Biomedicine & Pharmacotherapy 92 (2017) 58–68

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Original article

ACE-2/Ang1-7/Mas cascade mediates ACE inhibitor, captopril, protective effects in estrogen-deficient osteoporotic rats

Hatem M. Abuohashish[a,b,*], Mohammed M. Ahmed[a], Dina Sabry[c], Mahmoud M. Khattab[d], Salim S. Al-Rejaie[a]

[a] Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia
[b] Department of Biomedical Dental Sciences, College of Dentistry, University of Dammam, Dammam, Saudi Arabia
[c] Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Cairo University, Cairo, Egypt
[d] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Cairo University, Cairo, Egypt

ARTICLE INFO

Article history:
Received 14 March 2017
Received in revised form 25 April 2017
Accepted 12 May 2017

Keywords:
Captopril
Ovariectomized
Ang1-7
Mas receptor
Micro-CT
Minerals
Bone metabolism
Osteoclastogenesis

ABSTRACT

The local role of the renin angiotensin system (RAS) was documented recently beside its conventional systemic functions. Studies showed that the effector angiotensin II (AngII) alters bone health, while inhibition of the angiotensin converting enzyme (ACE-1) preserved these effects. The newly identified Ang1-7 exerts numerous beneficial effects opposing the AngII. Thus, the current study examines the role of Ang1-7 in mediating the osteo-preservative effects of ACEI (captopril) through the G-protein coupled Mas receptor using an ovariectomized (OVX) rat model of osteoporosis. 8 weeks after the surgical procedures, captopril was administered orally (40 mg kg^{-1} d^{-1}), while the specific Mas receptor blocker (A-779) was delivered at infusion rate of 400 ng kg^{-1} min^{-1} for 6 weeks. Bone metabolic markers were measured in serum and urine. Minerals concentrations were quantified in serum, urine and femoral bones by inductive coupled plasma mass spectroscopy (ICP-MS). Trabecular and cortical morphometry was analyzed in the right distal femurs using micro-CT. Finally, the expressions of RAS peptides, enzymes and receptors along with the receptor activator of NF-κB ligand (RANKL) and osteoprotegerin (OPG) were determined femurs heads. OVX animals markedly showed altered bone metabolism and mineralization along with disturbed bone micro-structure. Captopril significantly restored the metabolic bone biomarkers and corrected Ca^{2+} and P values in urine and bones of estrogen deficient rats. Moreover, the trabecular and cortical morphometric features were repaired by captopril in OVX groups. Captopril also improved the expressions of ACE-2, Ang1-7, Mas and OPG, while abolished OVX-induced up-regulation of ACE-1, AngII, Ang type 1 receptor (AT1R) and RANKL. Inhibition of Ang1-7 cascade by A-779 significantly eradicated captopril protective effects on bone metabolism, mineralization and micro-structure. A-779 also restored OVX effects on RANKL expression and ACE-1/AngII/AT1R cascade and down-regulated OPG expression and ACE-2/Ang1-7/Mas pathway. In line with the clinical observations of the bone-preservative properties following ACE-1 inhibition, local activation of ACE-2/Ang1-7/Mas signaling and suppressed osteoclastogenesis seem responsible for the osteo-preservative effect of captopril, which could offers a potential therapeutic value in treatment of disabling bone and skeletal muscular diseases.

1. Introduction

The well-known endocrinal renin angiotensin system (RAS) has different local and systemic physiological and pathological effects. RAS mainly controls blood pressure via regulating body fluids and electrolytes balance. In the liver, angiotensinogen (AGT) is secreted and then cleaved to angiotensin I (AngI) by the action of kidney-derived renin. AngI is, henceforth, transformed to the effector classical RAS peptide AngII by the effect of angiotensin-converting enzyme (ACE-1). AngII plays a vital role in various biological actions via binding to its specific membrane receptors including angiotensin type 1 and type 2 receptors (AT1R and AT2R; respectively) [1]. The systematic RAS gained a vital therapeutic

* Corresponding author at: Dept. Biomedical Dental Sciences, College of Dentistry University of Dammam, P.O. Box 1982, Dammam 31441, Saudi Arabia.
E-mail addresses: hatem.abuohashish@yahoo.com, habuohashish@uod.edu.sa (H.M. Abuohashish).

http://dx.doi.org/10.1016/j.biopha.2017.05.062
0753-3322/

value as a target of antihypertensive medications including ACE-1 inhibitors (ACEI) and angiotensin receptor blockers (ARB) [2]. In recent decades, another effective component for RAS was suggested after the introduction of the new ACE-1 homolog, namely ACE-2 [3]. ACE-2 favors cleaving AngII to a heptapeptide. ACE-2 can also convert AngI to Ang1–9, which is then cleaved by either neutral endopeptidase (NEP) or ACE to yield Ang1-7. AngI can be also directly cleaved to Ang1-7 by the action of NEP [4]. Ang1-7 exerts its effects via binding to an orphan G-protein coupled receptor called Mas, which was identified as a functional receptor for Ang1-7 in 1986 by Young et al. [5] who described the oncogene receptor and cloned its cDNAs. Studies using the selective Ang1-7 antagonist A-779 provided a clear evidences that Mas receptor is an Ang1-7 receptor distinct from the classical AngII [6,7]. The ACE-2/Ang1-7/Mas pathway often opposes the vasoconstriction, inflammatory and proliferative functions of ACE-1/AngII/AT1R cascade and hence it is considered the beneficial RAS cascade especially in the cardiovascular system.

Numerous investigations reported the local expressions of RAS components such as renin, ACE-1 and AngII receptors in the skeletal system, where they play a vital role in local bone remodeling [1,8,9]. The expression of AngII receptors was primary identified in osteoblasts produced from newborn mouse calvaria in an *in vitro* study [1]. Animal studies also revealed that the local bone expression of RAS could be involved in the skeletal deteriorations associated with age [10], obstructive nephropathy [11] and type 1 diabetes [12]. In addition, RAS was reported to participate in the healing process in fractured femur mouse model [13], and the development of osteonecrosis by steroids in rabbits [9] along with post-menopausal and glucocorticoids-linked osteoporosis in OVX animals [14–16]. In addition, production of AngII was reported in osteoblasts or osteoclasts by the action ACE-1 on AngI [17]. AngII also inhibited osteoblasts differentiation in an *in vitro* study [18]. Findings of Hiruma et al. study revealed that bone remodeling and metabolism may be regulated by the local RAS [19]. AngII was found to provoke osteoporosis and the osteoclastic activity through the receptor activator of nuclear factor kappa-B ligand (RANKL) [14]. Of note, Ang1-7 was found in Krishnan et al. study [20] to attenuate the osteoclastogenesis process of tibias derived bone marrow cells in mice, while Mas receptor expression was reported in bone marrow-derived cells [21].

Notably, clinical studies showed that patients who are on ACEI therapy may have greater bone mineral density (BMD) and reduced risk of fractures [22–27]. In one clinical study, post-menopausal women with hypertension who were on fosinopril (an ACEI) therapy did not face the physiological bone mass loss, which was present in post-menopausal women without ACEI therapy [25]. In addition, a large case–control analysis, which took place in the UK, advocated a possibly lowered risk of fractures with long-term ACEI treatment [22]. Animal studies also showed the potential importance of ACE-1 in bone health and metabolism. The ACEI enalapril was not able to positively influence bone functions in OVX mice [28] or spontaneously hypertensive rats [29]. Nevertheless, enalapril improved bone health of Tsukuba hypertensive mouse [1]. Similarly, OVX rats showed markedly enhanced trabecular structure and strength with the treatment of ACEI captopril [15,30].

It is well-documented that Ang1-7 might mediate most of ACEI therapeutic effects and the level of Ang1-7 is usually increased following ACEIs treatment [31,32]. Taken together, the present study aimed to document the contribution of ACE-2/Ang1-7/Mas cascade in the osteo-preservative properties reported clinically and experimentally following ACE-1 inhibition by captopril. Using an OVX rat model, captopril markedly protected bone metabolism, mineralization and microstructure via local stimulation of Ang1-7/Mas signaling and restraint of osteoclastogenesis process.

2. Material and methods

2.1. Experimental animals and ethical approval

In the existing study, adult female Wistar rats with similar ages and weighting approximately 220–250 g were supplied from Experimental Animal Care Center at college of Pharmacy, King Saud University (KSU). Animals were subject to standard and controlled experimental conditions. The experimental protocol of this study was in agreement with the National Institute of Health Guidelines (NIH Publications No. 80-23; 1996). In addition, an 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. Induction of osteoporosis

The OVX-rat model was employed in the current study to induce post-menopausal osteoporosis. In brief, animals were generally anesthetized using IP mixture of ketamine (80 mg kg^{-1}) and xylazine (5 mg kg^{-1}). Then, two longitudinal incisions were made on the dorsolateral body wall inferior to the rib cage. The right and left ovaries were exposed, ligated and excised. Animals in sham groups had similar procedure without ligation and excision of the ovaries. Topical application of fusidic acid cream was provided twice weekly for 4 weeks to prevent postoperative infection.

2.3. Study design and animals grouping

Animals were accumulated for 8 weeks after the OVX or sham procedure. Then, animals were grouped into six groups (n = 10) as follow: Group 1: Sham, Group 2: Sham + Cap, Group 3: Sham + Cap + A-779, Group 4: OVX, Group 5: OVX + Cap and Group 6: OVX + Cap + A-779. At the same time, Sham + Cap + A-779 and OVX + Cap + A-779 groups had a subcutaneous implantation of osmotic pumps (model 2006, Alzet, Durect Corporation, Minneapolis, USA) supplying the specific mass receptor antagonist (D-Ala^7)-Angiotensin I/II (1–7) trifluoroacetate salt (A-779, Bachem AG, Hauptstrasse, Bubendorf, Switzerland) at infusion rate 400 ng/kg/min for 6 weeks [33,34]. Meanwhile, Sham + Cap, Sham + Cap + A-779, OVX + Cap and OVX + Cap + A-779 groups started the oral captopril treatment (Cat # C175750, Toronto Research Chemicals, Inc., Ontario, Canada). Captopril was dissolved in distilled water and administered with gastric gavage in 40 mg kg^{-1} d^{-1} dose for 6 weeks [35]. Animals general health and body weights were monitored during the treatment periods and the dose of captopril were weekly adjusted. All groups were provided controlled experimental conditions (temperature 22 ± 1 °C, 12/12 dark and light cycles and humidity 50–55%) as well as a free access to purina rat chow and water *ad libitum*. At the last day of the sixth week, all groups were located in metabolic cages fastened for 16hr and urine samples were obtained and kept at −70 °C. Blood was collected by cardiac puncture under ketamine and xylazine anesthesia and serum samples were provided at 4000 RPM. Next, the femoral bones samples were removed and cleaned from soft tissues and weighted, then, expressed as grams. The left femoral bone samples were preserved at −70 °C until analyzed, while the right femoral bones were kept in 10% formalin for mico-CT and minerals analysis. Moreover, uterine tissues were excised and cleaned from fats and