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Plasma 8-isoprostane levels are associated with endothelial dysfunction in resistant hypertension

Ana Paula Cabral de Faria, Vanessa Fontana, Rodrigo Modolo, Natália Ruggeri Barbaro, Andréa Rodrigues Sabbatini, Isabella Fagian Pansani, Silvia E. Ferreira-Melo, Heitor Moreno *

Laboratory of Cardiovascular Pharmacology, Faculty of Medical Sciences, University of Campinas, Campinas, SP, Brazil

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ABSTRACT

Background: Impaired endothelial function and arterial stiffness are associated with hypertension and are important risk factors for cardiovascular events. Reactive oxygen species reduce nitric oxide bioavailability and have a pivotal role in endothelial function. Resistant hypertension (RHTN) is characterized by blood pressure (BP) above goal (140/90 mmHg) in spite of the concurrent use of ≥3 antihypertensive drugs of different classes. This study evaluated the association between 8-isoprostane levels, an oxidative stress marker, endothelial function and arterial stiffness, in RHTN.
Methods: Ninety-four RHTN and 55 well-controlled hypertensive (HT) patients were included. Plasma 8-isoprostane levels were determined by ELISA. Also, flow-mediated dilation (FMD) and pulse wave velocity (PWV) were evaluated to determine endothelial function and arterial stiffness, respectively.
Results: Levels of 8-isoprostane were markedly higher in RHTN compared to HT patients (22.5 ± 11.2 vs. 17.3 ± 9.8 pg/ml, $p < 0.05$, respectively). A significant inverse correlation was observed between FMD and 8-isoprostane ($r = -0.35$, $p = 0.001$) in RHTN. Finally, multiple logistic regression revealed that 8-isoprostane was a significant predictor of endothelial dysfunction (FMD ≤ median) in RHTN group.
Conclusion: RHTN showed markedly higher oxidative stress measured by 8-isoprostane, compared to HT patients. Taken together, our findings suggest the involvement of oxidative stress in endothelial function in RHTN.

1. Introduction

Resistant hypertension (RHTN) is defined as patients whose blood pressure (BP) remains above goal (140/90 mmHg) in spite of the concurrent use of three or more antihypertensive drugs of different classes. Ideally, one of the agents should be a diuretic, and all agents should be prescribed at optimal doses. Also the definition includes patients whose BP is controlled using four or more antihypertensive medications [1]. The pathophysiology of RHTN is multifactorial and comprises hyperactivity of sympathetic nervous and renin–angiotensin–aldosterone (RAAS) systems [2,3], endothelial dysfunction [4] and arterial stiffness [5]. Therefore, endothelial function and arterial stiffness may have predictive value since they are markers of future cardiovascular events [6,7].

Endothelial dysfunction consists in impaired endothelium-dependent relaxation due to decreased vascular nitric oxide (NO) bioavailability. An imbalance between reactive oxidative species (ROS) production and antioxidant capacity characterizes oxidative stress status [8]. Increased BP may enhance vascular production of ROS [9], which in turn reduces NO bioavailability and impairs endothelial function [10]. Indeed, arterial stiffness may be directly or indirectly explained by cellular injury mediated by oxidative stress [11]. In this context, 8-isoprostane is a chemically stable lipid peroxidation product of arachidonic acid, and its quantification provides a reliable approach to the assessment of oxidative stress in vivo [12]. Moreover, isoprostanes have been revealed as potential biomarkers mediating cardiovascular diseases and indicating increased cardiovascular risk [13,14].

2. Methods

2.1. Study population

A cross-sectional study was performed in 94 patients diagnosed with RHTN and 55 well-controlled hypertensive (HT) patients from Outpatient Resistant Hypertension Clinic of the University of Campinas. Clinical follow-up was conducted for a 6-month period with ambulatory BP; screening for secondary causes of hypertension (pheochromocytoma, coarctation of the aorta, Conn's or Cushing's syndrome, renal artery stenosis) and pseudoresistant hypertension (white coat hypertension and adherence assessment).

Inclusion criteria comprised male or female >35 y diagnosed with true resistant hypertension according to American Heart Association

* Corresponding author at: Laboratory of Cardiovascular Pharmacology, FCM 10 Building, 1st Floor, University of Campinas, Campinas, SP 13083-970, Brazil. Tel.: +55 19 3521 9538.
E-mail address: hmoreno@uol.com.br (H. Moreno).

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Statement [1]. We excluded patients with symptomatic ischemic heart disease, liver disease, impaired renal function and history of stroke and peripheral vascular disease. The patients signed the written informed consent form before enrolling in the study. This study was approved by local Research Ethics Committee and performed in accordance with the Declaration of Helsinki.

2.2. Office BP measurements

Brachial systolic BP (SBP) and diastolic BP (DBP) were measured in the right arm using a validated digital sphygmomanometer (HEM-907XL, OMRON Healthcare Inc.) with the patient in seated position. Measurements were assessed twice from each patient by a trained health professional.

2.3. Ambulatory BP monitoring

The assessment of ambulatory blood pressure monitoring is essential for the accurate diagnosis of true resistant hypertension, in order to avoid misleading diagnosis of pseudoresistance hypertension [15]. According to the latest Guidelines of The European Society of Hypertension, the limit to determine altered 24-h-BP values is 130 × 80 mmHg [16]. ABPM (SBP and DBP) was evaluated using automatic oscillometric monitor (SpaceLabs). The patients were instructed to keep normal daily activities and to take notes of the 24-h-time period in a personal diary.

2.4. Laboratory measurements

Venous blood samples were collected in tubes containing heparin, with further centrifugation for determination of plasma 8-isoprostane by an enzyme immunoassay EIA kit (Cayman Chemical Company) and aldosterone levels were measured by radioimmunoassay-RIA (Immunotech S.A.S.), both according to the manufacturer's instructions. Intra- and inter-assay coefficients of variance of ELISA 8-isoprostane kits were below 8.4% and 10.8%, respectively.

2.5. Flow-mediated dilation assessment

Endothelial function was evaluated by flow-mediated dilation (FMD) method in accordance with the current guidelines [17,18]. We used a linear vascular transducer (7–12 MHz, Toshiba PowerVision 6000) synchronized with electrocardiogram signals. Subjects in a supine position in a quiet, air-conditioned room (22–24 °C) were submitted to a brachial artery occlusion for 5 min, using an aneroid sphygmomanometer after 8 h of fasting. Brachial artery diameter was recorded before and after cuff compression. All studies were initiated at 08:00 h, after overnight fasting). The change in the brachial artery diameter in dependent procedure was expressed as a percentage change relative to the vessel diameter immediately before cuff inflation. All FMD tests were preceded by endothelium-independent vasodilation test (glyceryl-trinitrate-mediated) to guarantee that the non-endothelial function was normal (vasodilation > 20%, data not shown). The vascular function examination was performed by only one experienced blinded examiner. The intraobserver CVs was 1.6%.

2.6. Pulse wave velocity assessment

Pulse wave velocity (PWV), a noninvasive and reproducible method to determine arterial stiffness, was measured by SphygmoCor system (Artcor). Pulse waves were obtained transcutaneously, using the right common carotid and femoral arteries with patients in supine position. PWV was calculated from the distance directly covered by the waves, between the femoral recording site and the supra-sternal notch minus the distance from the supra-sternal notch to the carotid recording site, and the transit time (distance in meters/Δtime in seconds) [19]. Three consecutive readings were assessed and the average of PWV values was used in the analyses.

Table 1
General characteristics of study groups.

	HT (n = 55)	RHTN (n = 94)
Age (y)	56 ± 13	58 ± 11
Gender (F/M)	29/26	64/30
BMI (kg/m^2)	30.9 ± 6.2	29.7 ± 5.3
Office SBP (mmHg)	131 ± 10	148 ± 20*
Office DBP (mmHg)	81 ± 9	87 ± 15*
Office PP (mmHg)	50 ± 7	61 ± 15*
ABPM SBP (mmHg)	116 ± 11	128 ± 17*
ABPM DBP mmHg)	69 ± 7	79 ± 13*
ABPM PP (mmHg)	47 ± 13	53 ± 10*
FMD (%)	–	6.9 ± 1.8
PWV (m/s)	–	10.1 ± 2.4
Glucose (mg/dl)	98 ± 19	104 ± 41
HbA1c (%)	6.0 ± 0.6	6.6 ± 2.3
Cholesterol (mg/dl)	195 ± 44	194 ± 38
HDL-c (mg/dl)	42 ± 11	48 ± 9*
LDL-c (mg/dl)	122 ± 38	117 ± 28
Triglycerides (mg/dl)	155 ± 55	146 ± 74
Creatinine (mg/dl)	0.97 ± 0.37	0.98 ± 0.38
Creatinine clearance (ml per min per 1.73 m^2)	79 ± 26	97 ± 22*
Urea (mg.dl)	38 ± 13	37 ± 16
Microalbuminuria (mg/g)	17 ± 22	53 ± 95*
Sodium excretion (mEq/l)	159 ± 35	168 ± 56
PAC (pg/ml)	79.3 ± 48.6	123.8 ± 92.9*
Renin (pg/ml)	35 ± 39	55 ± 68

Data are expressed as mean ± SD. F: female; M: male; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; PP: pulse pressure; ABPM: ambulatory blood pressure monitoring; FMD: flow-mediated dilation; PWV: pulse wave velocity; HbA1c: glycated hemoglobin A1c; LDL and HDL: low- and high-density lipoproteins, respectively; PAC: plasma aldosterone concentration.
* $p < 0.05$ vs. HT.

2.7. Statistical analyses

Data were expressed as mean and standard deviation (SD). Normality of distribution was assessed by Kolmogorov–Smirnov test. Differences between the study groups were determined using the Student's *t*-test or Mann–Whitney test for independent samples, according to the distribution of the data. The chi-square test was used for categorical variables. Study variables were correlated by Pearson correlation test and a multiple logistic regression analysis was performed to predict endothelial dysfunction (FMD ≤ median (6.9%)), adjusted for age, gender, body mass index (BMI), presence of type 2 diabetes, aldosterone, SBP levels, LDL-cholesterol and smoking status. Endothelial dysfunction was considered the primary endpoint, since it was the dependent variable of the logistic regression. The level of statistical significance accepted was 0.05.

Table 2
Medication used in the HT and RHTN groups.

	HT (n = 55)	RHTN (n = 94)
Number of AH drugs (daily)	2.3 ± 0.6	4.3 ± 1.2*
Diuretics (%)	93	100*
ACEIs (%)	60	43*
ARBs (%)	25	77*
CCBs (%)	25	84*
β-blockers (%)	46	84*
Spironolactone (%)	5	35*
Other AH drugs (%)	0	36*
Statins (%)	35	70*
Glucose-lowering drugs (%)	30	35

Data are expressed as mean ± SD. AH drugs: antihypertensive drugs; ACEIs: angiotensin-converting enzyme inhibitors; ARBs: angiotensin receptor blockers; CCBs: calcium channel blockers.
* $p < 0.05$ vs. HT.