronic auto-immune condition. CFA causes hyper-immunization through stimulating T-helper cells with up-regulation of cytokines (Billiau and Matthys, 2001). Moreover, CFA administration initiates localized immunological inflammatory reactions in a pattern simulating clinical RA (Stills and Bailey, 1991; Duan et al., 2014; Smith et al., 2015).

Ramipril, a well known angiotensin converting enzyme (ACE) inhibitor, is suggested to have anti-inflammatory and

* Corresponding author.
E-mail address: drbasimanwar2006@yahoo.com (B.A. Shehata Messiha).

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immunomodulatory potentials since the renin angiotensin system (RAS) was reported to have an important role in inducing inflammatory and autoimmune responses. Angiotensin II exerts proinflammatory effects via stimulation of NADPH oxidase and uncoupling of endothelial nitric oxide synthase (Benigni et al., 2010; De Cavanagh et al., 2011). Accordingly, suppression of RAS was considered as an anti-inflammatory strategy (Sandmann et al., 2006; Ding et al., 2014; Ochodnicky et al., 2014).

Haloperidol, a dopaminergic receptor antagonist, was selected here as peripheral dopamine receptor blockade was reported to have immunomodulatory effects (Nakano et al., 2011; Nakashioya et al., 2011). Side by side, blockade of peripheral dopamine receptors was coupled with suppressed pro-inflammatory cytokines production (Laengle et al., 2006). Additionally, up-regulated dopamine D_1 receptors were reported to have an important role in RA inflammatory progression (Capellino et al., 2014).

Accordingly, we aimed to evaluate the modulatory effects of ramipril and haloperidol, compared to the standard drugs dexamesathone and methotrexate, on experimental RA in rats. Serum RF, MMP-3 and COMP as specific rheumatoid biomarkers, IgG and ANA as immunological biomarkers, TNF-α and IL-10 as immunomodulatory cytokines, MPO and CRP as inflammatory biomarkers, as well as MDA and GSH as oxidative stress biomarkers were measured, together with a histopathological study of joint and spleen.

2. Material and methods

2.1. Material

2.1.1. Animals

Adult female albino rats, weighing 200–220 g were used in the present study. Animals were obtained from El-Nasr Company, Abo-Zabaal, Egypt and were kept in the animal room for two weeks for adaptation before being subjected to laboratory experiments. During the adaptation and the experimental periods, animals were allowed free access to standard forage and tap water *ad libitum*. Animals were kept under stable temperature and relative humidity in an air-conditioned animal house with specific pathogen-free conditions, with 12- h/12- h dark/light cycles. Handling of animals and animal care were done according to the guidelines of Beni-Suef Animal House approved by the Pharmacology and Toxicology Department, Faculty of Pharmacy, Beni-Suef University, which were based on the guidelines suggested by the recommendations of the National Institutes of Health (NIH) Guide for Care and Use of Laboratory Animals (Publication no. 85-23, revised 1985).

2.1.2. Drugs, chemicals and reagent kits

Dexamesathone, methotrexate, ramipril, haloperidol and CFA were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Serum RF, MMP-3, COMP, IgG and ANA ELISA kits, and MPO and MDA colorimetric kits were purchased from CliniLab Company (Cairo, Egypt). Serum TNF-α ELISA kit was obtained from Glory Science Company (St. Del Rio, USA). Serum IL-10 ELISA kit was obtained from Cusabo Biotech Co. (China). Serum CRP colorimetric kit was obtained from Assaypro LLC (St. Charles, MO, USA). Serum GSH colorimetric reagent kit was purchased from BioDiagnostic Company (Giza, Egypt). All the used chemicals, solvents and reagents were of analytical grade.

2.2. Experimental design

Sixty weight-matched healthy acclimatized rats were divided into 6 groups, each of 10 rats. Allocation of animals in their groups was completely random, where rats were numbered from 1–60 and divided into groups 1–6 using random digits table in a two-digit manner. Doses of test agents were determined using pilot trials guided with published literature. The first group was kept as a normal control group receiving vehicles only. The second group was kept as an arthritis control group and received CFA only. Groups 3 and 4 were kept as reference treatment groups and received dexamesathone (1.5 mg/kg/day; Gretzer et al., 2001; Gramoun et al., 2014; Wu et al., 2014) and methotrexate (1 mg/kg/day; Kim and Kang, 2015). Groups 5 and 6 were kept as test treatment groups and received ramipril (0.9 mg/kg/day; Liebetrau et al., 2005; Agrawal and Gupta, 2013) and haloperidol (1 mg/kg/day; Zahorodna et al., 2004). Reference standards or test agents were administered orally for seven consecutive days, starting from day 13 through day 19. Blood, spleen and joint samples were withdrawn on day 20 (24 h after the last drug administration).

2.3. Methods

2.3.1. Induction of arthritis

Arthritis was induced with three subcutaneous doses of CFA, each of 0.4 ml, injected in three different limbs, on days 1, 4 and 7. This is an aggressive modified model of arthritis, as previously-reported RA models relied on a single CFA dose (Newbound, 1963; Kripa et al., 2010; Singh et al., 2015), or two CFA doses administered 4 days apart (Snekhalatha et al., 2013).

To ensure chronic arthritis induction in this model, and exclude the presence of a just temporary inflammatory reaction, setting of the model in pilot trials was performed by the aid of a histopathological study on joint and spleen sections isolated 60 days after the last CFA dose.

2.3.2. Serum sampling

On the 20th day, animals were lightly anaesthetized with ether, and blood was collected from the retro-orbital plexus using heparinized micro-tubes. Blood was left to coagulate at room temperature, then centrifuged at 3000 rpm for 10 min using a cooling centrifuge (Sigma 3-30k, USA). The supernatant clear serum layer was withdrawn and stored in a −80 °C deep freezer (Als Angelantoni Life Science, Italy) for the time of assay of serum levels of RF, MMP-3, COMP, IgG, ANA, TNF-α, IL-10, MPO, CRP, MDA and GSH.

2.3.3. Assessment of serum biomarkers

Serum RF and IgG were assessed using ELISA reagent kits (minimal detectable limits are 1.0 μ/ml and 6.0 mg/l, intra-assay variabilities are below 4.9% and 15.0%, and inter-assay variabilities are below 4.0% and 15%, respectively) according to the methods described by kits manufacturer instructions based on the principles described by Waaler (2007) and by Kim et al. (2010). Serum MMP-3 was measured according to ELISA kit manufacturer instructions (minimal detectable limit is 19 pg/ml, intra-assay variability is lower than 5.5%, and inter-assay variability is lower than 9.7%) based on the principles described earlier (Brown, 1998; Nagase; 1998; Haro et al., 2000). Serum COMP was assessed using ELISA reagent kits (minimal detectable limit is 5 ng/ml, intra-assay variability is lower than 8.0%, and inter-assay variability is lower than 10%) as previously described (Paulsson and Heinegård, 1981; Petersen et al., 2010). Serum ANA level was measured using ANA ELISA kits (minimal detectable limit is 0.00078 μ/ml, intra-assay and inter-assay variabilities are lower than 15.0%) as indicated (Von Mühlen and Tan, 1995). Serum TNF-α was assessed using ELISA reagent kits (minimal detectable limit is 1 pg/ml, intra-assay and inter-assay variabilities are lower than 10.0%) as described earlier (Brouckaert et al., 1993). Serum IL-10 was assessed using ELISA reagent kits (minimal detectable limit is 3 pg/ml, intra-assay and inter-assay variabilities are lower than 10.0%) as previously described (Khozeimeh et al., 2014). Serum MPO was measured

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