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The ACE-2/Ang1-7/Mas cascade enhances bone structure and metabolism following angiotensin-II type 1 receptor blockade

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ABSTRACT

The renin angiotensin system (RAS) regulates numerous systemic functions and is expressed locally in skeletal tissues. Angiotensin1-7 (Ang1-7) is a beneficial member of the RAS, and the therapeutic effects of a large number of angiotensin receptors blockers (ARBs) are mediated by an Ang1-7-dependent cascade. This study examines whether the reported osteo-preservative effects of losartan are mediated through the angiotensin converting enzyme2 (ACE-2)/Ang1-7/Mas pathway in ovariectomized (OVX) rats. Sham and OVX animals received losartan (10 mg/kg/d p.o.) for 6 weeks. A specific Mas receptor blocker (A-779) was delivered via mini-osmotic pumps during the losartan treatment period. Serum and urine bone metabolism biomarker levels were measured. Bone trabecular and cortical morphometry were quantified in distal femurs, whereas mineral contents were estimated in ashed bones, serum and urine. Finally, the expression of RAS components, the receptor activator of NF-κB ligand (RANKL) and osteoprotegerin (OPG) was determined. Losartan significantly improved the elevated bone metabolism marker levels and altered trabecular and cortical structures in OVX animals, and restored normal urinary and skeletal mineral levels. Mas receptor inhibition significantly abolished all osteo-protective effects of losartan and enhanced the deleterious effects of OVX. Losartan enhanced OVX-induced up-regulation of ACE-1, AngII, angiotensin type 1 (AT_1) receptor and RANKL expression, and increased ACE-2, Ang1-7, Mas and OPG expression in OVX animals. However, A-779 significantly eradicated the effects of losartan on RAS components and RANKL/OPG expression. Thus, Ang1-7 are involved in the osteo-preservative effects of losartan via Mas receptor, which may add therapeutic value to this well-known antihypertensive agent.

1. Introduction

The well-recognized renin angiotensin system (RAS) regulates body fluids, electrolytes and hence blood pressure (Peach, 1977). The effector octapeptide angiotensin II (AngII) is produced systemically and locally mainly by angiotensin converting enzyme (ACE-1)-mediated cleavage of the decapeptide AngI. AngII exerts its actions by interacting with its specific membrane receptors, angiotensin II type 1 (AT_1) receptor and AT_2 receptor. The ACE-1/AngII/AT_1 receptor axis is responsible for diverse pathological effects of the RAS, including vasoconstriction, proliferation and inflammation, making it an important target for different therapeutic agents, particularly antihypertensive medications, including ACE-1 inhibitors (ACEIs) and AngII receptor blockers (ARBs). The introduction of ACE-2 in 2000 (Donoghue et al., 2000) as a new homolog of ACE-1, which promotes AngII degradation to the heptapeptide Ang1-7, was the cornerstone for the identification of the newly discovered RAS axis. The majority of the reported effects of Ang1-7 are mediated via the activation of a G-protein coupled receptor, the Mas receptor (Nie et al., 2009). The ACE-2/Ang1-7/Mas cascade usually opposes the well-documented effects of the ACE-1/AngII/ AT_1 receptor axis and is considered the beneficial RAS pathway, particularly during cardiovascular events. Accordingly, numerous investigators have recommended the use of Ang1-7 to preserve and even eliminate the AngII-induced damage observed in cardiovascular diseases (Shi et al., 2010).

The linkage between the RAS and bone structure and metabolism has recently received attention. RAS influences bone density, micro-environments and fracture risks. The major proteins and receptors of

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the RAS are expressed locally within bone cells. AT_1 receptor is expressed in cultured osteoblasts and osteoclasts, suggesting the existence of a local RAS in bone (Izu et al., 2009). However, AngII is postulated to be generated from AngI by ACE-1 in osteoblasts or osteoclasts (Hatton et al., 1997). In an in vitro study, AngII blocked the differentiation and bone formation properties of osteoblasts (Schurman et al., 2004). Furthermore, according to the study by Hiruma et al. (1997) the local expression of the RAS in bone significantly regulates bone remodeling and metabolism. AngII was also reported to accelerate osteoclastic functions by activating RANKL (Shimizu et al., 2008). Thus, strategies that counterbalance AngII in bones might be a valuable therapeutic approach to prevent bone loss, particularly in hypertensive patients.

The therapeutic value of the well-known antihypertensive agents ARBs has been extended to bone health. ARBs have been reported to enhance bone health and decrease the risk of fractures in the clinic (Solomon et al., 2011) and in experimental models (Donmez et al., 2012; Kang et al., 2013; Ma et al., 2010; Rajkumar et al., 2013; Shimizu et al., 2008). Notably, ARBs increase Ang1-7 expression systemically and locally, and the majority of the effects of ARBs are reminiscent of Ang1-7 functions (Shi et al., 2010). Ang1-7 was shown to directly interact with AT_1 receptor in one study, leading to functional down-regulation (Shi et al., 2010). Moreover, A779 (a specific Mas receptor blocker) inhibits the antihypertensive effect of ARBs (Iusuf et al., 2008). Thus, the present study explores the potential role of the ACE-2/Ang1-7/Mas cascade in mediating the osteo-preservative effects on an OVX rat model following AT_1 receptor blockade using the ARB drug losartan.

2. Materials and methods

2.1. Experimental animals and ethical approval

Forty-eight adult female Wistar albino rats (three months old) were supplied by the Experimental Animal care Center, College of Pharmacy, King Saud University (KSU). They were housed in a room with controlled humidity (50–55%), temperature (22 ± 1 °C) and light (12/12 dark and light h). Animals had a free access to Purina rat chow (Grain Silos & Flour Mills Organization, Riyadh, Saudi Arabia) and water ad libitum. The experimental protocol of this study was consistent with the National Institute of Health Guidelines (NIH Publications No. 80-23; 1996). In addition, ethical approval was provided for the present study by the Research Ethical Committee, College of Pharmacy, KSU, as well as the Ethical Committee, Faculty of Pharmacy, Cairo University, Cairo, Egypt (ethical approval No. PT-208).

2.2. Study design

After a 2 week habituation period, all animals were separated into the following six groups (n=8): Group 1: Sham, Group 2: Sham+Los, Group 3: Sham+Los+A-779, Group 4: OVX, Group 5: OVX+Los and Group 6: OVX+Los+A-779. Osteoporosis was induced by a bilateral OVX operation in all OVX groups. Animals were maintained under general anesthesia using single IP injection of a ketamine and xylazine mixture (80 and 5 mg/kg; respectively). The bilateral longitudinal incisions were generated in the dorsolateral body wall at the region located below the rib cage. The ovaries were then exteriorized, ligated and excised. Sham groups received the incisions without the ligation and excision steps. A topical fusidic acid cream was applied twice per week for 4 weeks to prevent the risk of postoperative infection. Animals in the Sham+Los+A-779 and OVX+Los+A-779 groups received subcutaneously implanted osmotic pumps (model 2006, Alzet, Durect Corporation, Minneapolis, USA) containing the specific mass receptor antagonist (D-Ala^7)-Angiotensin I/II (1-7) trifluoroacetate salt (A-779, Bachem AG, Bubendorf, Switzerland), which was infused at a rate of

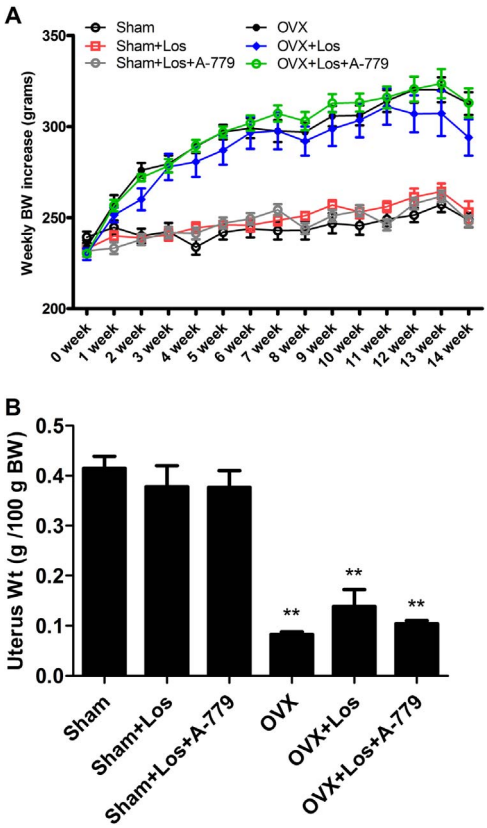

Fig. 1. Effects of the losartan treatment alone or in combination with the Mas receptor blocker A-779 on (A) the weekly body weight (BW) increase and (B) final uterus weights in OVX Wistar albino rats. The mean weights of all OVX groups were compared to their respective Sham groups weekly. The mean uterus dry weights per 100 g of final BW of all OVX groups were compared to their respective Sham groups. Results are presented as the mean ± S.E.M. of each group (n=8/group). Data were statistically evaluated using one-way ANOVA followed by the Student-Newman-Keuls multiple comparisons post hoc test. Differences were considered statistically significant when $^{*}P < 0.05$ and $^{**}P < 0.01$ compared to the Sham group and $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ compared to the OVX group.

400 ng/kg/min for 6 consecutive weeks. Animals in the Sham+Los, Sham+Los+A-779, OVX+Los and OVX+Los+A-779 groups were administered losartan potassium salt (Cat # L470500, Toronto Research Chemicals, Inc., Ontario, Canada) by gastric gavage at a dose of 10 mg/kg/d. The Sham and OVX animals received infusions and oral treatments that lasted for 6 consecutive weeks beginning 8 weeks after the sham and OVX operations. Doses of losartan were selected based on previous studies (Bayar et al., 2015) and dissolved in distilled water. The overall health and body weights of all experimental groups were carefully observed during the study, and the dose of losartan was adjusted accordingly each week. At the end of experiment, all groups were fasted in metabolic cages for 16 h to collect urine samples that were then frozen at −70 °C. Blood samples were acquired under ketamine and xylazine anesthesia by cardiac puncture and then sera were obtained by centrifuging the samples at 1800 g. The femoral bone samples were then removed, cleaned of soft tissues and weighed. The mean femoral bone weights were expressed in grams. Uterine tissues also were removed, cleaned of fat and the wet weights were determined and expressed as g/100 g body weight.

2.3. Determination of bone turnover biomarkers

The serum concentrations of bone-specific alkaline phosphatase (BALP), osteocalcin (OC), tartrate-resistant acid phosphatase 5b (TRACP-5b) and cross-linked C-telopeptides of type I collagen (CTX) and the urinary levels of deoxypyridinoline (DPD) were measured with a rat sandwich ELISA kit (Biotang Inc., Waltham, Massachusetts,