jraas

Journal of the Renin-Angiotensin-Aldosterone System
12(4) 446–455

DOI: 10.1177/1470320311415134
jra.sagepub.com
SAGE

Effects of antihypertensive treatment on endothelial function in postmenopausal hypertensive women. A significant role for aldosterone inhibition

Rosario Rossi, Annachiara Nuzzo, Daniele Iaccarino, Antonella Lattanzi, Giorgia Origliani, Daniel Enrique Monopoli and Maria Grazia Modena

Abstract

Introduction: Endothelial dysfunction is a well-demonstrated independent predictor of cardiovascular events in hypertensive postmenopausal women. Accordingly, it is plausible that improving endothelial function could represent an adjunctive target for antihypertensive treatment. The aim of our study was to evaluate the effect of pharmacologic treatment on endothelial function in the specific population of hypertensive postmenopausal women.

Methods: A total of 320 consecutive hypertensive postmenopausal women underwent a high-resolution ultrasound study of the brachial artery at baseline and after six months, while 'optimal' control of blood pressure (maintenance of blood pressure values below 140/90 mmHg at all follow-up visits) was achieved using antihypertensive therapy. Endothelial function was measured as flow-mediated dilation, using ultrasound method.

Results: After six months of treatment, flow-mediated dilatation (FMD) had significantly improved in the majority of patients (n = 257 [80.3% of the entire population]; FMD = 8.1 ± 1.0% at baseline *vs.* 10.6 ± 1.5% after follow-up; p < 0.001), but it had not changed or worsened in others (n = 63 [19.7%]; FMD = 8.2 ± 1.2% at baseline *vs.* 7.6 ± 1.0% after six months; p = ns). Improvement of endothelial function, at multivariate analysis, resulted independently associated with the use of aldosterone inhibitors (odds ratio = 2.15; 95% confidence interval: 1.55–2.75; p = 0.001).

Conclusions: This study demonstrates that a significant improvement in endothelial function may be obtained after six months of an optimal antihypertensive therapy. Among all hypertensive postmenopausal women that achieved an optimal control of blood pressure during follow-up, the use of drugs that inhibit aldosterone receptors was associated with an improvement of endothelial function, beyond the 'optimal' blood pressure control.

Keywords

Aldosterone, antihypertensive therapy, endothelium, hypertension, vasodilation

Introduction

Endothelium plays an important role in the modulation of vascular tone. The major endothelium-produced relaxing factor is nitric oxide (NO), which is derived from L-arginine by the activity of the enzyme NO-synthase.[1] Since 1992, the non-invasive measurement of brachial artery flow-mediated dilation (FMD) has been used to evaluate endothelial function, because FMD largely depends on the ability of the endothelium to produce NO.[2]

Essential hypertension is characterized by impaired endothelium-dependent vasodilation,[3,4] and it has been suggested that endothelial dysfunction could act as a promoter of hypertension in apparently healthy postmenopausal women.[5] Moreover, endothelial dysfunction resulted an independent predictors of cardiovascular events in patients with hypertension.[6-9] This fact was expressly demonstrated, by our group, in hypertensive postmenopausal women.[10] Accordingly, it is plausible that reversing endothelial dysfunction could represent an adjunctive target for antihypertensive treatment. However, it has been widely established that simple blood pressure reduction is not sufficient to improve or restore endothelial dysfunction.[10] This

'Early Atherosclerotic Clinic' (Bene Essere Donna & Cardiometabolic Center) of the Policlinico Hospital, Institute of Cardiology, University of Modena and Reggio Emilia, Italy

Corresponding author:
Rosario Rossi, Institute of Cardiology, Policlinico Hospital,
Via del Pozzo, 71–41100 Modena, Italy.
Email: rossi.rosario@unimore.it

beneficial effect can be achieved only by specific mechanisms not shared by all drug classes.[11-13]

In the present study we addressed the effect of six months' antihypertensive treatment with different antihypertensive drug classes, on flow-mediated, endothelium-dependent, vasodilatation of the brachial artery, in a cohort of postmenopausal women with essential hypertension.

Subjects and methods

Patients

Postmenopausal patients with newly diagnosed hypertension, according to the European Guidelines for the management of arterial hypertension,[14] were eligible for the study.

Menopause was defined by the absence of menstruation for at least 12 months or by the dosage of hormone serum level (follicle-stimulating hormone > 40 IU/L and 17 beta-estradiol < 110 pmol/L).

The patients' history, physical examination, 12-lead electrocardiogram, and echocardiogram were used to exclude past or present heart disease. Three hundred and thirty-two consecutive women were enrolled. All gave their informed consent to participate in this prospective cross-sectional study, which had been approved by the Science and Ethic Committee of our university.

Study protocol

Enrolled postmenopausal women underwent a study of the endothelial function (ultrasound study of the brachial artery) at baseline. A second evaluation was scheduled for six months later. In the interim, patients were to receive antihypertensive treatment. Therapies were assigned to maintain systolic BP < 140 mmHg and diastolic BP < 90 mmHg. Patients were visited every four weeks. The choice of antihypertensive drug used was at the discretion of the study investigators, and was made on the basis of a step-by-step approach, when the BP values were unsatisfactory (≥ 140/≥ 90 mmHg). Women were excluded from the study for the following reasons: hypertension due to identifiable cause; a failure to achieve a BP < 140/< 90 mmHg after six months of treatment; achieved the desired values of BP with only changes in lifestyle, therefore, no use of antihypertensive drugs; an improbability of remaining geographically accessible for study visits; current participation in other ongoing clinical trials.

Evaluation of endothelial function

Endothelial function was assessed by the measurement of the flow-mediated (endothelial-dependent) dilatation performed on the brachial artery. FMD of the brachial artery was measured without knowledge of the patient's clinical data.

The technique for assessing brachial artery FMD has been described in detail elsewhere.[2,10,15,16] Briefly, FMD was assessed in the subject's right arm in the recumbent position after a 15-min equilibration period in a temperature-controlled room (22°C to 25°C) by a high resolution ultrasound instrument GE Vivid E, with a 5- to 12-MHz linear array multifrequency transducer (General Electric Company, Fairfield, CT, USA). The brachial artery was longitudinally imaged ~ 5 cm proximal to the antecubital crease, where the clearest image was obtained, and the diameter at end diastole was measured (the mean of three measurements was used in the analysis). A pressure cuff, placed on the forearm (distal to the target artery), was inflated until no blood flow was detected through the brachial artery with the Doppler probe. After 5 min, the cuff was released, and this was followed by an increase in blood flow. This phenomenon increased shear stress, which served as the stimulus to induce dilatation. After cuff release, the diameter of the brachial artery was measured at 45, 60, 90, 180, and 300 s. The maximum diameter in any of these measurements was used in the calculation of FMD according to the following formula: (maximum diameter during reactive hyperaemia - diameter at baseline)/diameter at baseline %.

The same procedure, performed after administration of 0.4 mg of sublingual nitroglycerin, allowed us to calculate the nitroglycerin-mediated dilatation.

Intrareader reproducibility for baseline diameter, maximum diameter, and FMD was evaluated by comparing an original and a blinded quality control reread of ultrasounds from 50 consecutive women in our centre. The intraclass correlation coefficients were 0.99, 0.99, and 0.89, respectively. Intrasubject variability was evaluated by comparing results from repeated examinations of 50 women on two days a week apart. The intraclass correlation coefficients for baseline diameter, maximum diameter, and FMD were 0.94, 0.92, and 0.78, respectively. Per cent technical error of measurement was 0.45% for baseline diameter measurement, 0.78% for maximum diameter measurement, and 7.4% for FMD measurement.

Statistical analysis

Statistical analysis was performed using SPSS software version 15.0 (SPSS Inc., Chicago, IL, USA). Data were expressed as mean ± one SD for normally distributed continuous variables. Dichotomous parameters were summarized as a percentage. The two studied groups were compared, at baseline, by the Student *t* test for unpaired data and the chi-square test with Yate's correction for continuity, as appropriate. Correlation between continuous variables were tested using the Pearson *r* test.

To assess the independent correlates of improvement of endothelial function during follow-up, we constructed a

model of multivariate binary logistic regression analysis including FMD as a dependent variable (FMD improvement *vs.* FMD non-improvement during follow-up), as well as the other parameters related to the brachial artery ultrasound study (brachial artery diameter and nitroglycerin-mediated vasodilatation), a series of traditional risk factors (age, heart rate, smoking habit, body mass index, hypercholesterolemia, type II diabetes, and serum levels of fasting glucose, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides), liver and renal function derived parameters, serum electrolytes, one indicator of effectiveness of antihypertensive therapy (systolic and diastolic BP decline during follow-up), and the use of antihypertensive and non-antihypertensive drugs during follow-up, as a covariate. In addition, we evaluated the power of the dual-blockade of the renin–angiotensin–aldosterone system (RAAS) (concomitant use of an aldosterone inhibitor + ACE-inhibitor or angiotensin receptor blocker) in the ability to influence FMD improvement.

All probability values are two-tailed, and statistical significance was considered as rejection of the null hypothesis with > 95% confidence.

Results

The study included 320 consecutive postmenopausal women with essential hypertension. All of these patients, at baseline, underwent an ultrasound study of the brachial artery, with the first determination of FMD. At the second evaluation (six months after antihypertensive drug treatment), FMD had significantly improved in the majority of patients ($n = 257$ [80.3% of the entire population]; group 1: improved FMD); but it had not changed or worsened in others ($n = 63$ [19.7%]; group 2: not improved FMD). In patients of the latter group we observed that FMD values were unchanged (with respect to baseline) in 33 cases (10.3% of the entire population), whereas they were reduced in 30 patients (9.4% of the entire population).

Demographics, clinical, and brachial artery characteristics of the two study groups are shown in Table 1.

With regard to the assumed drugs, we noted 71 women (22.2% of the entire population) taking only one antihypertensive medication; 176 (55.0%) two; 52 (16.3%) three; and 21 (6.5%) four or more. One hundred fifty-six of 320 patients (48.7%) were taking (alone or associated) a thiazide diuretic; 121 (37.8%) an angiotensin-converting enzyme (ACE) inhibitor; 120 (37.5%) an angiotensin receptor blocker (ARB); 102 (31.9%) a calcium channel blocker; 83 (25.9%) a beta adrenergic blocker; 49 (15.3%) an aldosterone receptor blocker; 36 (11.2%) a loop diuretic; and 35 (10.9%) an 'other' antihypertensive drug. The antihypertensive regimens and proportion of subjects who received ACE inhibitors, ARBs, calcium channel blockers, beta-blockers and diuretics, alone or combined, or other drugs, are shown in Table 2.

The results of multivariate regression analysis are shown in Table 3. Improvement of endothelial function, expressed as FMD, resulted independently associated with the use of aldosterone inhibitors. Such drugs affect endothelial function beyond BP decline. Among all used drugs, only this class of antihypertensive medication is able, in our experience, to independently influence endothelial function during follow-up. The power of the single potential prognostic factor, evaluated using regression analysis, is shown in Table 4. There are other parameters that influence the outcome of the study. i.e. systolic BP change during follow-up, and the two-level blockade of the RAAS. These parameters, although statistically significant, are not independently able to influence the improvement of FMD during follow-up.

The relationship between systolic BP decline and FMD improvement during follow-up is shown in Figure 1.

Regarding the 49 patients receiving aldosterone inhibitors, only two patients were taking spironolactone, at a dose of 25 mg/day (these patients were those included in the group that did not improve their endothelial function during follow-up); 25 patients assumed potassium canrenoate, at a dose of 50 mg/day; and 22 canrenone, at a dose of 50 mg/day. All hypertensive women included in these last two subgroups significantly improved their endothelial function during follow-up. In Italy eplerenone, a selective aldosterone receptor blocker, is not commercially available. All of the aldosterone inhibitors used in the present study are non-selective.

In the present study aldosterone inhibitors were assumed in association with diuretics (loop or thiazide) in all 49 treated patients. These drugs were included in the pharmacological scheme of treatment in the second step in 10/49 patients (20.4%); in the third step in 31 (63.3%); and in the fourth step in eight patients (16.3%).

To better evaluate the characteristics of patients taking aldosterone receptor inhibitors, we further divided our population into two groups: patients taking versus patients not taking aldosterone receptor inhibitors. The results of this comparison are shown in Table 5. It is important to emphasize a) that the dual blockade of the RAAS (concomitant use of an aldosterone inhibitor + ACE-inhibitor or ARB) was used in a significantly greater proportion of patients in the group treated with aldosterone inhibitors. No patient in our series has been treated using the association of ACE-inhibitor + ARB or all the three RAAS-blocking agents. b) Patients taking aldosterone inhibitors do not show significant elevation of serum potassium nor significant deterioration of renal function during follow-up, compared with other patients. These facts are probably due to the low dose of prescribed medications and, above all, because in our experience the aldosterone inhibitor is always used in association with (thiazide or loop) diuretics. c) The relatively low-dose of aldosterone inhibitors used accounts for the fact that none of our patients have experienced potentially drug-related side effects, such as gynaecomastia or hirsutism.

Table 1. Main demographic, anthropometric, clinical and brachial artery characteristics of the patients, divided according to the behaviour of endothelial-mediated vasodilation during follow-up

Parameter	Group 1: improved FMD (n = 257)	Group 2: non-improved FMD (n = 63)
Demographic, anthropometric, clinical variables and cardiovascular risk factors, at baseline		
Age, years	56 ± 7	57 ± 6
Current smokers	21.4% (n = 55)	23.8% (n = 15)
Total cholesterol, mg/dl	206 ± 46	207 ± 40
HDL cholesterol, mg/dl	48 ± 14	54 ± 16
LDL cholesterol, mg/dl	112 ± 40	110 ± 37
Hypercholesterolemia	32.7% (n = 84)	31.7% (n = 20)
Triglycerides, mg/dl	152 ± 90	156 ± 98
Glucose, mg/dl	102 ± 28	103 ± 26
Type 2 diabetes mellitus	8.2% (n = 21)	7.9% (n = 5)
BMI, kg/m^2	27.5 ± 4.3	27.8 ± 4.6
Obesity[a] (BMI ≥ 30 kg/m^2)	7.0% (n = 18)	6.3% (n = 4)
Heart rate, bpm	80 ± 14	77 ± 11
Framingham Risk Score[b]	7.4 ± 6.3	7.3 ± 6.5
Liver function-related parameters, at baseline		
Total bilirubin, mg/dl	0.72 ± 0.37	0.75 ± 0.39
AST, IU/L	23 ± 10	22 ± 7
ALT, IU/L	28 ± 18	28 ± 16
γ-GT, IU/L	37 ± 29	36 ± 28
Renal function-related parameters, at baseline		
Creatinine, mg/dl	0.92 ± 0.26	0.91 ± 0.21
BUN, mg/dl	31 ± 10	31 ± 10
Uric acid, mg/dl	5.6 ± 1.3	5.4 ± 1.3
Serum electrolytes, at baseline		
Sodium, mEq/L	138 ± 5	139 ± 4
Potassium, mEq/L	3.9 ± 0.4	4.1 ± 0.5
Medication, other than antihypertensive drugs, at baseline		
Statins	31.1% (n = 80)	30.1% (n = 19)
Oral hypoglycaemic drugs	8.2% (n = 21)	7.9% (n = 5)
Brachial artery characteristics, at baseline		
BAD, mm	3.8 ± 0.6	3.9 ± 0.5
FMD, %	8.1 ± 1.0	8.2 ± 1.2
NTG-MD, %	17.2 ± 2.0	16.9 ± 2.1
Brachial artery characteristics, after six months of follow-up		
BAD, mm	3.9 ± 0.7	3.9 ± 0.6
FMD, %	10.6 ± 1.5[c]	7.6 ± 1.0[d]
NTG-MD, %	17.5 ± 2.2	17.4 ± 2.3

Data are presented as mean value ± one SD, or number (%) of patients. [a]According to the World Health Organization definition. [b]http://www.mdcalc.com/framingham-cardiac-risk-score. [c]$p < 0.001$ intra-group (*vs.* baseline) comparison. [d]$p = 0.02$ intra-group (*vs.* baseline), and $p < 0.0001$ inter-groups (*vs.* group 1) comparisons. ALT: alanine aminotransferase, AST: aspartate aminotransferase, BAD: brachial artery diameter, BMI: body mass index, BUN: blood urea nitrogen, FMD: flow-mediated dilation, HDL: high-density lipoprotein, LDL: low-density lipoprotein, NTG-MD: nitroglycerin-mediated dilation, γ-GT: γ-glutamil transferase.

Discussion

One of the main results of the present study is that not all hypertensive postmenopausal women treated with antihypertensive drugs improved their endothelial function, although their BP levels were effectively controlled. In other words, a proportion of hypertensive postmenopausal women (about one in five, in our study) that did not improve, or even worsened, their FMD despite achieving target BP. We do not know the true reason why this happens, but we know that these subjects have a worse prognosis, having a higher risk (compared with those who improve their endothelial function) of suffering cardio- and cerebro-vascular events.[9,10,12]

An important finding of our study is that treatment with ACE inhibitors and/or ARBs has not emerged as an independent factor able to significantly influence FMD in hypertensive postmenopausal women. These agents have been shown to improve endothelial function in several studies,[17-20]

Table 2. Blood pressure values at baseline and after six months of treatment, and the antihypertensive regimen: a comparison between groups

Parameter	Group 1: improved FMD (n = 257)	Group 2: non-improved FMD (n = 63)
Baseline blood pressure (systolic/diastolic), mmHg	157 ± 15 / 94 ± 10	156 ± 14 / 93 ± 9
Blood pressure after 6 months (systolic/diastolic), mmHg	134 ± 12 / 83 ± 9[a]	133 ± 13 / 84 ± 9[a]
Regimen		
Single-drug therapy	22.3% (n = 58)	22.3% (n = 13)
Two antihypertensive drugs	55.2% (n = 142)	53.9% (n = 34)
Three antihypertensive drugs	15.9% (n = 41)	17.5% (n = 11)
Four or more antihypertensive drugs	6.6% (n = 17)	6.3% (n = 4)
Antihypertensive drugs used[b]		
ACE inhibitors	39.6% (n = 102)	36.5% (n = 23)
ARB	36.9% (n = 95)	39.6% (n = 25)
Calcium channel blockers	31.9% (n = 82)	31.7% (n = 20)
Beta adrenergic blockers	26.0% (n = 67)	25.4% (n = 16)
Thiazide diuretics	48.6% (125)	49.2% (n = 31)
Loop diuretics	11.3% (n = 29)	11.1% (n = 7)
Aldosterone receptor blockers	18.3% (n = 47)	3.2% (n = 2)[c]
Dual blockade of RAAS (aldosterone receptor blocker + ACE-inhibitor or ARB)	15.2% (n = 39)	3.2% (n = 2)[c]
Other antihypertensive drugs	10.5% (n = 27)	9.5% (n = 6)

Data are presented as mean value ± one SD or number (%) of patients. [a]$p < 0.0001$ *vs.* baseline. [b]Alone or combined. [c]$p < 0.0001$, inter-group comparison. ACE: angiotensin-converting enzyme, ARB: angiotensin II receptor blockers, RAAS: renin–angiotensin–aldosterone system.

Table 3. The independent parameters correlated with 'improvement of the flow-mediated dilation' during follow-up, as resulted in the multivariate regression analysis

Parameter	B	SE	Wald	p	Exp(B)	CI for Exp(B)
Use of aldosterone receptor blockers	1.87	0.73	6.4	0.001	2.15	1.55–2.75
Constant	2.21	0.79	7.7	0.0001	3.18	–

Use of aldosterone receptor blockers is expressed as categorical variables (use *vs.* non-use). CI: confidence interval, Exp(B): odds ratio, SE: standard error.

but in our population, because they have been used in the vast majority of cases (about 75% of our patients received or an ACE inhibitor or an ARB), were not able, statistically, to significantly influence FMD. These important drugs appear to be, rather, the 'standard of care' of antihypertensive drug treatment of postmenopausal women, in our experience.

The only class of drugs that clearly emerged as an independent factor able to significantly improve endothelial function in hypertensive postmenopausal women is aldosterone receptor blockers.

Aldosterone, aldosterone inhibition, and endothelial function

Normalization of elevated BP in hypertensive individuals is associated with reduced target-organ damage and incident cardiovascular morbidity and mortality. Endothelial dysfunction may precede hypertension,[5] and many clinical manifestations of end-organ damage,[21] and predict the occurrence of future cardiovascular events in hypertensive postmenopausal women.[9,10,12] Activation of the renin–angiotensin–aldosterone system has been associated with increased cardiovascular morbidity and mortality. Antihypertensive drugs that interfere with the RAAS exert, in fact, beneficial effects on vascular structure in hypertensive patients at variable risk for cardiovascular diseases.[18,19,22]

Aldosterone is a mineralocorticoid synthesized not only in the adrenal cortex but also in blood vessels.[23] Aldosterone exerts significant pathophysiological effects on the cardiovascular system and causes vascular injury in the brain, heart, and kidneys.[24]

Several studies has reported that aldosterone contributes to endothelial dysfunction. Acute short-term systemic administration of aldosterone to young healthy subjects at a dose that increases aldosterone to plasma levels similar to those observed in patients with chronic heart failure

Table 4. Potential predictors of FMD progression evaluated using regression analysis

Parameter	Score	*p*
Age, years	1.61	0.20
Heart rate, bpm	0.12	0.74
Baseline systolic BP, mmHg	0.51	0.48
Baseline diastolic BP, mmHg	0.50	0.41
Smoking habits (yes/no)	0.41	0.53
Type II diabetes mellitus (yes/no)	0.48	0.62
Total cholesterol, mg/dl	0.12	0.72
HDL cholesterol, mg/dl	0.26	0.61
LDL cholesterol, mg/dl	0.19	0.66
Triglycerides, mg/dl	0.17	0.68
Glucose, mg/dl	0.12	0.72
Body mass index, kg/m^2	0.61	0.83
Creatinine, mg/dl	0.71	0.36
BUN, mg/dl	0.93	0.82
Systolic BP change during follow-up, mmHg	9.8	0.05
Diastolic BP change during follow-up, mmHg	2.41	0.11
Use of ACE-inhibitors during follow-up (yes/no)	2.58	0.10
Use of ARBs during follow-up (yes/no)	0.98	0.65
Use of aldosterone inhibitors during follow-up (yes/no)	24.2	0.001
Use of double-blockade of RAAS (simultaneous use of an aldosterone inhibitor + an ACE-inhibitor or ARB) during follow-up (yes/no)	16.7	0.01
Use of diuretics during follow-up (yes/no)	0.42	0.45
Use of calcium channel blockers during follow-up (yes/no)	0.64	0.41
Use of beta adrenergic blockers during follow-up (yes/no)	0.41	0.54
Use of statins during follow-up (yes/no)	1.99	0.21
Use of oral hypoglycaemic drugs during follow-up (yes/no)	0.14	0.15
Brachial artery diameter at baseline, mm	0.23	0.81
Baseline FMD, %	0.66	0.78
Baseline NTG-MD, %	0.65	0.74

Abbreviations: see Tables 1 and 2.

reduced the response to acetylcholine, a well-known endothelial-dependent vasodilator.[25] This detrimental effect was observed without changes in blood pressure levels, suggesting that aldosterone is able to impair endothelial function through non-haemodynamic mechanisms. Treatment with the mineralocorticoid receptor antagonist ameliorated endothelial responses to acetylcholine in rats infused with angiotensin II, suggesting that aldosterone mediates vascular reactivity alterations induced by angiotensin II.[26] In addition, aldosterone has been shown to be involved in the pathogenesis of hypertension.[27] Several mechanisms can underlie the deleterious effects of aldosterone on endothelium, with one important effect being decreased nitric oxide availability.[28] However, in patients with hypertension and/or left ventricular hypertrophy/dysfunction, treatment with mineralocorticoid receptor antagonists have shown a beneficial effect in improving cardiac performance and cardiovascular mortality.[29]

Mineralocorticoid antagonism attenuates cardiovascular damage by mechanisms that seem to be in part independent of changes in BP values and that involve direct blockade of aldosterone's cardiovascular pro-inflammatory and pro-fibrotic effects.[30] Aldosterone induces fibrosis in the heart, blood vessels, and kidney, particularly in the presence of high salt,[31] and the antagonism of mineralocorticoid receptors has been associated with reduced fibrosis in human myocardium, limited ventricular remodelling and improved survival in patients with ventricular dysfunction and heart failure.[30-34] Our data suggest an additional mechanism by which aldosterone may exert its negative effect on the development of cardiovascular events, namely endothelial dysfunction.

Potassium canrenoate is a competitive non-selective inhibitor of aldosterone receptor; its pharmacologic activity is due to a rapid conversion to canrenone, which is the active metabolite. Potassium canrenoate is rapidly and completely absorbed from the gastrointestinal tract and bypasses the liver unchanged. Canrenone and canrenoate are in enzymatic equilibrium in vivo, in which canrenoate represents the derived γ-hydroxycarboxylic acid.[35] The bioavailability and water solubility of potassium canrenoate are greater than those of spironolactone; therefore the number of active metabolites of the latter is considerably greater.[35] These two drugs (potassium canrenoate and canrenone), used in hypertensive postmenopausal women, significantly improve endothelial function, independently of the reduction of BP values.

Study limitations

Our study has some limitations in that it is an observational, non-randomized study.

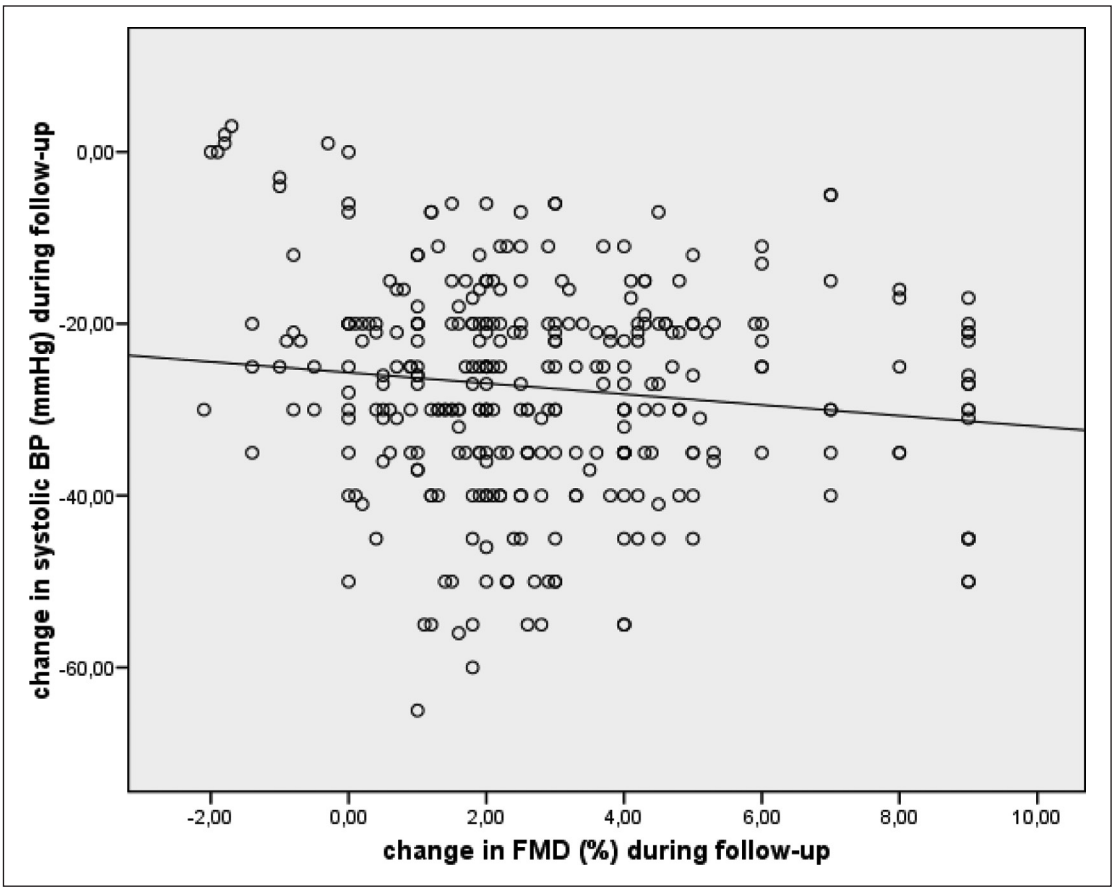

Figure 1. Regression analysis between change in flow-mediated dilatation (FMD) and change in blood pressure (BP) values during follow-up ($r = -0.12$, $p < 0.05$).

The initial aim of the present study was to investigate the role of the various classes of antihypertensive drugs on the improvement of endothelial function in a specific population of hypertensive postmenopausal women. The main result of the study, i.e. the importance of aldosterone inhibition, was not a pre-specified objective, but emerged from post-hoc data analysis. This is why our patients did not undergo evaluation of circulating blood concentrations of aldosterone at baseline, nor evaluation of the aldosterone–renin ratio. Moreover, none of our patients had symptoms suggestive of hyperaldosteronism, and, in addition, the plasma potassium levels showed similar levels in the two study groups at baseline, and none of our women had significant hypokalaemia (potassium levels below 3.5 mEq/L).

An important consideration is that aldosterone inhibitors have been reported as effective in treating resistant hypertension, especially in obese patients and in patients with sleep apnoea syndrome.[36] In our series, baseline BP are comparable between groups and, in addition, the number of drugs useful in achieving optimal BP values is statistically not different between groups. No patient in our cohort suffered from sleep apnoea syndrome, and there were no significant differences, relative to all studied parameters (including the prevalence of obesity), between groups.

The present study presents several strengths, including the prospective design, the homogeneous sample, and the strict follow-up of the participants. All of these factors support us to hypothesize, in postmenopausal hypertensive women, a significant influence of antihypertensive pharmacological treatment on the recovery of endothelial dysfunction. In addition, we highlight the importance of inhibiting aldosterone receptors in order to additionally improve endothelial function.

Although the present data are encouraging, it is opportune to specify that the routine clinical use of the non-invasive evaluation of endothelial function on an individual basis may be premature, because the test is still not completely standardized, and is costly and rather labour intensive. Moreover, the value of the present study is to provide biological insight rather than practical implications. The encouraging results of the present study, for the significant impact on endothelial function, may be used for planning new randomized trials to assess whether the addition of an aldosterone inhibitors actually translates into a significant clinical impact in hypertensive populations.

Table 5. Main demographic, anthropometric, clinical and brachial artery characteristics of the patients, divided according to whether or not taking aldosterone inhibitors

Parameter	Group 1: patients taking aldosterone inhibitors (n = 49)	Group 2: patients NOT taking aldosterone inhibitors (n = 271)
Demographic, anthropometric, clinical variables and cardiovascular risk factors, at baseline		
Age, years	57 ± 7	57 ± 8
Current smokers	22.4% (n = 11)	21.8% (n = 59)
Total cholesterol, mg/dl	205 ± 45	209 ± 42
HDL cholesterol, mg/dl	47 ± 12	51 ± 15
LDL cholesterol, mg/dl	113 ± 38	112 ± 38
Hypercholesterolaemia	32.6% (n = 16)	32.5% (n = 88)
Triglycerides, mg/dl	159 ± 89	162 ± 88
Glucose, mg/dl	100 ± 18	104 ± 25
Type 2 diabetes mellitus	8.1% (n = 4)	8.1% (n = 22)
BMI, kg/m^2	26.7 ± 4.2	27.5 ± 4.5
Obesity* (BMI ≥ 30 kg/m^2)	6.1% (n = 3)	7.0% (n = 19)
Heart rate, bpm	81 ± 13	79 ± 13
Framingham Risk Score†	7.6 ± 6.3	7.7 ± 6.6
Blood pressure		
Baseline (systolic / diastolic), mmHg	156 ± 16 / 93 ± 11	157 ± 15 / 94 ± 10
After 6 months (systolic / diastolic), mmHg	133 ± 14 / 82 ± 10[a]	134 ± 15 / 83 ± 10[a]
Liver function-related parameters, at baseline		
Total bilirubin, mg/dl	0.78 ± 0.38	0.77 ± 0.39
AST, IU/L	25 ± 9	23 ± 9
ALT, IU/L	28 ± 15	28 ± 16
γ-GT, IU/L	36 ± 27	37 ± 30
Renal function-related parameters, at baseline		
Creatinine, mg/dl	0.90 ± 0.35	0.99 ± 0.29
BUN, mg/dl	34 ± 9	30 ± 11
Uric acid, mg/dl	5.5 ± 1.2	5.5 ± 1.4
Renal function-related parameters, after six months of follow-up		
Creatinine, mg/dl	1.02 ± 0.38	0.98 ± 0.31
BUN, mg/dl	39 ± 9	38 ± 10
Uric acid, mg/dl	5.9 ± 1.5	5.6 ± 1.3
Serum electrolytes, at baseline		
Sodium, mEq/L	141 ± 6	139 ± 5
Potassium, mEq/L	4.0 ± 0.5	4.1 ± 0.4
Serum electrolytes, after six months of follow-up		
Sodium, mEq/L	140 ± 8	140 ± 6
Potassium, mEq/L	4.5 ± 0.8	4.3 ± 0.5
Antihypertensive medications		
Dual blockade of RAAS (aldosterone receptor blocker + ACE-inhibitor or ARB)	75.5% (n = 37)	0[b]
Use of ACE-inhibitor	55.1% (n = 27)	36.2% (n = 98)[c]
Use of ARB	20.4% (n = 10)	40.6% (n = 110)[d]
Medications, other than antihypertensive drugs		
Statins	30.6% (n = 15)	29.9% (n = 81)
Oral hypoglycaemic drugs	8.1% (n = 4)	8.1% (n = 22)
Brachial artery characteristics, at baseline		
BAD, mm	3.9 ± 0.5	3.9 ± 0.7
FMD, %	8.0 ± 1.3	8.3 ± 1.1
NTG-MD, %	18.9 ± 3.2	18.1 ± 2.8
Brachial artery characteristics, after six months of follow-up		
BAD, mm	4.0 ± 0.8	3.9 ± 0.7
FMD, %	14.3 ± 1.7[e]	11.5 ± 1.4[a]
NTG-MD, %	18.5 ± 2.2	17.9 ± 2.6

[a]$p < 0.0001$ intra-group (*vs.* baseline) comparison. [b]$p < 0.0001$ inter-group comparison. [c]$p < 0.01$ inter-group comparison. [d]$p < 0.001$ inter-group comparison. [e]$p < 0.0001$ intra-group (*vs.* baseline), and $p < 0.01$ inter-group (*vs.* group 2) comparisons. Abbreviations: see Table 1. *: according to the world health organization criteria, †: http://hp2010.nhlbihin.net/atpiii/calculator.asp?usertype=prof.

Acknowledgement

This study is part of the 'Progetto Strategico Salute della Donna' sponsored by Istituto Superiore di Sanità (ISS), Rome, Italy.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of interest

The authors declare that they have no conflicts of interest.

References

1. Lüscher TF and Vanhoutte PM. *The endothelium: modulator of cardiovascular function*. Boca Raton, FL: CRC Press, 1990, pp.1–215.
2. Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet* 1992; 340: 1111–1115.
3. Panza JA, Quyyumi AA, Brush JE Jr and Epstein SE. Abnormal endothelium dependent vascular relaxation in patients with essential hypertension. *N Engl J Med* 1990; 323: 22–27.
4. Taddei S, Virdis A, Mattei P and Salvetti A. Vasodilation to acetylcholine in primary and secondary forms of human hypertension. *Hypertension* 1993; 21: 929–933.
5. Rossi R, Chiurlia E, Nuzzo A, Cioni E, Origliani G and Modena MG. Flow-mediated vasodilation and the risk of developing hypertension in healthy postmenopausal women. *J Am Coll Cardiol* 2004; 44: 1636–1640.
6. Taddei S and Salvetti A. Endothelial dysfunction in essential hypertension: clinical implications. *J Hypertens* 2002; 20: 1671–1674.
7. Brunner H, Cockcroft JR, Deanfield J, Donald A, Ferrannini E, Halcox J, et al.; Working Group on Endothelins and Endothelial Factors of the European Society of Hypertension. Endothelial function and dysfunction. Part II: Association with cardiovascular risk factors and diseases. A statement by the Working Group on Endothelins and Endothelial Factors of the European Society of Hypertension. *J Hypertens* 2005; 23: 233–246.
8. Perticone F, Ceravolo R, Pujia A, Ventura G, Iacopino S, Scozzafava A, et al. Prognostic significance of endothelial dysfunction in hypertensive patients. *Circulation* 2001; 104: 191–196.
9. Muiesan ML, Salvetti M, Paini A, Monteduro C, Galbanini G, Poisa P, et al. Prognostic role of flow-mediated dilatation on the brachial artery in hypertensive patients. *J Hypertens* 2008; 26: 1612–1618.
10. Modena MG, Bonetti L, Coppi F, Bursi F and Rossi R. Prognostic role of reversible endothelial dysfunction in hypertensive postmenopausal women. *J Am Coll Cardiol* 2002; 40: 505–510.
11. Muiesan ML, Salvetti M, Monteduro C, Rizzoni D, Zulli R, Corbellini C, et al. Effect of treatment on flow-dependent vasodilation of the brachial artery in essential hypertension. *Hypertension* 1999; 33: 575–580.
12. Taddei S, Virdis A, Ghiadoni L, Sudano I and Salvetti A. Effects of antihypertensive drugs on endothelial dysfunction: clinical implications. *Drugs* 2002; 62: 265–284.
13. Ghiadoni L, Magagna A, Versari D, Kardasz I, Huang Y, Taddei S, et al. Different effect of antihypertensive drugs on conduit artery endothelial function. *J Hypertens* 2003; 41: 1281–1286.
14. Mancia G, De Backer G, Dominiczak A, Cifkova R, Fogard R, Germano G, et al. 2007 guidelines for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *J Hypertens* 2007; 25: 1751–1762.
15. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol* 2002; 39: 257–265.
16. Rossi R, Nuzzo A, Origliani G and Modena MG. Prognostic role of flow-mediated dilation and cardiac risk factors in postmenopausal women. *J Am Coll Cardiol* 2008; 51: 997–1002.
17. Osto E, Coppolino G, Volpe M and Cosentino F. Restoring the dysfunctional endothelium. *Curr Pharm Des* 2007; 13: 1053–1068.
18. Landmesser U and Drexler H. Effect of angiotensin II type 1 receptor antagonism on endothelial function: role of bradykinin and nitric oxide. *J Hypertens Suppl* 2006; 24: S39–S43.
19. Savoia C and Schiffrin EL. Inhibition of the renin angiotensin system: implications for the endothelium. *Curr Diab Rep* 2006; 6: 274–278.
20. Neutel JM. Effect of the renin–angiotensin system on the vessel wall: using ACE inhibition to improve endothelial function. *J Hum Hypertens* 2004; 18: 599–606.
21. Park JB and Schiffrin EL. Small artery remodeling is the most prevalent (earliest?) form of target organ damage in mild essential hypertension. *J Hypertens* 2001; 19: 921–930.
22. Rizzoni D, Porteri E, De Ciuceis C, et al. Effect of treatment with candesartan or enalapril on subcutaneous small artery structure in hypertensive patients with noninsulin-dependent diabetes mellitus. *Hypertension* 2005; 45: 659–665.
23. Schiffrin EL. Effects of aldosterone on the vasculature. *Hypertension* 2006; 47: 312–318.
24. Joffe HV and Alder KA. Effect of aldosterone and mineralocorticoid receptor blockade on vascular inflammation. *Heart Fail Rev* 2005; 10: 31–37.
25. Farquharson CA and Struthers AD. Aldosterone induces acute endothelial dysfunction in vivo in humans: evidence for an aldosterone-induced vasculopathy. *Clin Sci* 2002; 103: 425–431.
26. Virdis A, Neves MF, Amir F, Viel E, Touyz RM and Schiffrin EL. Spironolactone improves angiotensin-induced vascular changes and oxidative stress. *Hypertension* 2002; 40: 504–510.

27. Freel EM and Connell JM. Mechanisms of hypertension: the expanding role of aldosterone. *J Am Soc Nephrol* 2004; 15: 1993–2001.
28. Cachofeiro V, Miana M, de Las Heras N, Martın-Fernandez B, Ballesteros S, Fernandez-Tresguerres J, et al. Aldosterone and the vascular system. *J Steroid Biochem Mol Biol* 2008; 109: 331–335.
29. Pitt B, Reichek N, Willenbrock R, Zannad F, Phillips RA, Roniker B, et al. Effects of eplerenone, enalapril, and eplerenone/enalapril in patients with essential hypertension and left ventricular hypertrophy: the 4E-left ventricular hypertrophy study. *Circulation* 2003; 108: 1831–1838.
30. Zannad F, Alla F, Dousset B, Perez A and Pitt B. Limitation of excessive exracellular matrix turnover may contribute to survival benefit of spironolactone therapy in patients with congestive heart failure: insight from the randomized aldactone evaluation study (RALES). *Circulation* 2000; 102: 2700–2706.
31. Modena MG, Aveta P, Menozzi A and Rossi R. Aldosterone inhibition limits collagen synthesis and progressive left ventricular enlargement after anterior myocardial infarction. *Am Heart J* 2001; 141: 41–46.
32. Struthers AD. Aldosterone blockade in heart failure. *J Renin Angiotensin Aldosterone Syst* 2004; 5(Suppl. 1): S23–S27.
33. O'Keefe JH, Abuissa H and Pitt B. Eplerenone improves prognosis in postmyocardial infarction diabetic patients with heart failure: results from EPHESUS. *Diabetes Obes Metab* 2008; 10: 492–497.
34. Boccanelli A, Mureddu GF, Cacciatore G, Clemenza F, Di Lenarda A, Gavazzi A, et al.; AREA IN-CHF Investigators. Anti-remodelling effect of canrenone in patients with mild chronic heart failure (AREA IN-CHF study): final results. *Eur J Heart Fail* 2009; 11: 68–76.
35. Sadee W, Dagcioglu M and Schroder R. Pharmacokinetics of spironolactone, canrenone and canrenoate-K in humans. *J Pharmacol Exp Ther* 1973; 185: 686–695.
36. Serafidis PA and Bakris GL. Resistant hypertension: an overview of evaluation and treatment. *J Am Coll Cardiol* 2008; 52: 1749–1757.