ALTERED RELEASE OF CYTOCHROME P450 METABOLITES OF ARACHIDONIC ACID IN RENOVASCULAR DISEASE.

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Short title: CYTOCHROME P450 METABOLITES IN RENOVASCULAR DISEASE

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MATERIALS AND METHODS

Clinical characteristics of patients and control subjects. Selection and exclusion criteria. Three groups of subjects, pair-matched for gender and age, were studied in patients with renovascular disease (RVD) (n=10), patients with essential hypertension (EH) (n=10), and normotensive subjects (C) (n=10). Hypertensive patients were recruited among those admitted to the Hypertension Unit of the Department of Internal Medicine, University Hospital of Verona. Diagnosis of RVD was based on angiographic evidence of severe stenosis (exceeding 70% of the lumen diameter) of a renal artery in hypertensive patients [1]. The diagnosis of RVD with hypertension was supported by positive renal scintigraphy and magnetic resonance angiography of the renal arteries and then confirmed by percutaneous renal artery angiography.

Angiography revealed the presence of significant stenosis of a single renal artery related to atherosclerotic plaques in 8 patients (aged 60 to 85 years) and fibromuscular dysplasia in the remaining two male patients (aged 26 and 75 years). None of the patients had bilateral stenosis. EH was diagnosed on the basis of negative results of the investigation for secondary hypertension, which included in most cases a negative renal magnetic resonance angiography.

All patients were on antihypertensive treatment at the time of study to obtain normalization of the arterial pressure. Angiotensin-converting enzyme inhibitors (ACE-I) and antagonists of the angiotensin receptor (AT_1) were replaced with other antihypertensive drugs. All subjects with RVD and essential hypertension were on antihypertensive treatment to normalize blood pressure. Those

drugs, having a direct effect on the activity of the RAS, ACE-I and AT1 receptor antagonists, were withdrawn 2 months or more before the study.

Antihypertensive drugs were calcium antagonists (n=7), α-receptor blockers (n=5), ß-receptor blockers (n=4), diuretics (n=5), spironolactone (n=1) and clonidine (n=1) at the time of study (median number of drugs prescribed per patient 2, range 1-5).

Essential hypertensive patients were being treated with calcium antagonists (n=8), α-receptor blockers (n=3), β-receptor blockers (n=3), or diuretics (n=1) at the time of study (median number of drugs prescribed per patient 1.5, range 1-3).

Ten normotensive and healthy subjects were studied and matched with hypertensive patients for gender and age. Two RVD patients had diabetes mellitus, treated with oral anti-diabetic drugs. Two RVD patients, one with EH and one control were receiving a statin. None of the subjects was receiving any other cardiovascular drug.

Exclusion criteria for all subjects were: heart failure, previous cardiovascular events, renal atrophy, end stage renal disease, neoplasia, chronic inflammatory disease. Non steroidal anti-inflammatory drugs, including aspirin, were not permitted or withdrawn at least 2 months before study. All the studied subjects were Caucasian.

Measurement of flow-mediated vasodilatation

Endothelial function was investigated according to the model of flow-mediated vasodilatation (FMD) [2]. FMD was evaluated using a high resolution ultrasound echo Doppler (AU5; Esaote Ultrasound System) with a 7.5 MHz linear transducer. The axial resolution of this probe was 0.05 mm, and ultrasonic calipers were accurate to 0.05 mm. We measured brachial artery diameters and

flow velocity (at a fixed position: 1 cm above the elbow fold). Hemodynamic measurements related to FMD were obtained 30 s and 1, 2, 4, 6 and 8 min after the beginning of distal hyperemia and related increase in flow velocity at the level of investigated arteries. Diameter variations were expressed as percentage of variation with respect to the basal diameter.

Analysis of carotid artery structure and stiffness.

All subjects underwent an echo-Doppler evaluation of the carotid arteries for the measurement of intima-media thickness and identification of atherosclerotic plaques.

Intima media thickness of the common carotid artery (cIMT) was measured in the segment 1 cm long proximal to the bulb (the average of two measurements in the distal wall). Plaques were defined as focal structure encroaching into the arterial lumen at least 0.5 mm or 50% of the surrounding cIMT values or demonstrated thickness >1.5 mm.

Applanation tonometry was performed at carotid level using PulsePen (Dia-Tecne) to calculate augmentation index (AI) and augmentation pressure (AP) [3].

REFERENCES

1. Morganti A, Marana I, Airoldi F, Alberti C, Nador B, Palatresi S. Renovascular hypertension. Clinical and diagnostic clues. *Ann Urol (Paris).* 1999;33:137-143.
2. Joannides R, Haefeli WE, Linder L, Richard V, Bakkali EH, Thuillez C, Luscher TF. Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo. *Circulation.* 1995;91:1314-1319.
3. Salvi P, Lio G, Labat C, Ricci E, Pannier B, Benetos A. Validation of a new non-invasive portable tonometer for determining arterial pressure wave and pulse wave velocity: the PulsePen device. *J Hypertens.* 2004;22:2285-2293.

TABLE S1. Anthropometric and biochemical characteristics of Hypertensive Patients and Healthy Subjects

Variables	RVD	EH	Controls
Age, years	75 (25-85)	73 (33-86)	71 (25-81)
Gender, male/female	7/3	7/3	7/3
BMI, kg/m^2 (table 2)	27.5 (21.2-33.1)	26.0 (17.5-35.7)	24.9 (21.5-31.3)
p-Cholesterol, mmol/L	4.8 (4.0-6.9)	5.1 (3.8-5.7)	5.48 (4.2-6.0)
p-HDL-Cholesterol, mmol/L	1.5 (0.9-2.2)	1.61 (1.1-2.1)	1.36 (1.1-1.6)
p-Triglycerides, mmol/L	1.7 (0.4-3.7)	1.02 (0.5-1.4)	1.26 (0.6-2.3)
p-Homocysteine, μmol/L	18.1 (8.9-30.3)	15.3 (11.5-74.1)	15.2 (8.7-21.0)
p-Glucose, mmol/L	5.61 (4.3-11.6)	5.06 (4.5-11.3)	5.27 (4.7-6.4)
Creatinine, μmol/L	109.17 (71.6-163.5)*	80.8 (52.1-95.4)	67.6 (60.0-102.5)
Creatinine clearance, mL/min	50.1 (29.3-168.7)*	79.9 (51.5-134.5)	84.8 (42.0-170.3)
p-TNF-α, pg/mL	10.1 (1.8-113.4)	9.9 (0.7-44.2)	10.6 (2.9-38.3)
s-C-RP, mg/L	1.6 (0.5-6.5)	1.1 (0.3-13.8)	2.0 (0.2-6.4)
PRA, ng/mL per hour	0.55 (0.05-5)	0.20 (0.05-1)	0.20 (0.05-2.7)

Data are presented as median (range); * indicates P <0.05 vs normotensive controls

RVD, renovascular disease patients; EH, essential hypertensive patients; BMI, body mass index; HDL, high density lipoprotein; GFR, glomerular filtration rate.

TABLE S2: Haemodynamic and vascular characteristics of Hypertensive Patients and Healthy Subjects

Variables	RVD	EH	Controls
Carotid artery stenosis, n	9/10 *	6/10	2/10
IMT, mm	0.93 (0.75-1.5)	0.89 (0.44-1.15)	0.88 (0.72-1.03)
SBP, mmHg	143 (124-170)	154 (128-170)	143 (122-154)
DBP, mmHg	73 (67-74)	77 (67-89)	83 (74-93)
FMD, %	-0.34 (-2.38-5.57)	0.24 (-9.35-7.5)	1.37 (-1.0-4.81)
AI, %	30 (9-50)	25 (9-46)	20 (9-54)
AP, mmHg	16 (5-64)	11 (3-27)	8 (4-22)

* indicates P <0.05 vs normotensive controls

RVD, renovascular disease patients; EH, essential hypertensive patients; IMT, intima media thickness; SBP, systolic blood pressure; DBP, diastolic blood pressure; FMD, flow mediated dilatation; AI, augmentation index; AP augmentation pressure.

Figure S1: Plasma concentration of EETs, DHETs, EETs/DHETs ratio and urinary excretion of DHETs

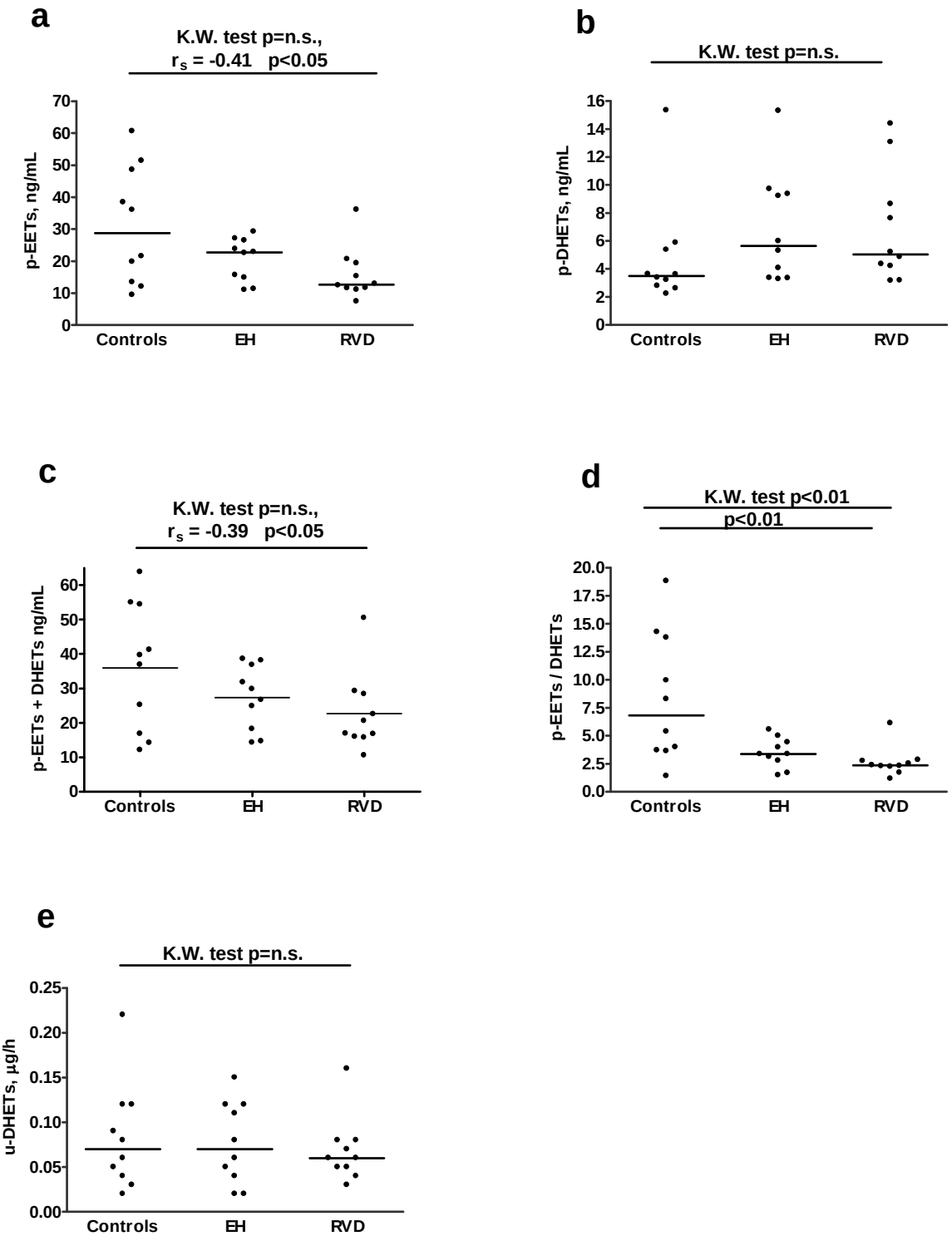

Figure S2: Correlation between plasma 20-HETE and plasma DHETs