

Research Article

Effects of Candesartan versus Amlodipine on Capillary Rarefaction, Pulse Wave Velocity and Central Blood Pressure in Patients with Essential Hypertension

Rajendra P Raghuraman¹, Christine Carney², Helen Mullahy², Oluwabusola Ogunseitan², Duolao Wang³ and Tarek F Antonios^{1,2*}

¹Department of Clinical Sciences, Vascular Biology Research Centre, Molecular & Clinical Sciences Research Institute, St George's University Hospitals NHS Foundation Trust, London, UK

²Blood Pressure Unit, St George's University Hospitals NHS Foundation Trust, London, UK

³Biosstatistics Unit, Liverpool School of Tropical Medicine, UK

Abstract

Background: A reduction in the density of capillaries (rarefaction) is known to occur in many tissues in patients with essential hypertension and play a role in increasing Blood Pressure (BP). The aim of this trial was to assess in a randomized, double blind, design the effects of treatment of hypertension with candesartan versus amlodipine on microvascular rarefaction and other indices of vascular function.

Methods: We recruited twenty-two individuals with mild-to-moderate hypertension. After a 2-week placebo run-in period, patients who remained hypertensive ($\geq 140/90$ mmHg) were randomized to 8-weeks treatment with Candesartan tablets 8mg daily (with forced titration to 16mg) or Amlodipine tablets 5mg daily (with forced titration to 10mg). The capillary microcirculation was studied using CapiScope system CAM1. Pulse wave velocity, central BP and aortic Augmentation Index were also measured.

Results: We observed significant reductions in brachial BP, and central BP after 4 and 8 weeks treatment with either candesartan or amlodipine but there was no significant effect on basal (functional) or maximal (structural) capillary densities, or pulse wave velocity.

*Corresponding author: Tarek F Antonios, Department of Clinical Sciences, University Hospitals NHS Foundation Trust, London, UK; Blood Pressure Unit, St George's University Hospitals NHS Foundation Trust Vascular Biology Research Centre, Molecular & Clinical Sciences Research Institute, St. George's, University of London, London SW17 0RE, UK, Tel: +44 208725 5627; Fax: +44 208 725 2722; Email: t.antonios@sgul.ac

Citation: Raghuraman RP, Carney C, Mullahy H, Ogunseitan O, Wang D, et al. (2017) Effects of Candesartan versus Amlodipine on Capillary Rarefaction, Pulse Wave Velocity and Central Blood Pressure in Patients with Essential Hypertension. J Non Invasive Vasc Invest 2: 008.

Received: June 29, 2017; **Accepted:** August 03, 2017; **Published:** August 21, 2017

Conclusion: Eight weeks treatment of hypertension with either amlodipine or candesartan significantly reduced brachial and central BP but was not sufficient to induce a regression in functional or structural microvascular abnormalities.

Keywords: Amlodipine; Candesartan; Capillary density; Capillary rarefaction; Hypertension; Microcirculation

List of Abbreviations

AI - Aortic Augmentation Index
ARB - Angiotensin Receptor Blocker
ACEi - Angiotensin Converting Enzyme inhibitor
BCD - Basal Capillary Density
BP - Blood Pressure
CCB - Calcium Channel Blocker
CI - Confidence Intervals
cSBP - Central Aortic Pressure
ECG - Electrocardiogram
MCD - Maximal Capillary Density
PWV - Pulse Wave Velocity
SHR - Spontaneously Hypertensive Rats
SE - Standard Error

Introduction

Increasing evidence suggests a crucial role for the microcirculation in the causation of hypertension and cardiovascular disease [1]. Impaired tissue perfusion secondary to microcirculatory abnormalities is also implicated in the pathogenesis of obesity, diabetes mellitus and insulin resistance [2]. A reduction in the density (rarefaction) of capillaries and arterioles is a consistent finding among the many functional and structural microcirculatory abnormalities that occur in human hypertension [3]. We have previously shown that much of the capillary rarefaction in hypertension is due to the structural (i.e., Anatomic) absence of capillaries [4]. We have also shown significant capillary rarefaction in patients with borderline intermittent hypertension as well as in normotensive offspring of hypertensive parents suggesting that capillary rarefaction may be a primary abnormality that precedes the onset of hypertension [5-7]. Furthermore we have recently demonstrated that alterations in structural capillary density in early pregnancy can accurately and independently predict preeclampsia [8,9]. Therefore the improvement of microvascular abnormalities represents pertinent therapeutic targets [10]. However few observational and open label studies have shown non-consistent results about the reversal of micro vascular rarefaction with antihypertensive treatments [10-13].

The aim of the CAMIRA trial was to assess in a randomized, double blind, placebo controlled two-arm parallel group clinical trial. The effects of treatment of hypertension with the Angiotensin Receptor Blocker (ARB) candesartan versus the dihydropyridine Calcium Channel Blocker (CCB) amlodipine on micro vascular rarefaction and other indices of vascular function in individuals with mild-to-moderate essential hypertension.

Methodology

Participants

The CAMIRA trial (Eudra CT number: 2008-005432-32, IS-RCTN 62554526) was a single center, randomized, double blind, placebo-controlled, parallel-group study performed at St. George's Healthcare NHS Trust hospital (London, UK). The London-Surrey Borders Research Ethics Committee approved the study (number 08/H0806/72). Written informed consent was obtained from all participants. We recruited Caucasian individuals with mild-to-moderate uncomplicated essential hypertension (sitting systolic BP ≥ 140 - <180 mmHg and/or sitting diastolic BP ≥ 90 - <110 mmHg) who have never been previously treated for their high BP or have been off their anti-hypertensive medications for at least 8 weeks. Patients were excluded from the study if they had diabetes mellitus (fasting plasma glucose ≥ 7.0 mmol/L, random plasma glucose ≥ 11.1 mmol/L or HbA1c $>6.5\%$) secondary hypertension (excluded hyperaldosteronism, renal artery stenosis, pheochromocytoma by measuring plasma renin, plasma aldosterone, 24 hour urinary metanephrines, magnetic resonance imaging of renal arteries), chronic kidney or liver disease (normal renal & liver function tests), ischemic heart disease (normal ECG), heart failure (normal echocardiogram), skin diseases, or cold hands (measuring skin temperature).

After a 2-weeks single-blind placebo run-in period, patients who remained hypertensive (systolic BP 140–180 mmHg and/or diastolic BP 90–110 mmHg) were randomized to 8-weeks treatment with either candesartan 8mg orally once daily (with forced titration to 16mg once daily after 2 weeks) or amlodipine 5mg orally once daily (with forced titration to 10mg once daily after 2 weeks). Total study duration was 10 weeks.

Blood pressure measurement

At each visit, subjects were seated with their backs supported for at least 10 minutes in a temperature-controlled room. All measurements were taken throughout the study from the same arm. Three sitting and 2 standing BP measurements were obtained at 2-minute intervals using appropriate cuff size using the Omron HEM705CP (Hutchings Health Care Ltd., Sussex, UK).

Intravital capillary microscopy studies (Capillaroscopy)

Previously validated methods 4-6, were used to assess capillary density in the skin of the dorsum of the fingers [14]. The studies were performed in a temperature-controlled laboratory (21°-24° C) after the study subjects had at least 20-minute rest for acclimatization. Subjects were seated with the left arm and hand supported at heart level. Microscopic images were obtained using the CapiScope system CAM1 and the number of capillaries was counted on-line using computer software (KK Technology, Exeter, UK). Basal Capillary Density (BCD) represents the number of capillaries that are "open" or "functioning" at the time of measurement and was calculated as the mean of 4 microscopic fields.

Maximization of capillaries

A miniature BP cuff was wrapped around the base of the finger and was then inflated to 60mmHg and further images were recorded using one of the above four fields chosen at random. Venous congestion was maintained for 2 minutes [14]. Skin and room temperatures

were monitored at each visit. Capillaroscopy measurements were performed at the end of the placebo-run in visit, after 4-weeks and 8-weeks of active drug treatment.

Carotid-Femoral Pulse Wave Velocity (PWV) measurements

PWV was measured non-invasively using the COMPLIOR device as previously described [15]. Two different pressure wave signals were recorded simultaneously using pressure sensitive transducers placed on the skin at two sites, the right common carotid artery and the right femoral artery. All calculations over 10-12 cardiac cycles were automated.

Aortic Augmentation Index (AI) & central Aortic Pressure (cSBP)

AI and cSBP were measured using the Omron HEM-9000AI device (Omron Healthcare, Kyoto, Japan) [16]. Radial artery tonometry was performed on the left arm of each participant, and BP was measured. All vascular measurements were performed by a single experienced operator (R.R.) in a quiet temperature-controlled laboratory.

Laboratory tests

Blood and urine samples were obtained at entry to the study, end of placebo run-in period and after 4 weeks and 8 weeks of active drug treatment. Variables measured were serum electrolytes, urea, creatinine, uric acid, glucose, total cholesterol, triglycerides and full blood count. 24 hour urinary excretion of sodium, potassium and creatinine were measured as well.

Statistical analysis

The primary outcome variable was the change in maximal (structural) skin capillary density during venous congestion after 8 weeks of active treatment. All other variables recorded were prospectively defined as secondary outcome criteria. The mean, Standard Deviation (SD) and range are reported for all continuous variables, which were normally distributed. For maximal capillary density during venous congestion, an analysis of covariance was performed adjusted on basal capillary density and age. Pair wise comparisons between groups and within groups were performed using this analysis of covariance model, without adjustment for multiple comparisons in this preliminary approach. All other parameters were analyzed similarly. Student's paired and unpaired t- tests were used to evaluate differences between and among groups. For the other criteria, difference between groups was assessed with a two tailed Student's T test for independent samples or with nonparametric test for nonnormal distribution. A type error of 5% was set for all test procedures.

Statistical significance was declared when the p-value was <0.05 . All statistical analysis was carried out using the IBM SPSS 22, USA.

Results

A total of 155 Caucasian subjects were invited to participate in the study and subsequently 114 subjects were screened for eligibility. Of these, 42 individuals did not meet the inclusion criteria (mainly because of normalization of their BP on the follow-up visit), 38 subjects withdrew consent, 8 were found to be receiving antihypertensive medications from their general practitioner and 4 had abnormal laboratory results. Eventually 22 subjects were recruited into the study but

1 subject withdrew consent during the run-in period. Table 1 shows the baseline characteristics of the recruited subjects. The average age of the subjects was 42.4 years (range 26-59 years) and the average BMI was 29.0 (range 20.0-43.2). At entry to the study mean±SD sitting and standing BP were 147/97±14/8mmHg and 147/96±16/8mmHg respectively.

Variable	All (N=21)
Age, years	42.4(9.4)
Sex, M/F	14/7
Weight, Kg	88.0(16.8)
BMI, kg/m ²	29.0(5.2)
Waist circumference, cm	101.4(13.0)
Hip circumference, cm	107.3(11.9)
Blood Pressure, mmHg	
Sitting	147/97(14/8)
Standing	147/96(16/8)
Mean Blood Pressure, mmHg	
Sitting	113.7(8.9)
Standing	110.3(10.0)
Heart rate, beats/m	72.1(10.1)
Smoking history	
Yes	4(19.0%)
No	13(61.9%)
Ex-smoker	4(19.0%)
Family History	
Ischaemic Heart Disease	10(47.6%)
Hypertension	13(61.9%)
Stroke	5(23.8%)
Laboratory results	
Haemoglobin, gm/dL	14.7(1.4)
Serum sodium, mmol/L	139.6(2.0)
Serum potassium, mmol/L	4.3(0.3)
Serum glucose, mmol/L	4.8(0.56)
Total cholesterol, mmol/L	5.1(1.0)
LDL-cholesterol, mmol/L	2.9(0.8)
HDL-cholesterol, mmol/L	1.4(0.3)
24h Urinary sodium, mmol	115(72)
24h Urinary potassium, mmol	53(18)

Table 1: Baseline characteristics of recruited subjects at the start of the placebo run-in period.

At the end of the 2 weeks single blind placebo run-in period mean sitting BP was 144/94±14/9mmHg (p=0.07 and p=0.057 respectively compared to baseline levels). In six subjects the BP dropped significantly on placebo, so much so they did not meet the inclusion criteria and were therefore excluded from the randomized phase. There were no significant changes in other clinical or laboratory parameters. Table 2 shows the baseline characteristics of the randomized subjects. In total 9 subjects were randomized to candesartan and 6 subjects were randomized to amlodipine. Table 3 shows the longitudinal changes in BMI, sitting and standing systolic and diastolic BP, pulse rate, central systolic BP, PWV, basal and maximal capillary densities and aortic augmentation from baseline.

Blood pressure changes

In paired comparisons of sitting systolic BP, there was a significant decrease from the end of the placebo run-in period to 4 weeks active treatment (mean change -19.0 mmHg; 95% CI -11.1 to -26.9, p<0.0001) and to 8 weeks active treatment (mean change -26.3 mmHg; 95% CI -17.5 to -35.0, p<0.0001) (Table 3). Similarly there was a significant decrease in sitting diastolic BP at 4 weeks active treatment (mean change -13.8 mmHg; 95% CI -9.8 to -17.7, p<0.0001) and to 8 weeks active treatment (mean change -16.1 mmHg; 95% CI -13.1 to -19.0, p<0.0001). For standing systolic BP, there was also a significant decrease at 4 weeks active treatment (mean change -21.9 mmHg; 95%

CI -11.1 to -32.7, p=0.001) and to 8 weeks active treatment (mean change -26.5 mmHg; 95% CI -15.8 to -37.3, p<0.0001). Standing diastolic BP decreased significantly after 4 weeks of active treatment (mean change -11.8 mmHg; 95% CI -6.4 to -17.3, p<0.0001) and to 8 weeks active treatment (mean change -16.1 mmHg; 95% CI -11.4 to -20.8, p<0.0001). There were no significant differences between the responses to amlodipine versus candesartan in all BP parameters.

Variable	All (N=15)	Amlodipine Group (N=6)	Candesartan Group (N=9)
Age, years	44.6(8.1)	40.8(7.6)	47.1(7.8)
Male, n (%)	5(33.3)	2(33.3)	3(33.3)
Weight, Kg	90.9(14.4)	88.5(7.9)	92.5(17.8)
BMI, kg/m ²	29.9(4.8)	29.2(2.7)	30.3(5.9)
Waist circumference, cm	101.5(12.3)	95.3(9.9)	105.7(12.4)
Hip circumference, cm	110.7(7.7)	109.0(1.5)	111.8(9.9)
Heart rate, b/m	72.6(8.3)	66.2(6.0)	76.9(6.8)
Smoking n (%)			
Yes	2(13.3)	0(0.0)	2(22.2)
No	11(73.3)	5(83.3)	6(66.7)
Ex-smoker	2(13.3)	1(16.7)	1(11.1)
Family History			
Ischaemic heart disease	7(46.7)	3(50.0)	4(44.4)
Hypertension	10(66.7)	6(100)	4(44.4)
Stroke	4(26.7)	2(33.3)	2(22.2)
Blood pressure, mmHg			
Sitting	149/96(14/7)	144/99(18/7)	150/95(12/8)
Standing	148/98(15/7)	143/99(18/8)	152/96(11/7)
Central blood pressure	144/92(14/10)	139/94(16/14)	148/91(13/6)
Pulse wave velocity, m/sec	9.7(1.2)	9.5(1.3)	9.9(1.2)
Augmentation Index	75.1(10.4)	74.0(5.9)	75.8(12.6)
Basal Capillary Density (per field)	49.6(6.2)	51.7(5.5)	48.1(6.5)
Maximal Capillary density (per field)	53.2(6.7)	53.8(7.8)	52.7(6.2)

Table 2: Baseline characteristics of randomized subjects.

Results are presented as Mean(SD)

Central systolic blood pressure and aortic augmentation changes

cSBP decreased significantly after 4 weeks of treatment (mean change -18.4 mmHg; 95% CI -8.0 to -28.8, p=0.002) and after 8 weeks (mean change -22.6 mmHg; 95% CI -13.6 to -31.6, p<0.0001). AI also decreased significantly after 4 weeks of treatment (mean change -7.6 mmHg; 95% CI -2.2 to -13.0, p=0.010) and after 8 weeks (mean change -9.2 mmHg; 95% CI -4.7 to -13.6, p=0.001). There were no significant differences between the responses to amlodipine versus candesartan.

Pulse wave velocity

The changes in PWV were not significant at any point in the study (Table 3). We could not rule out the possibility that we have not seen significant differences because of the small number of subjects.

Capillary density changes

There were no significant changes in basal (i.e., before venous congestion) capillary density after 4 weeks (mean change -0.4 cap/field; 95% CI -3.5 to 2.6, p=0.76) or after 8 weeks of active treatment (mean change 1.2 cap/field; 95% CI -2.3 to 4.7, p=0.48). Similarly, there were no significant changes in maximal (i.e., with venous congestion) capillary density after 4 weeks (mean change 1.5 cap/field; 95% CI -2.1 to 5.2, p=0.38) or after 8 weeks of active treatment (mean change 1.8 cap/field; 95% CI -1.1 to 1.8, p=0.20).

Variable	All	Candesartan	Amlodipine	Between Groups t test p-value	t-test Overall p-value
	Mean (SE) change	Mean (SE) change	Mean (SE) change		
Sitting Systolic BP (mmHg)					
4 weeks treatment	-19.0(3.7)	-19.4(5.6)	-18.5(4.8)	0.25	<0.0001
8 weeks treatment	-26.3(4.0)	-29.4(5.6)	-21.8(5.4)	0.94	<0.0001
Sitting Diastolic BP (mmHg)					
4 weeks treatment	-13.8(1.8)	-11.8(2.5)	-16.5(2.5)	0.93	<0.0001
8 weeks treatment	-16.1(1.3)	-15.9(1.8)	-16.4(2.3)	0.49	<0.0001
Sitting Mean BP (mmHg)					
4 weeks treatment	-15.0(2.4)	-14.5(3.3)	-15.8(3.8)	0.8	<0.0001
8 weeks treatment	-18.7(2.4)	-20.6(2.7)	-16.1(4.5)	0.39	<0.0001
Standing Systolic BP (mmHg)					
4 weeks treatment	-21.9(5.0)	-24.8(5.3)	-17.4(10.2)	0.63	<0.0001
8 weeks treatment	-26.5(4.8)	-30.1(4.0)	-20.3(11.6)	0.88	<0.0001
Standing Diastolic BP (mmHg)					
4 weeks treatment	-11.8(2.5)	-11.0(3.2)	-13.2(4.4)	0.99	<0.0001
8 weeks treatment	-16.1(2.1)	-16.4(1.8)	-15.5(5.4)	0.24	<0.0001
Basal Capillary Density (capillaries per field)					
4 weeks treatment	-0.4(1.4)	0(1.5)	-1.0(2.8)	0.73	0.76
8 weeks treatment	1.2(1.6)	2.2(2.1)	-0.2(2.7)	0.48	0.48
Maximal Capillary Density (capillaries per field)					
4 weeks treatment	1.5(1.7)	0.4(2.0)	3.0(3.0)	0.48	0.38
8 weeks treatment	1.8(1.3)	0.6(1.6)	3.5(2.3)	0.3	0.2
Pulse Wave Velocity (meters per second)					
4 weeks treatment	-0.1(0.3)	-0.3(0.5)	0.04(0.4)	0.8	0.67
8 weeks treatment	0.1(0.4)	0.5(0.5)	-0.5(0.7)	0.25	0.79
Central Systolic BP (mmHg)					
4 weeks treatment	-18.4(4.8)	-18.5(7.3)	-18.2(5.3)	0.98	0.002
8 weeks treatment	-22.6(4.0)	-24.9(6.0)	-18.8(4.2)	0.5	<0.0001
Augmentation Index (%)					
4 weeks treatment	-7.6(2.5)	-4.3(3.2)	-13.0(2.7)	0.09	0.01
8 weeks treatment	-9.2(2.0)	-8.4(3.1)	-10.5(1.7)	0.64	0.001

Table 3: Changes in variables from end of placebo run-in period to 4 and 8 weeks visit in randomized subjects.

Discussion

This is the first randomized, double blind, placebo controlled, parallelgroup study aimed at comparing the effects of treatment of hypertension with a CCB versus an ARB on skin capillary density in hypertensive patients using well-validated techniques. The study showed that after 4 weeks and 8 weeks treatment with either candesartan or amlodipine, there were significant reductions in both systolic and diastolic brachial BP and central systolic BP but there was no significant affection basal (functional) or maximal (structural) capillary densities. The study also showed that both candesartan and amlodipine significantly reduced Aortic Augmentation Index but had no significant effect on pulse wave velocity.

Preclinical studies have suggested that treatment with antihypertensive medications may reverse or even prevent microvascular rarefaction, although inconsistent results were obtained with different classes of drugs [17]. One study that compared the effects of different antihypertensive treatments on capillary density in Spontaneously Hypertensive Rats (SHR) led to the conclusion that despite their

similar effectiveness in decreasing BP, functional rarefaction was reversed by losartan, nifedipine and enalapril, while structural rarefaction was normalized only by losartan and enalapril while atenolol had

no noticeable effect [18]. It was therefore suggested that dihydropyridine CCB and rennin-angiotensin system blockers were effective in this regard, while data on diuretics and beta-blockers were inconclusive. These selective desirable effects on the microcirculation have been implicated in explaining the beneficial effects of these drugs in reducing micro albuminuria and preserving renal function.

Very little, however, is known about the effects of treatment of human essential hypertension on capillary rarefaction which may be related to end-organ damage, as suggested by the association between hypertensive myocardial disease and reduced myocardial capillary density [19]. Few observational and open label studies have shown inconsistent or even contradictory results about the reversal of microvascular rarefaction with antihypertensive treatments [10,13,20,21]. It was postulated that treatment of hypertension and a reduction in BP

represents a necessary but not a sufficient circumstance for inducing a regression in microvascular abnormalities, because drugs with similar hemodynamic profile may have dissimilar effects on small artery morphology [22]. In a previous open-label pilot study in individuals with treatment-naïve mild-to-moderate essential hypertension, we found that 6-weeks treatment with the ARB irbesartan resulted in significant lowering of both systolic and diastolic BP and significant increase in maximal capillary density (with venous congestion) [23]. In another observational study we reported that capillary density was 25-30% higher in treated compared with untreated hypertensive patients but was significantly lower than in age-matched normotensive controls [11].

Debbabi and colleagues, in an observational study, evaluated skin capillary density in hypertensive patients who were treated with different antihypertensive drugs and who had their BP well controlled below 140/90mmHg for at least 12 months. They found that basal (functional) and maximal (structural) skin capillary densities in treated hypertensive patients were significantly higher when compared to never-treated hypertensive patients or to normotensive subjects. 20 The finding of a higher capillary density in treated hypertensive patients is very curious and rather difficult to construe [21]. In another open-label cross-sectional study the same group reported that only hypertensive patients who were treated and adequately controlled with perindopril/indapamide combination for more than 6 months had significantly higher maximal capillary density than both patients treated and controlled by other drugs, and normotensive individuals [13]. They suggested that the normalization of the skin capillary density could be, at least partially, BP independent as equivalent BP control is not synonymous with equivalent microvascular benefits and suggest different long-term results for end-organ damage. Of interest in their study is that most of the patients in the controlled-other group (48%) were treated with an ARB [13].

Several other studies have shown similar negative effects of ARBs on capillary rarefaction. Kaiser and colleagues in an open-label randomized design studied skin capillary density in 44 subjects with mild to moderate essential hypertension before and after six months treatment with either a beta-blocker (metoprolol) or an ARB (olmesartan) [24]. Interestingly, they found that only treatment with metoprolol resulted in a significant reversal of rarefaction and an increase in capillary density whilst treatment with olmesartan had no significant effect on capillary density. Treatment with valsartan showed conflicting results as one study showing significant increase in capillary density after only 4 weeks while another study showed no significant effect on capillary density [25,26]. These results are interesting in that they contradict what has been suggested that drugs that block the rennin-angiotensin system e.g. ACEi and ARBs may be more efficacious than other drugs such as beta-blockers in reversing the microcirculatory abnormalities in hypertension [27].

On the other hand, several other studies have shown no effect of hypertension treatment on capillary rarefaction. Penna et al., evaluated functional and structural capillary density in treated and well-controlled patients with essential hypertension [12]. They found that treated hypertensive patients had lower mean functional capillary density at baseline, during post-occlusive reactive hyperemia and during venous congestion suggesting that treatment and control of BP, regardless of the type of therapy used, does not necessarily result in reversing capillary rarefaction. De Ciuceis and colleagues, in an open-label design, studied the effects of treatment of hypertension on

structural alterations of retinal arterioles, skin capillary density, arterial dispensability and oxidative stress with a CCB alone and the combination of a CCB with either an ACE inhibitor or a diuretic in patients with mild to moderate essential hypertension [10]. All their patients were treated with lercanidipine for 4 weeks and then either enalapril (n=10) or hydrochlorothiazide (n=10) was added for 6 months. They found no change in basal capillary density at any point in the study. After 4 weeks of treatment with lercanidipine alone, maximal capillary density was slightly but not significantly increased. After 6 months of treatment with the combination of lercanidipine and enalapril, the increase in maximal capillary density was statistically significant. The change in maximal capillary density with the combination of lercanidipine and hydrochlorothiazide was not statistically significant. Similar to our study, the authors found no change in PWV between groups or between any time points despite the fact that central SBP was significantly reduced. More recently, the same group, in another randomized open-label design, studied the effects of one-year treatment of hypertension with either ramipril or aliskiren and found no significant effects on capillary rarefaction [28]. The results from our present study and the aforementioned studies suggest that the reduction and normalization of BP values may not be sufficient for obtaining a regression of microvascular rarefaction in essential hypertension even after one year [29]. These findings also suggest that the improvement of capillary rarefaction and other microcirculatory abnormalities with selective ARBs or ACEi drugs may not represent a class effect but rather drug-specific additional properties of these particular drugs on the microcirculation [13]. Our finding of no significant change in PWV in treated hypertensive patients is not novel, as other groups have found similar results [10,30-32].

We acknowledge that our study has several limitations. Our inability to find a significant effect of both candesartan and amlodipine treatment on capillary rarefaction can be feasibly explained by the small number of subjects who completed the study (type 2 error) or the shorter duration of active treatment with these 2 drugs. Additionally we could not rule out any persisting effects of previous antihypertensive medications our subjects received before enrolling in this study. It is also conceivable that amlodipine and candesartan may genuinely lack substantial microvascular effects as other studies have shown that treatment with amlodipine did not improve coronary flow reserve in hypertensive patients [33,34]. Similarly in the spontaneously hypertensive rat amlodipine and candesartan did not show any significant reverse arteriolar remodeling compared to placebo [35].

In conclusion, this study has shown that 4 weeks and 8 weeks treatment with candesartan or amlodipine resulted in significant reductions of both systolic and diastolic brachial BP and central systolic BP but had no significant effect on basal (functional) or maximal (structural) capillary densities. These results indicate that normalization of BP with amlodipine or candesartan treatment does not necessarily result in reversal of capillary rarefaction in essential hypertension. The study also showed that both candesartan and amlodipine significantly reduced Aortic Augmentation Index but had no significant effect on pulse wave velocity.

Conflicts of Interest and Source of Funding

Takeda, UK funded the study. The authors declare no conflict of interest.

Details of ethics approval: The London-Surrey Borders Research Ethics committee approved this study. (REC: 08/H0806/72)

Trial registration: (EUDRACT: 2008-005432-32, ISRCTN 62554526)

References

1. Gavin JB, Maxwell L, Edgar SG (1998) Microvascular involvement in cardiac pathology. *J Mol Cell Cardiol* 30: 2531-2540.
2. Levy BI, Schiffrin EL, Mourad JJ, Agostini D, Vicaud E, et al. (2008) Impaired tissue perfusion: A pathology common to hypertension, obesity, and diabetes mellitus. *Circulation* 118: 968-976.
3. Boudier HA S, le Noble JL, Messing MW, Huijberts MS, le Noble FA, et al. (1992) The microcirculation and hypertension. *J Hypertens* 10: 147-156.
4. Antonios TFT, Singer DR, Markandu ND, Mortimer PS, MacGregor GA, et al. (1999) Structural skin capillary rarefaction in essential hypertension. *Hypertension* 33: 998-1001.
5. Antonios TFT, Singer DR, Markandu ND, Mortimer PS, MacGregor GA (1999) Rarefaction of skin capillaries in borderline essential hypertension suggests an early structural abnormality. *Hypertension* 34: 655-658.
6. Antonios TF, Rattray FM, Singer DR, Markandu ND, Mortimer PS, et al. (2003) Rarefaction of skin capillaries in normotensive offspring of individuals with essential hypertension. *Heart* 89:175-178.
7. Antonios TF, Raghuraman RP, D'Souza R, Nathan P, Wang D, et al. (2012) Capillary remodeling in infants born to hypertensive pregnancy: Pilot study. *Am J Hypertens* 25: 848-853.
8. Antonios TF, Nama V, Wang D, Manyonda IT (2013) Microvascular remodeling in preeclampsia: Quantifying capillary rarefaction accurately and independently predicts preeclampsia. *Am J Hypertens* 26: 1162-1169.
9. Nama V, Manyonda IT, Onwude J, Antonios TF (2012) Structural capillary rarefaction and the onset of preeclampsia. *Obstet Gynecol* 119: 967-974.
10. De Ciuceis C, Salvetti M, Rossini C, Muiesan ML, Paini A, et al. (2014) Effect of antihypertensive treatment on microvascular structure, central blood pressure and oxidative stress in patients with mild essential hypertension. *J Hypertens* 32: 565-574
11. Antonios TF, Kaski JC, Hasan KM, Brown SJ, Singer DR (2001) Rarefaction of skin capillaries in patients with anginal chest pain and normal coronary arteriograms. *Eur Heart J* 22: 1144-1148.
12. Penna GL, Garbero Rde F, Neves MF, Oigman W, Bottino DA, et al. (2008) Treatment of essential hypertension does not normalize capillary rarefaction. *Clinics (Sao Paulo)* 63: 613-618.
13. Debbabi H, Bonnin P, Levy BI (2010) Effects of blood pressure control with perindopril/indapamide on the microcirculation in hypertensive patients. *Am J Hypertens* 23: 1136-1143.
14. Antonios TFT, Rattray FE, Singer DR, Markandu ND, Mortimer PS, et al. (1999) MacGregor GA. Maximization of skin capillaries during intravital video-microscopy in essential hypertension: Comparison between venous congestion, reactive hyperaemia and core heat load tests. *Clin Sci (Lond)* 97: 523-528.
15. Asmar R, Topouchian J, Pannier B, Benetos A, Safar M, et al. (2001) Pulse wave velocity as endpoint in large-scale intervention trial. The complior study. Scientific, quality control, coordination and investigation committees of the complior study. *J Hypertens* 19: 813-818.
16. Kips JG, Schutte AE, Vermeersch SJ, Huisman HW, Van Rooyen JM, et al. (2011) Comparison of central pressure estimates obtained from sphygmocor, omron hem-9000ai and carotid applanation tonometry. *J Hypertens* 29: 1115-1120.
17. Rizzoni D, Pasini E, Flati V, Rodella LF, Paiardi S, et al. (2008) Angiotensin receptor blockers improve insulin signaling and prevent microvascular rarefaction in the skeletal muscle of spontaneously hypertensive rats. *J Hypertens* 26: 1595-1601.
18. Sabino B, Lessa MA, Nascimento AR, Rodrigues CA, Henriques Md, et al. (2008) Effects of antihypertensive drugs on capillary rarefaction in spontaneously hypertensive rats: Intravital microscopy and histologic analysis. *J Cardiovasc Pharmacol* 51: 402-409.
19. Strauer BE (1990) Significance of coronary circulation in hypertensive heart disease for development and prevention of heart failure. *Am J Cardiol* 65: 34-41.
20. Debbabi H, Uzan L, Mourad JJ, Safar M, Levy BI, et al. (2006) Increased skin capillary density in treated essential hypertensive patients. *Am J Hypertens* 19: 477-483.
21. Antonios TF (2006) Microvascular rarefaction in hypertension--reversal or over-correction by treatment? *Am J Hypertens* 19: 484-485.
22. Agabiti-Rosei E, Heagerty AM, Rizzoni D (2009) Effects of antihypertensive treatment on small artery remodelling. *J Hypertens* 27: 1107-1114.
23. Rattray FE, Coltart RA, Markandu ND, MacGregor GA, Antonios TFT (2004) Effect of an angiotensin ii antagonist; irbesartan on blood pressure and skin capillary density in patients with essential hypertension. *Journal of Hypertens* 22:25.
24. Kaiser SE, Sanjuliani AF, Estado V, Gomes MB, Tibiriçá E (2013) Antihypertensive treatment improves microvascular rarefaction and reactivity in low-risk hypertensive individuals. *Microcirculation* 20: 703-716.
25. Jumar A, Harazny JM, Ott C, Kistner I, Friedrich S, et al. (2016) Improvement in retinal capillary rarefaction after valsartan treatment in hypertensive patients. *J Clin Hypertens (Greenwich)* 18: 1112-1118.
26. van der Zijl NJ, Serné EH, Goossens GH, Moors CC, Ijzerman RG, et al. (2011) Valsartan-induced improvement in insulin sensitivity is not paralleled by changes in microvascular function in individuals with impaired glucose metabolism. *J Hypertens* 29: 1955-1962.
27. Olsen MH, Fossum E, Høiegggen A, Wachtell K, Hjerkin E, et al. (2005) Long-term treatment with losartan versus atenolol improves insulin sensitivity in hypertension: Icarus, a life substudy. *J Hypertens* 23: 891-898.
28. De Ciuceis C, Savoia C, Arrabito E, Porteri E, Mazza M, et al. (2014) Effects of a long-term treatment with aliskiren or ramipril on structural alterations of subcutaneous small-resistance arteries of diabetic hypertensive patients. *Hypertension* 64: 717-724.
29. Schiffrin EL, Deng LY (1996) Structure and function of resistance arteries of hypertensive patients treated with a beta-blocker or a calcium channel antagonist. *J Hypertens* 14: 1247-1255.
30. Rajzer M, Klocek M, Kawecka-Jaszcz K (2003) Effect of amlodipine, quinapril, and losartan on pulse wave velocity and plasma collagen markers in patients with mild-to-moderate arterial hypertension. *Am J Hypertens* 16: 439-444.
31. Mackenzie IS, McEniery CM, Dhakam Z, Brown MJ, Cockcroft JR, et al. (2009) Comparison of the effects of antihypertensive agents on central blood pressure and arterial stiffness in isolated systolic hypertension. *Hypertension* 54: 409-413.
32. Agnoletti D, Zhang Y, Borghi C, Blacher J, Safar ME (2013) Effects of antihypertensive drugs on central blood pressure in humans: a preliminary observation. *Am J Hypertens* 26: 1045-1052.
33. Naya M, Tsukamoto T, Morita K, Katoh C, Furumoto T, et al. (2007) Olmesartan, but not amlodipine, improves endothelium-dependent coronary dilation in hypertensive patients. *J Am Coll Cardiol* 50: 1144-1149.
34. Toyama T, Sato C, Koyama K, Kasama S, Murakami J, et al. (2012) Olmesartan improves coronary flow reserve of hypertensive patients using coronary magnetic resonance imaging compared with amlodipine. *Cardiology* 122: 230-236.
35. Mancini M, Scavone A, Sartorio CL, Baccaro R, Kleinert C, et al (2017) Effect of different drug classes on reverse remodeling of intramural coronary arterioles in the spontaneously hypertensive rat. *Microcirculation* 24.