European Journal of Pharmacology 809 (2017) 231–241

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Cardiovascular pharmacology

Oleanolic acid modulates the renin-angiotensin system and cardiac natriuretic hormone concomitantly with volume and pressure balance in rats

You Mee Ahn, Yoon Hee Choi, Jung Joo Yoon, Yun Jung Lee, Kyung Woo Cho, Dae Gill Kang*, Ho Sub Lee*

Department of Herbal Resources, Hanbang Body Fluid Research Center, Professional Graduate School of Oriental Medicine, Wonkwang University, Republic of Korea

ARTICLE INFO

Chemical compound studied in this article:
Oleanolic acid (PubChem CID: 10494)

Keywords:
Atrial natriuretic peptide
Cardiovascular
Hypertension
Renin-angiotensin system
Triterpenoid

ABSTRACT

Oleanolic acid is known to possess beneficial effects on the regulation of the cardiovascular homeostasis. However, the exact nature of the role of oleanolic acid on the regulation of body fluid balance and blood pressure homeostasis and its mechanisms involved are not well defined. Experiments were performed to identify the effects of oleanolic acid on the renin-angiotensin system and cardiac natriuretic hormone (ANP) system, and also renal function and blood pressure in normotensive and renovascular hypertensive rats. The change in the plasma levels of hormones and the expressions of renin, angiotensin II receptors, ANP, natriuretic peptide receptor-C, M_2 muscarinic receptor and GIRK4 were determined in the kidney, heart and aorta. Oleanolic acid was administered orally for 1 or 3 weeks. Here, we found that oleanolic acid suppressed plasma levels of renin activity and aldosterone and intrarenal levels of renin and angiotensin II type 1 receptor expression and increased angiotensin II type 2 receptor in normotensive and hypertensive rats. Also, oleanolic acid increased plasma levels of ANP. Further, oleanolic acid suppressed angiotensin II type 1 receptor and natriuretic peptide receptor-C expression and increased angiotensin II type 2 receptor and ANP expression in the heart and aorta. Along with these changes, oleanolic acid accentuated urinary volume, electrolyte excretion and glomerular filtration rate in normotensive rats and suppressed arterial blood pressure in hypertensive rats. These findings suggest that beneficial effects of oleanolic acid on the cardiorenal system are closely associated with its roles on the renin-angiotensin system and cardiac natriuretic hormone system.

1. Introduction

Oleanolic acid (OA) is a biologically active triterpenoid found in edible, medicinal, and other plants, and elicits anti-inflammatory, antioxidative and antiproliferative effects (Liu, 1995; Somova et al., 2003). Also, OA is one of bioactive ingredients contained in olive oil, which is consumed in Mediterranean diet (Romero et al., 2010). Although the mechanisms are not yet clearly defined, OA is known to protect the heart and kidney from inflammatory and oxidative stress. OA has been known to possess antihypertensive (Bachhav et al., 2011; Mapanga et al., 2009; Somova et al., 2003) and cardiorenal protective properties (Mapanga et al., 2009; Patil et al., 2010; Senthil et al., 2007), in which the mechanisms involved are suggested to be related to its antioxidant property (Bachhav et al., 2011; Senthil et al., 2007; Somova et al., 2003). Therefore, the role of OA on the regulation of body fluid balance and blood pressure homeostasis and its mechanisms involved are to be clarified.

It was previously reported that OA increased glomerular filtration rate (GFR) and urinary excretion of Na^+ (Mapanga et al., 2009). Furthermore, it was recently shown that OA increased plasma levels of atrial natriuretic peptide (ANP) via accentuation of cardiac synthesis and secretion (Kim et al., 2013a). These reports suggest that OA affects the regulation of body fluid balance and blood pressure homeostasis. The natriuretic peptide system (NPS), consists of natriuretic peptides and its selective receptors, is closely involved in the regulation of body fluid balance and blood pressure homeostasis directly and indirectly via inhibition of the renin-angiotensin system (RAS). ANP increases urinary excretion of salt and water through an increase in GFR and blockade of tubular reabsorption of Na^+ (McGrath et al., 2005). The NPS is an endogenous negative regulator for the RAS. ANP inhibits renin release from the kidney and aldosterone synthesis and release from the adrenal gland (McGrath et al., 2005; Potter et al., 2006),

* Corresponding authors.
E-mail addresses: dgkang@wku.ac.kr (D.G. Kang), host@wku.ac.kr (H.S. Lee).

http://dx.doi.org/10.1016/j.ejphar.2017.05.030
Received 22 April 2017; Received in revised form 12 May 2017; Accepted 12 May 2017
Available online 14 May 2017
0014-2999/

angiotensin (Ang) II generation (Kawaguchi et al., 1990), and Ang II type 1 (AT_1) receptor activation by Ang II (Li et al., 2002). The roles of AT_1 receptor located on the renal tubules and glomerular arterioles are closely involved in the regulation of the salt reabsorption and glomerular filtration rate (Kobori et al., 2007; Navar et al., 1996; Navar, 2014; Stockand and Sansom, 1998).

In the present study, we hypothesized that OA modulates body fluid and salt balance and blood pressure homeostasis through suppression of the RAS and accentuation of cardiac natriuretic hormone. The purpose of the present study was to identify the effects of OA on the systemic and renal RAS and cardiac NPS along with the renal function and arterial blood pressure in sham-operated normotensive and Goldblatt (two-kidney, one-clip, 2K1C) hypertensive rats. Because ANP is a negative regulator for the RAS and OA increased the levels of ANP in the circulation (Kim et al., 2013a), experiments were performed in the renin-dependent model of hypertension.

2. Materials and methods

2.1. Animals

All animal procedures were approved by the Institutional Animal Care and Utilization Committee for Medical Science of Wonkwang University in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. Age-matched male Sprague-Dawley rats [140–150 (5 weeks old) or 270–300g (8 weeks old), Samtako Inc, Osan, Korea] were maintained in an air-conditioned and 12 h light-dark cycle animal care room. They were given free access to water and food (Cargillagripurina, Kunsan, Korea). For the groups devoted to the water and salt balance, rats were housed individually in metabolic cages (Tecniplast, Buguggiate, Italy). At the end of the experiments, all rats were killed with guillotine and trunk blood was collected in prechilled ethylenediaminetetraacetic acid (EDTA)-coated or heparinized tubes. The blood was centrifuged at 990×*g* for 15 min at 4 °C, and plasma samples were separated, and kept at −70 °C until used. Tissue samples were snap frozen in liquid nitrogen and stored at −70 °C until used.

2.2. Experimental protocols

Experiments were performed in two separate series of experiments: individually housed normotensive and group housed two-kidney, one-clip (2K1C, Goldblatt) renal hypertensive rats. To avoid possible isolation stress (Gomez-Galan et al., 2016; Liu et al., 2013), except normotensive rats which necessarily need individualized housing in metabolic cages, Sham and 2K1C Goldblatt hypertensive rats were group housed. In one series of experiments, OA was tested to identify the effects on the systemic and intrarenal RAS and renal function. Three groups of male Sprague-Dawley rats were housed individually in metabolic cages and acclimatized to the environment for three days prior to the study. Three consecutive control days were followed by 7 days of OA (20 mg/kg/day, n=8), OA (30 mg/kg/day, n=8), and vehicle (n=8) administration. OA was given orally by oral gavage once a day for 7 days at 6 PM in a volume of 1 ml of 5% dimethyl sulfoxide (DMSO). Urine samples for day- and night-time period were collected separately (night-time period, 6 PM through 9 AM next day; day-time period, 9 AM through 5 PM) for the measurement of the levels of urine volume, osmolality, Na^+, K^+, Cl^-, and creatinine. In the other series of experiments, the effects of OA were tested in 2K1C hypertensive rats. Rats were group housed in regular rat cages (4 rats in a cage). Experiments were performed to identify the effects of OA on the RAS and plasma levels of ANP, and blood pressure in 4 separate groups: sham-operated (Sham, vehicle, n=8), OA in Sham (OA, 30 mg/kg/day, n=8), 2K1C (vehicle, n=7), and OA in 2K1C (OA, 30 mg/kg/day, n=8). OA or vehicle was administered for 3 weeks after 4 weeks of surgery. In another two sets of 4 separate groups as above with the same experimental protocol, Sham (vehicle, n=4), OA in Sham (OA, 30 mg/kg/day, n=4), 2K1C (vehicle, n=4), and OA in 2K1C (OA, 30 mg/kg/day, n=4), were used to identify the effects of OA on the protein expression of the components of the RAS and NPS in the kidney, heart and vessel. The doses of OA used in the present study were in the range of previous reports (Dubey et al., 2013; Kim et al., 2013a).

2.3. Preparation of 2K1C renal hypertensive rats

Two-kidney, one-clip (2K1C) hypertensive rat model was produced by a method previously reported (Kang et al., 2002) with slight modifications. Briefly, rats were anesthetized with Ketamine (25 mg/kg, intramuscularly) and Rompun (5 mg/kg, intramuscularly). For the 2K1C rats, a silver clip (0.2 mm slit) was applied on the left renal artery. For the sham-operated (Sham) rats, all procedures were the same as the 2K1C rats except silver clip application. Systolic blood pressure was measured weekly by tail-cuff method (MK2000, Muromachi Kikai, Tokyo, Japan).

2.4. Chemical assays

Urine samples were centrifuged at 2000×*g* for 15 min (4 °C) and the supernatants were kept in a refrigerator until used. All chemical assays were completed within 12 h. The concentration of ions was measured using Electrolyte Analyzer (NOVA Biochemical, Waltham, MA, USA), osmolality using Advanced Osmometer (Advanced Instruments Inc., Norwood, MA, USA). Na^+ balance was calculated as follow:

Na^+ balance=Na^+ intake−urinary excretion of Na^+(UNaV). Here, Na^+ intake=food consumed (g/23 h)×Na^+ content in food (μEq/g), and UNaV (μEq/23 h)=urine volume (ml/15 h)×Na^+ concentration in night-time urine (μEq/ml)+urine volume (ml/8 h)×Na^+ concentration in day-time urine (μEq/ml). Creatinine concentration of plasma and urine was measured by colorimetric method (Jaffe reaction) using spectrophotometer (Miloton Roy, Rochester, NY, USA).

2.5. Radioimmunoassay of renin activity, aldosterone and ANP

Plasma renin activity (PRA) and renal renin content were measured by the method previously reported (Ahn et al., 2012; Kang et al., 2002). Results were expressed as nanograms of Ang I generated per milliliter of plasma per hour (ng Ang I/ml/h). Renal renin content was measured by measuring Ang I generated with enough renin substrate and small amount of whole kidney extract. Results were expressed as nanograms of Ang I generated per milligram of wet kidney weight per hour (ng Ang I/mg/h). Plasma levels of aldosterone (Aldo) were measured using Aldo assay kit (ALDOCTK-2, DiaSorin Inc, Stillwater, MN, USA), respectively. Plasma levels of ANP were measured by the method previously reported (Kim et al., 2013a).

2.6. Protein preparation and Western blot analysis

The cortex of the kidney, cardiac tissues and thoracic aorta was homogenized with a lysis buffer {50 mM Tris-HCl (pH 7.5), Triton X-100 1%, 150 mM sodium chloride, SDS 1%, sodium deoxycholate 0.5%, 2 mM EDTA}. The homogenate was centrifuged at 600×*g* for 10 min at 4 °C, and then the supernatant was centrifuged at 1000×*g* for 5 min at 4 °C. The proteins (40 μg/lane) were separated on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a nitrocellulose membrane (Amersham™ Protran™, Buckinghamshire, UK). The membrane was washed with TBS-T {10 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% Tween-20} and blocked with 5% BSA for 1 h and incubated with the appropriate primary antibody to AT_1 receptor, AT_2 receptor, natriuretic peptide receptor (NPR)-C and β-actin (Santa Cruz Biotechnology, Santa Cruz,