Journal of the American Society of Hypertension 8(3) (2014) 146–151

Research Article

Modulation of aldosterone levels by −344 C/T *CYP11B2* polymorphism and spironolactone use in resistant hypertension

Vanessa Fontana, PhD[a,*], Ana Paula Cabral de Faria, MSc[a], Natália Ruggeri Barbaro, MSc[a], Andréa Rodrigues Sabbatini, MSc[a], Rodrigo Modolo, MD[a], Riccardo Lacchini, PhD[b], and Heitor Moreno, PhD, MD[a]

[a]*Laboratory of Cardiovascular Pharmacology, Faculty of Medical Sciences, University of Campinas, Campinas, SP, Brazil; and*
[b]*Laboratory of Cardiovascular Pharmacology, Faculty of Medicine of Ribeirao Preto, University of Sao Paulo, Ribeirao Preto, SP, Brazil*

Manuscript received September 4, 2013 and accepted December 1, 2013

Abstract

Interindividual variability in plasma aldosterone levels comprises environmental and genetic sources. Increased aldosterone levels have been associated with higher risk of hypertension and target-organ damage related to hypertension. Aldosterone excess and intravascular volume expansion are implicated in pathophysiology of resistant hypertension (RH). We sought to investigate whether −344 C/T polymorphism (rs1799998) in aldosterone synthase gene (*CYP11B2*) is associated with plasma aldosterone levels in patients with resistant hypertension. Sixty-two patients with resistant hypertension were enrolled in this cross-sectional study. Genotypes were obtained by allelic discrimination assay using real time polymerase chain reaction. Multivariable linear regression was used to identify whether TT genotype was a predictor of aldosterone levels. No differences in clinical and laboratorial parameters were found among genotype groups. We found an additive effect of the T allele on plasma aldosterone concentration in RH. Also, there was higher aldosterone levels in TT homozygous under use of spironolactone compared with C carriers and compared with TT subjects who was not under use of spironolactone. TT genotype and the use of spironolactone were significant predictors of aldosterone levels in RH subjects. Plasma aldosterone concentration is significantly associated with −344 C/T *CYP11B2* polymorphism and with the treatment with spironolactone in resistant hypertensive subjects. J Am Soc Hypertens 2014;8(3):146–151.

Keywords: Mineralocorticoid receptor antagonists; refractory hypertension; aldosterone synthase and aldosterone breakthrough.

Introduction

The effects of aldosterone in the cardiovascular system are mediated by mineralocorticoid receptors (MR) activation, promoting extracellular volume expansion, cardiac remodeling, endothelial dysfunction, arterial stiffness, inflammation, and production of reactive oxygen species.[1–3] Higher circulating aldosterone levels were associated with risk of hypertension in normotensive subjects[4] and with target-organ damage in essential and resistant hypertension.[5,6] Moreover, about 30% of patients with resistant hypertension (RH), defined as lack of blood pressure (BP) control despite the use of three antihypertensive drugs or any BP level requiring four or more antihypertensive drugs,[7] have elevated plasma aldosterone concentration and intravascular volume expansion.[8] The optimal fourth-line drug in the treatment of resistant hypertension has been extensively discussed, and the efficacy of MR antagonists was demonstrated in observational and prospective studies.[9,10]

Genetic polymorphisms in aldosterone synthase gene (*CYP11B2)* were associated with BP and hypertension.[11,12] Also, higher urinary aldosterone excretion was observed in

This work was supported by Sao Paulo Research Foundation (FAPESP) and National Council for Scientific and Technological Development (CNPq).

All authors have declared no financial or other relationship that might lead to a conflict of interest.

*Corresponding author: Vanessa Fontana, PhD, Laboratory of Cardiovascular Pharmacology, Faculty of Medical Sciences, University of Campinas, PO Box 6111, Campinas, SP, Brazil 13083-970. Tel.: (+55) 19-3521-9539; fax: (+55) 19-3289-2968.

E-mail: fontana@unicamp.br

1933-1711/$ - see front matter
http://dx.doi.org/10.1016/j.jash.2013.12.001

T carriers for −344 C/T polymorphism in *CYP11B2*.[13] Recently, functional analysis revealed that haplotypes that include allele C reduce aldosterone synthase transcription.[14]

Therefore, the aim of this cross-sectional study was to evaluate the impact of −344 C/T polymorphism in *CYP11B2* on plasma aldosterone concentration (PAC) in patients with resistant hypertension (RH).

Methods

Study Subjects

A total of 62 resistant hypertensive subjects from Outpatient Resistant Hypertension Clinic of the University of Campinas (Campinas, Brazil) were enrolled in this study. RH was defined according to *American Heart Association Statement*.[7] Patients with a systolic BP ≥140 mm Hg and/or a diastolic BP ≥90 mm Hg in spite of the use of three antihypertensive drugs including a diuretic were considered resistant hypertensive. Also, patients who use four or more drugs, regardless of BP control, are classified as RH subjects. Office and ambulatory BP monitoring (ABPM) measurements were recorded using a digital sphygmomanometer (Omron HEM-711DLX; OMRON Healthcare Inc, Bannockburn, IL) and an ambulatory BP monitor (Spacelabs Inc, Redmon, WA). For ABPM assessment, patients were instructed to continue normal daily activities and record their sleep period in a personal diary. Secondary hypertension (renal artery stenosis, pheochromocytoma, and primary hyperaldosteronism) and pseudoresistance (white coat effect and lack of adherence) causes were excluded by clinical and laboratorial evaluation. Patients with suspected primary aldosteronism (aldosterone/renin ratio >50 pg/mL*(pg/mL)$^{-1}$)[15] and sleep apnea (''high risk'' by the Berlin sleep questionnaire)[16] were not enrolled in this study. This cross-sectional study was approved by the Research Ethics Committee at the Faculty of Medical Sciences, University of Campinas (Sao Paulo, Brazil) and all participants signed written informed consent form before enrolling in the study (approval no. 222/2011).

Laboratory Assessments

Blood samples were collected at early morning after a fasting time of 8 hours and with the patients in the seated position. Plasma aldosterone and renin concentration was measured by radioimmunoassay (Immunotech SAS, Marseille, France) according to the manufacturer's instructions.

−344 T/C CYP11B2 Genotyping

Blood samples were collected into tubes containing EDTA after a fasting time of 8 hours. Genomic DNA was extracted using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). *CYP11B2* genotype −344 T/C (rs1799998) was determined using the TaqMan allelic discrimination assay (Applied Biosystems, Foster City, CA). In brief, polymerase chain reaction (PCR) was performed with 10 ng of DNA and conditions were as follows: one step of 10 minutes at 95°C, followed by 40 cycles of DNA denaturation at 92°C for 15 seconds, and annealing/extension at 60°C for 1 minute. Fluorescence signals were detected using StepOnePlus (Applied Biosystems) and analyzed with manufacturer's software.

Statistical Analysis

Continuous variables were expressed as mean and standard deviation. Clinical data of the three genotypic groups (CC, CT, and TT) were compared using the Kruskal-Wallis test followed by Dunn's multiple comparison test. Aldosterone levels between C carriers and TT homozygous were compared using the Mann Whitney test. Categorical data were presented in percentages and compared by χ^2 test. A multiple linear regression analysis was performed to evaluate the effects of TT genotype and mineralocorticoid receptor antagonists on log aldosterone concentration, adjusted for gender, ambulatory mean BP, and body mass index. Hardy-Weinberg equilibrium was evaluated using χ^2 test. The level of significance accepted was 0.05.

Results

General characteristics of resistant hypertensive subjects enrolled in the study are listed in Table 1. No differences were observed in demographic and laboratorial variables among −344 C/T *CYP11B2* genotype groups (CC, CT, and TT). Left ventricular mass index and microalbuminuria were similar among genotype groups. There were no differences in number of antihypertensive drugs used by the three groups (CC, 4.2 ± 1.1; CT, 4.5 ± 0.9; and TT, 4.2 ± 0.9 [mean ± SD]). Moreover, all patients were under use of thiazide diuretics, and no differences were observed in antihypertensive class agents used among subgroups, ACE inhibitors (CC = 60%, CT = 40%, TT = 46%; P = .66); ARB II (CC = 40%, CT = 61%, TT = 42%; P = .32); calcium channel blocker (CC = 100%, CT = 85%, TT = 88%; P = .64); β-blockers (CC = 60%, CT = 76%, TT = 79%; P = .66); centrally acting drug (CC = 20%, CT = 36%, TT = 25%; P = .57); mineralocorticoid receptor antagonist (CC = 20%, CT = 52%, TT = 42%; P = .38); and others (CC = 20%, CT = 03%, TT = 08%; P = .32).

Table 2 shows allele and genotype frequencies. No deviation from Hardy-Weinberg equilibrium was found (P < .05). We found higher plasma aldosterone concentration in TT group compared with CT and CC groups (Figure 1). In addition, within a subgroup of RH patients under use of spironolactone (n = 28), a MR antagonist,