Pharmacological Reports 68 (2016) 692–702

Contents lists available at ScienceDirect

Pharmacological Reports

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

Original research article

Peroxisome proliferator-activated receptor-α stimulation by clofibrate favors an antioxidant and vasodilator environment in a stressed left ventricle

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ARTICLE INFO

Article history:
Received 27 August 2015
Received in revised form 3 March 2016
Accepted 4 March 2016
Available online 21 March 2016

Keywords:
Target organ damage
PPAR-α
Stressed left ventricle

ABSTRACT

Background: Arterial high blood pressure is a risk factor for target organ damage; the most susceptible organs are the arteries, brain, kidneys, and heart. The damage mechanisms include oxidative stress and renin-angiotensin system (RAS) overactivity. Therefore, our aim was to study whether clofibrate-induced peroxisome proliferator-activated receptor-alpha (PPAR-α) stimulation is able to prevent alterations in cardiac functioning derived from RAS overstimulation in the left ventricle of rats with hypertension secondary to aortic coarctation and to improve antioxidant defenses.
Methods: Male Wistar rats were assigned to Control (Sham)- or aortic coarctation-surgery and further divided to receive (1 or 21 days) vehicle, clofibrate (100 mg/kg), captopril (20 mg/kg), or clofibrate + captopril. The left ventricle was obtained to measure: angiotensin II and -(1–7), AT_1 and AT_2 receptors, angiotensin converting enzyme (ACE)-1 and -2, and MAS receptor; the activity and expression of superoxide dismutase, catalase, endothelial nitric oxide synthase, the production of reactive oxygen species (ROS) and peroxidated lipids; as well as *ex vivo* cardiac functioning.
Results: Clofibrate decreased angiotensin II, AT_1 receptor and ACE expression, and raised angiotensin-(1–7), AT_2 receptor, ACE-2 expression, superoxide dismutase and endothelial nitric oxide synthase participation. These effects promoted lower coronary vascular resistance and improved mechanical work compared to aortic coarctated vehicle-treated rats.
Conclusions: Clofibrate-induced PPAR-α stimulation changes the angiotensin II receptor profile, favors the ACE2/angiotensin-(1–7)/AT_2 receptor axis decreasing the vasoconstrictor environment, activates the antioxidant defense, and facilitates endothelial nitric oxide synthase activity favoring vasodilation. This may represent a protection for the stressed heart.

Introduction

Arterial hypertension has been identified as a major cause for developing left ventricular hypertrophy, coronary heart disease, myocardial infarction, and heart failure [1]. The array of factors involved in the hypertension-induced pathogenesis of cardiac damage includes overproduction of reactive oxygen species (ROS) [2]; which interact with proteins, lipids, and DNA, preventing them from carrying out their function and canceling the actions of nitric oxide (NO) [3–5]. ROS also causes overstimulation of vasoconstrictor pathways such as the adrenergic [6] and the renin-angiotensin system (RAS) [7], leading to arterial stiffness, endothelial dysfunction, loss of contractile function, and ventricular remodelation [8].

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http://dx.doi.org/10.1016/j.pharep.2016.03.002
1734-1140/

The RAS is a highly complex pathway able to exert cardiovascular effects. Angiotensin II (AngII), the main effector molecule, promotes its effects by interaction with at least two kinds of receptors: AT_1 and AT_2. Angiotensin AT_1 receptor has been related to vasoconstriction, hypertrophy, promotion of ROS formation, and cell proliferation. On the other hand, the AT_2 receptor is known to promote vasodilation, decline in cell proliferation, anti-inflammation, anti-fibrosis, anti-apoptosis, and neuroregeneration [9]. The pathway includes several enzymes and metabolites causing the detrimental or protective actions exerted by RAS components. Likewise, the relevance of the angiotensin-(1–7) [Ang-(1–7)] and the MAS receptor have increased due to their ability to counteract AT_1 receptor-mediated AngII actions.

Peroxisome proliferator-activated receptors (PPAR) are nuclear receptors that function as transcription factors. Three isoforms have been described: α, β/δ, and γ [10,11]. Activation of PPAR can be achieved by the agonist-receptor interaction. In the case of PPAR-α, a family of pharmacologically related compounds named fibrates (fenofibrate, bezafibrate, clofibrate, *etc.*) may activate their signaling pathway [12]. A highly relevant pathway affected by PPAR-α stimulation is RAS. Banks and Oyekan reported that their activation counters AT_1-mediated pressor and vasoconstrictor effects, modifying in an opposite manner AT_1- and AT_2-receptor expression in rat renal cortex [5]. Also, it was reported that PPAR-α stimulation increased antioxidant defenses, decreased ROS in kidneys from aortic coarctation (AoCo)-induced hypertensive rats and changed RAS participation [13].

Therefore, our aim was to study whether clofibrate-induced PPAR-α stimulation is able to lower AoCo-induced RAS overstimulation in the left ventricle (LV) and to improve antioxidant defenses in rats with AoCo-associated hypertension, helping to prevent alterations in cardiac functioning.

Material and methods

Materials

All reagents were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). They were of the best quality available.

Animals

All animal procedures were conducted in accordance with our Federal Regulations for Animal Experimentation and Care (Ministry of Agriculture, SAGARPA, NOM-062-ZOO-1999, Mexico) and were approved by the Institutional Animal Care and Use Committee. In order to fulfill our objective, we carried out 2 protocols.

Protocol 1. This experimental part was designed to test if clofibrate administration lowers the vasocontractil/pro-hypertensive/pro-oxidant branch of RAS in aortic coarctation (AoCo)-induced hypertensive rats. As a positive control for ACE inhibition we used captopril (20 mg/kg/d). Male Wistar rats (250–300 g, $n = 12–20$ per group) were randomly assigned to one of the following groups: (1) sham-operated clofibrate's vehicle-treated [canola oil, Vclof, 100 μL intraperitoneally (*ip*)], (2) sham-operated captopril's vehicle-treated (NaCl 0.9%, Vcap, 1 mL/100 g body weight, *gavage*), (3) sham-operated clofibrate (100 mg/kg/d in 100 μL *ip*)-treated (sham-clofibrate), (4) sham-operated captopril (20 mg/kg/d, in 1 mL/100 g body weight *gavage*)-treated (sham-captopril), (5) AoCo Vclof-treated (AoCo-Vclof, *ip*), (6) AoCo clofibrate (100 mg/kg/d, *ip*)-treated (AoCo-Clof), (7) AoCo captopril (20 mg/kg/d)-treated (AoCo-Cap, *gavage*), and (8) AoCo captopril (20 mg/kg/d, *gavage*) + clofibrate (100 mg/kg/d, *ip*)-treated (AoCo captopril + clofibrate). Protocol 2 was designed to establish the relationship between clofibrate and AoCo-induced oxidative stress. Therefore parameters were analyzed in Sham-Vclof, AoCo-Vclof, and AoCo-Clof. The dose of clofibrate and captopril were taken from previous studies [13,14] wherein clofibrate and captopril lowered blood pressure in AoCo and spontaneously hypertensive rats (SHRs), respectively. Treatments were applied once a day (9:00–10:00 AM), including the experimental day.

For both protocols, rats were further divided to be treated either 1 or 21 days. The animals were housed under standard conditions of temperature, humidity, and 12/12-h dark–light cycles. They had free access to water and standard rodent chow (Laboratory Rodent Diet 5001, PMI Nutrition International, LLC. Brentwood, MO, USA).

At the end of the pharmacological treatment (1 or 21 days), animals were anesthetized, instrumented for blood pressure measurement and blood sample collection from carotid artery, and euthanized to obtain heart. LV were rapidly separated, frozen at −80 °C and maintained until the protein determination was performed. Blood samples were centrifuged to 12,000 rpm RT for 10 min for plasma separation. Plasma samples were frozen and maintained at −80° C until the antioxidant capacity, tetrahydrobioterin (BH_4), dihydrobiopterin (BH_2), AngII, Ang-(1–7) and malondialdehyde (MDA) determinations were performed.

Aortic coarctation

Rats were anesthetized with isofluorane, underwent abdominal laparotomies to expose the abdominal aorta and were partially ligated with silk (3–0) at a point between the right and left renal arteries. The rats were sutured and allowed to recover. Sham surgery was performed following same procedure except the aorta ligation [15].

Blood pressure measurement

At the end of pharmacological treatment (1 or 21 days), animals were anesthetized (sodium pentobarbital, 50 mg/kg, *ip*) and systolic blood pressure (SBP) was measured in the carotid artery [16].

Electrophoretical determination

Plasmatic and LV AngII, Ang-(1–7), BH_2, BH_4, and bradykinin production was evaluated by capillary zone electrophoresis (CZE) [17–19]. Data are expressed as pmol of metabolite *per* mg of protein or *per* mL.

Detection of AT_1- and AT_2-receptor, MAS receptor, ACE, ACE2, SOD-1, and eNOS by Western blot

Protein expression was analyzed in the LV of rats from the different experimental groups. Primary antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and secondary horseradish peroxidase-labeled antibodies were from Bio-Rad. Protein was detected with the Immobilon Chemiluminescent System (Millipore, MA, USA) [19].

Measurement of ROS

Total ROS production was evaluated in homogenized LV from Sham-Vclof, AoCo-Vclof, or AoCo-Clof rats, using 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) (50 mM) as previously reported [13,20].

Quantification of MDA in blood samples

The plasmatic samples (50 μL) were deproteinized with cold methanol (1:1 v/v) and centrifuged at 16,000 × *g* for 15 min at 10 °C. The supernatants (80 μL) were filtered with a nitrocellulose