Environmental Toxicology and Pharmacology 52 (2017) 54–61

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

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

Research Paper

Combined oral contraceptive and nitric oxide synthesis inhibition synergistically causes cardiac hypertrophy and exacerbates insulin resistance in female rats

Lawrence A. Olatunji[a,*], Kehinde S. Olaniyi[a], Taofeek O. Usman[a,b], Bilikis A. Abolarinwa[a], Caleb J. Achile[a], In-kyeom Kim[c]

[a] Cardiovascular Research Laboratory, Department of Physiology, College of Health Sciences, University of Ilorin, Ilorin, Nigeria
[b] Cardiovascular Unit, Department of Physiology, College of Health Sciences, Osun State University, Osogbo, Nigeria
[c] Department of Pharmacology & Cardiovascular Research Institute, Kyungpook National University School of Medicine, Daegu 700-842, Republic of Korea

ARTICLE INFO

Keywords:
Cardiac hypertrophy
Inflammation
Nitric oxide
Oral contraceptive
Profibrotic biomarker

ABSTRACT

Combined oral contraceptive (COC) use or inhibition of nitric oxide (NO) synthesis has been shown to cause hypertension and insulin resistance. However, the concomitant effects of COC and NO deficiency on the heart and glucose regulation are not well known. We therefore hypothesized that COC treatment during NO deficiency would lead to the development of cardiac hypertrophy that is associated with aggravated glucose deregulation, pro-inflammatory and pro-fibrotic biomarkers. Eight-week-old female Wistar rats were randomly allotted into control, NO deficient (N^G-nitro-L-arginine methyl ester: L-NAME; 20.0 mg/kg *b.w.*), COC-treated (1.0 μg ethinylestradiol + 5.0 μg levonorgestrel, p.o) and L-NAME + COC-treated groups. The animals were treated daily for 6 weeks. Systolic blood pressure was estimated by tail-cuff plethysmography, insulin resistance (IR) and β-cell function were estimated by homeostatic model of assessment (HOMA-IR and HOMA-β). Pro-inflammatory (C-reactive protein; CRP and uric acid) and pro-fibrotic (plasminogen activator inhibitor-1; PAI-1) biomarkers were estimated in the plasma. Cardiac histological examination was also done. Results show that COC or L-NAME treatments led to increased blood pressure, HOMA-IR, impaired β-cell function, PAI-1, CRP and uric acid, without significant effect on cardiac mass. L-NAME + COC-treated group had significantly higher blood pressure, HOMA-IR, impaired β-cell function, PAI-1, CRP and cardiac mass than COC- or L-NAME-treated groups. Histological examination validated that COC use during NO deficiency causes cardiac hypertrophy. The present study demonstrates that COC treatment and NO deficiency synergistically causes cardiac hypertrophy that is associated with aggravated glucose deregulation, atherogenic dyslipidemia, pro-inflammatory and pro-fibrotic markers.

1. Introduction

In spite of increasing efforts to reduce cardiovascular disease (CVD) risk factors, cardiac and vascular diseases remain an enormous global burden, both in terms of health and costs (Heidenreich et al., 2011; Bhatnagar et al., 2015). The global rise in cardiovascular morbidity and mortality are attributable to increasing incidence of inflammatory CVD risk factors, perhaps due to poor early detection/prognosis (Canto et al., 2011). Cardiac hypertrophy (CH) is a compensatory asymptomatic independent CVD risk factor, which is strongly associated with hypertension, increased risk of heart failure, myocardial infarction and sudden cardiac death (Maulik and Kumar, 2012). The complex and dynamic pathophysiological mechanisms of cardiac hypertrophy has been the focus of intense scientific investigation, in an effort to design preventive and curative measures (Nishimura and Ommen, 2007). Elevated inflammatory CVD risk factors that are associated with metabolic disturbances (Tanno et al., 2012) have been identified as a key contributing factor in the development of cardiac hypertrophy (Maulik and Kumar, 2012; Kudo et al., 2009).

Insulin resistance (IR) has been shown to be a major metabolic disturbance among hypertension, dyslipidemia, type 2 diabetes, obesity, and attendant inflammatory CVD (Fonseca 2010). Also, the compensatory hyperinsulinemia is associated with inflammatory CVD and mortality independent of other risk factors such as obesity or diabetes (Abdul-Ghani et al., 2006). Considerable clinical evidence has suggested a role for IR in the pathogenesis of cardiac hypertrophy

* Corresponding author at: Department of Physiology, University of Ilorin, P.M.B. 1515 Ilorin, 240001, Nigeria.
E-mail address: tunjilaw@unilorin.edu.ng (L.A. Olatunji).

http://dx.doi.org/10.1016/j.etap.2017.03.012
Received 30 August 2016; Received in revised form 18 February 2017; Accepted 18 March 2017
Available online 20 March 2017
1382-6689/

(Rutter et al., 2003). However, cardiac hypertrophy has been associated with glucose deregulation in several epidemiological investigations (Devereux et al., 2000).

Nitric oxide (NO) is crucial in the regulation of blood pressure, body fluid, electrolytes, immune modulation, vascular reactivity, thrombotic and inflammatory responses (Leclercq et al., 2002). Many of NO-induced effects are considered to be cardio-protective (Umar and Laarse, 2009). Uncoupling of NO synthase (NOS), formation of reactive oxygen species (ROS) in combination with a low NO bioavailability predisposes an individual to cardiac injury (Umar and Laarse, 2009). Earlier studies have observed reduced NO bioavailability in CVD risk factors such as hypertension (Rajeshwari et al., 2014), cigarette smoking (Rocha and Libby, 2009), polycystic ovarian syndrome (PCOS) (Glintborg et al., 2009), obesity (Choudhury and Levi, 2011), metabolic disorders (Brevetti et al., 2003) among others. Also, long-term inhibition of NO synthesis in rats by N^G-nitro-L-arginine methyl ester (L-NAME) treatment has been reported to result in hypertension and increased plasma plasminogen activator inhibitor-1 (PAI-1), which is a pro-fibrotic marker (Katoh et al., 2000). Increased PAI-1 immunoreactivity has been detected in the endothelium and media of the aorta and coronary arteries of rats treated with L-NAME, which has been shown to cause vascular structural changes, which contributed to increasing CVD risk factors (Oestreicher et al., 2003).

Combined oral contraceptive (COC) containing ethinyl estradiol and levonorgestrel remains the most widely used method of contraception (Charlton et al., 2014), and also used for non-contraceptive reasons. COC has been shown to ameliorate ailments, including dysmenorrhea, fibroid-related symptoms, acne, and premenstrual dysphoric disorder (Maguire and Westhoff, 2011). COC use also drastically reduces maternal mortality (Ahmed et al., 2012) in several ways, including lowering the chance of pregnancy and its complications as well as reducing the risk of having an unsafe abortion. Currently, over 100 million women of fertile age use oral contraceptives worldwide (United Nations Department of Economic and Social Affairs, Population division (2010) 2011). However, the use of COC has been associated with increased cardiovascular events, such as arterial hypertension and coronary artery disease (Kang et al., 2001) especially among women who have concomitant risk factors such as smoking (Roy, 1999), aging (Lidegaard, 1999) and diabetes mellitus (Ahmed et al., 2005). Severe hypertension has been reported in 5% of COC users (Stachenfeld and Taylor, 2004), and previous studies have demonstrated pathophysiological changes in the heart of postmenopausal women (Harman et al., 2011). It has also been shown that depletion of ovarian hormones through ovariectomy causes hypertension and left ventricular fibrosis in rat model (Sun and Ren, 2012) and studies have revealed that COC users with concomitant risks such as PCOS are at increased risk of cardiac hypertrophy (Sassarini and Lumsden, 2015). However, the effect of COC containing ethinyl estradiol and levonorgestrel on the heart and glucose regulation during a condition with impaired NO synthesis is not well known. We therefore hypothesized that COC use during NO deficiency would lead to cardiac hypertrophy that is associated with aggravated glucose deregulation, pro-inflammatory and pro-fibrotic biomarkers.

2. Materials and methods

2.1. Animals and grouping

Eight-week-old female Wistar rats were obtained from the animal house of the College of Health Sciences, University of Ilorin (Ilorin, Nigeria). Rats were maintained under standard environmental conditions of temperature, relative humidity, and dark/light cycle. Rats had unrestricted access to standard rat diet and water. After acclimatization for two weeks, the animals were randomly allotted to: Group 1 (vehicle-treated; n = 8) which served as control, group 2 (L-NAME-treated; n = 8), group 3 (COC-treated; n = 8) and group 4 (L-NAME + COC-treated; n = 8). The investigation was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and was approved by the Institutional Review Board of University of Ilorin, and every effort was made to minimize both the number of animals used and their suffering.

2.2. Protocol

The vehicle and L-NAME-treated rats received distilled water as vehicle (p.o.) whereas COC-treated and COC+ L-NAME-treated rats received vehicle containing a combination of 1.0 μg of ethinyl estradiol and 5.0 μg of levonorgestrel (p.o.) daily (Bayer Schering pharmaceutical AG Germany). Vehicle and COC-treated rats had unlimited access to drinking water alone, whereas L-NAME- and L-NAME + COC-treated rats had unrestricted access to drinking water containing L-NAME (20.0 mg/kg *b.w;* Sigma Chemical, St Louis, MO, USA). The animals were treated daily for six weeks (Olatunji and Soladoye, 2008a). In addition to the treatments, body weight and food intake were monitored for the duration of the experiment. Systolic blood pressure was measured by the tail-cuff plethysmography using pressure detecting equipment (AD Instruments, Castle Hill, NSW, Australia).

2.3. Oral glucose tolerance test (OGTT), insulin resistance (IR) and pancreatic β-cell function

Glucose challenge test was performed 48 h before the end of the experiment. The rats had 12 h overnight fast. Glucose (2 g/kg *b.w.*) was given (p.o.). Blood sample was obtained from the tail before glucose load and then sequentially after 30, 60, 90 and 120 min. Blood glucose levels were determined with a glucometer (ONETOUCH®-LifeScans, Inc., Milpitas, CA, USA). Glucose tolerance was represented with glucose tolerance curve as previously described (Olatunji et al., 2012). The IR and pancreatic β-cell function was determined using the homeostasis model assessment methods: HOMA-IR = fasting glucose (mmol/l) * fasting insulin (μIU/l)/22.5 and HOMA-β cell function (%) = 20 * fasting insulin (μIU/l)/fasting glucose (mmol/l) – 3.5 respectively.

2.4. Sample preparation

At the end of treatment, the rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.). Blood was collected by cardiac puncture into heparinized bottle and was centrifuged at 3000 rpm for 5 min. Plasma was stored frozen until needed for biochemical assay. Heart was excised, blotted and weighed immediately. The heart was fixed in 4% formalin for histological examination.

2.5. Biochemical assays

Fasting insulin (Ray Biotechnology, Canada; kit number ELR-Insulin-1), PAI-1, C-reactive protein (CRP) and 17β-estradiol were determined using ELISA kits (Cloud Clone Corp. USA; kit numbers SEA532Ra, SEA821Ra and CEA461Ge respectively) respectively. Fasting plasma total cholesterol (TC), triglyceride (TG) and high-density lipoprotein cholesterol (HDL-C) were measured by standardized enzymatic colorimetric methods using reagents obtained from Randox Laboratory Ltd. (Co. Antrim, UK) while low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) were estimated. TC/HDL-C, TG/HDL-C and LDL-C/HDL-C ratios were estimated as atherogenic lipid indices.

2.6. Histological studies

For hematoxylin and eosin (H & E) stains, heart tissues were fixed in 4% formalin overnight, dehydrated, and embedded in paraffin. The paraffin-embedded samples were sectioned at 3-μm thickness. The