Original Article

Effect of antihypertensive treatment on microvascular structure, central blood pressure and oxidative stress in patients with mild essential hypertension

Carolina De Ciuceis[a], Massimo Salvetti[a], Claudia Rossini[a], Maria Lorenza Muiesan[a], Anna Paini[a], Sarah Duse[b], Elisa La Boria[a], Francesco Semeraro[b], Anna Cancarini[b], Claudia Agabiti Rosei[a], Annamaria Sarkar[a], Giuseppina Ruggeri[c], Luigi Caimi[c], Doris Ricotta[c], Damiano Rizzoni[a], and Enrico Agabiti Rosei[a]

Background: It has been previously demonstrated that dihydropyridine calcium channel blockers may possess antioxidant properties and might improve vascular structure. Combination treatment with an angiotensin-converting enzyme inhibitor may have additional advantages, compared with a thiazide diuretic, in this regard. The aim of the present study was, therefore, to investigate the effects of a short-term treatment with lercanidipine, and to compare two combination treatments: lercanidipine + enalapril vs. lercanidipine + hydrochlorothiazide on structural alterations in retinal arterioles, on skin capillary density and on large artery distensibility.

Patients and methods: Twenty essential hypertensive patients were included in the study and treated for 4 weeks with lercanidipine 20 mg per day orally. Then they were treated for 6 months with lercanidipine + enalapril ($n = 10$) or lercanidipine + hydrochlorothiazide ($n = 10$) combinations. Investigations were performed in basal condition, after appropriate washout of previous treatments, after 4 weeks of lercanidipine monotherapy treatment, and at the end of the combination treatment. Non-invasive measurements of wall-to-lumen ratio (W/L) and other morphological parameters of retinal arterioles using scanning laser Doppler flowmetry were performed (Heidelberg Retina Flowmeter, Heidelberg Engineering). Capillary density was evaluated by capillaroscopy, whereas pulse wave velocity and central blood pressure were assessed by the Sphygmo-Cor device (AtCor Medical West Ryde, Australia).

Results: A significant improvement of W/L and of other indices of retinal artery structure was observed after treatment with lercanidipine alone, with a further improvement after treatment with lercanidipine + enalapril, whereas after treatment with lercanidipine + hydrochlorothiazide the improvement was no longer observed. A similar behaviour was observed for central SBP and DBP. Capillary density was increased only after treatment with lercanidipine + enalapril.

Conclusion: Lercanidipine both in monotherapy and in combination with enalapril, was able to improve microvascular structure and to decrease central blood pressure, being thus a useful approach for both reducing blood pressure and improving vascular alterations in hypertension.

Keywords: capillary density, central blood pressure, distensibility, hypertension, microcirculation, oxidative stress, remodeling, small artery

Abbreviations: ABPM, 24-h ambulatory blood pressure monitoring; ACE, angiotensin-converting enzyme; AIx, augmentation index; CRP, C-reactive protein; IL-18, interleukin-18; LPO, lipid peroxidation; MCP-1, macrophage chemotactic factor-1; MDA, malonyldialdehyde; PAI-1, plasminogen activator inhibitor-1; PVW, pulse wave velocity; RAS, renin–angiotensin system; sICAM-1, soluble inter-cellular adhesion molecule 1; sVCAM-1, soluble vascular cell adhesion molecule 1

INTRODUCTION

Essential hypertension has been extensively reported to be associated with alterations in the microvascular structure, both in terms of an increased media-to-lumen ratio of small resistance arteries [1–3] and of capillary rarefaction [4–6]. It was also previously demonstrated that alterations in capillary density are correlated with the morphology of small resistance arteries, suggesting that capillary rarefaction and increase in the media-to-lumen ratio of subcutaneous small arteries may occur in parallel [7]. Also, an altered distensibility of large arteries is a common accompaniment of human essential hypertension

Journal of Hypertension 2014, 32:565–574

[a]Clinica Medica, Department of Clinical and Experimental Sciences, [b]Chair of Ophthalmology and [c]Chair of Clinical Biochemistry, University of Brescia, Italy

Correspondence to Professor Damiano Rizzoni, Clinica Medica, Department of Clinical and Experimental Sciences, University of Brescia, c/o 2a Medicina, Spedali Civili, 25100 Brescia, Italy. Tel: +39 030 396044; fax: +39 030 3384348; e-mail: rizzoni@med.unibs.it

Received 16 July 2013 **Revised** 31 October 2013 **Accepted** 31 October 2013

DOI:10.1097/HJH.0000000000000067

[8,9]; recently it has been demonstrated that the presence of structural alterations of small resistance arteries may be associated with the increase in stiffness of large arteries and may possibly contribute to the increase of central pressure by increasing the magnitude of wave reflections [10].

Both an increased media-to-lumen ratio of subcutaneous small resistance arteries [11,12] and an increased pulse wave velocity (PWV) [13] were demonstrated to be potent predictors of cardiovascular events, hence both the improvement of microvascular structural alterations and the reduction of aortic stiffness represent relevant therapeutic targets. Various antihypertensive drug classes may have different effects of structural alterations of small resistance arteries, since both dihydropyridine calcium channel blockers and inhibitors of the renin-angiotensin system (RAS) activity seem to be more effective than diuretics and beta blockers in terms of reduction of media-to-lumen ratio of subcutaneous small arteries [14]. Similarly, an association between a dihydropyridine calcium channel blocker and an angiotensin-converting enzyme (ACE) inhibitor was demonstrated to be more effective than an association between a diuretic and a beta blocker in improving large artery distensibility and reducing central blood pressure, with possible consequent prognostic relevance [15].

Since in essential hypertension, oxidative stress is increased, and this might play a role in the development of micro and macrovascular structural alterations [16], a possible explanation of the particularly pronounced effects of dihydropyridine calcium channel blockers and of ACE inhibitors on micro and macrovascular alterations could be related to their antioxidant effect [17–19].

Whereas alterations in aortic stiffness [9] and changes in microvascular density [4–6] may be evaluated non-invasively, a reliable evaluation of morphological alterations of small resistance arteries in humans, in particular, of the media-to-lumen ratio, requires an invasive approach: in fact, small resistance arteries are dissected from biopsies of subcutaneous tissue, usually from the gluteal region, and directly investigated using micromyographic methods [1–3]. Recently, however, a non-invasive method of evaluation of retinal artery morphology was demonstrated to be equally informative, in comparison with the invasive approach [20].

In the past decade, particular interest was focused on pharmaceutical properties of lercanidipine, a widely used and well tolerated dihydropyridine calcium channel blocker [21] with pronounced antioxidant properties [22,23]. The therapeutic association between lercanidipine and the ACE inhibitor enalapril seems particularly advantageous, especially in comparison with the association of a diuretic, since it might combine and potentiate the beneficial effects on microvascular structure, arterial distensibility, and oxidative stress of a calcium channel blocker and a RAS blocker [24,25].

Therefore, the aim of the present study was to investigate the effects of treatment with the combination of lercanidipine and either an ACE inhibitor (enalapril) or a diuretic (hydrochlorothiazide), after 4 weeks of lercanidipine alone, on structural alterations of retinal arterioles, capillary density, indices of arterial distensibility, and of oxidative stress.

PATIENTS AND METHODS

Twenty patients with mild to moderate hypertension were included in the study. All patients were treated with lercanidipine 20 mg per day for 4 weeks. Then, patients were randomized to receive lercanidipine 20 mg per day + enalapril (up to 20 mg per day) or lercanidipine 20 mg per day + hydrochlorothiazide (up to 25 mg per day) for 6 months. Random allocation to treatment was performed by generating a computerized list. The procedure was performed in an external institution. The dose of enalapril and hydrochlorothiazide was up-titrated if blood pressure was not at target (140/90 mmHg), starting from 10 mg of enalapril/12.5 mg hydrochlorothiazide.

Patients with previous cardiovascular event, clinic or laboratory evidence of heart or renal failure, diabetes mellitus, malignant disease or active inflammation, as well as patients previously treated with statins or acetylsalicylic acid, were excluded from the study. Previous antihypertensive treatment was withdrawn at least 2 weeks before study entry. Clinic blood pressure was measured by standard mercury sphygmomanometer according to European Society of Hypertension–European Society of Cardiology Guidelines [26] and patients were included in the study if their SBP ranged between 140 and 179 mmHg and/or their DBP ranged between 90 and 109 mmHg in sitting position. All patients underwent 24-h ambulatory blood pressure monitoring (ABPM) (Spacelab 90207/90217; Spacelab Medical, Issaquah, Washington, USA). Details about timing of application, intervals between measurements and data processing are provided elsewhere [27].

Venous blood samples were obtained from each patient, after overnight fasting, for standard laboratory tests [total cholesterol, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, triglycerides, and plasma glucose] at baseline and after 1 month of treatment with lercanidipine alone and 6 months of treatment with the drug combination.

The protocol of the study was approved by the Ethics Committee of our institution (Medical School, University of Brescia), and informed consent was obtained from each participant. The procedures followed were in accordance with institutional guidelines.

Evaluation or retinal arteriolar morphology

All patients underwent an evaluation of the retinal arteriolar morphology. Wall-to-lumen ratio of retinal arterioles was assessed using scanning laser Doppler flowmetry (SLDF) at 670 nm (Heidelberg Retina Flowmeter; Heidelberg Engineering, Heidelberg, Germany), an established method to investigate retinal perfusion [20,28,29]. Briefly, an arteriole with a size between 80 and 140 μm of the superficial retinal layer in a retinal sample of $2.56 \times 0.64 \times 0.30$ mm was scanned within 2 s, at a resolution of 256 points × 64 lines × 128 lines. Measurements were performed in the juxtapapillary area of the right eye, 2–3 mm temporal superior to the optic nerve; the mean of three measurements was taken [20,28]. Only arterioles that could unambiguously be discriminated and clearly be identified on the temporal superior side of the optic nerve were

selected. Images of arterioles without sharp contrast to the retina or with crossing and overlapping of venules, curved arterioles, or arterioles with more than one bifurcation on the image and images with more than four eye movements were excluded. The examination was performed without mydriasis, in sitting position after 20 min of rest, at room temperature and daylight conditions between 0800 and 1400 h, but before lunch. Analyses of diameters were performed offline with automatic full-field perfusion imaging analysis program (Nirox Optoelectronics, Brescia, Italy). Outer arteriole diameter was measured in reflection images, and lumen diameter was measured in perfusion images [20,28,29]. Wall-to-lumen ratio was calculated using the formula (AD − LD)/LD [20,28,29].

Evaluation of capillary density

Skin capillary density was assessed by capillaroscopy before and after venous congestion, as described elsewhere [6,30,31]. Briefly, after a period of rest in sitting position in a quiet and temperature-controlled room (21–22°C), capillaries from nail-fold and the dorsum of the fourth finger of the non-dominant hand were visualized by using an epi-illuminated microscope containing a 100 W mercury vapour lamp light source, and pictures (final magnification of 200) were obtained by video-microscopy (Videocap 3.0 D1 200; DS Medica, Milan, Italy) in baseline conditions (baseline capillary density) and after venous congestion (total capillary density), in order to visualize functionally excluded capillaries. Venous congestion was induced by inflating up to 60 mmHg for 2 min a miniature blood pressure cuff applied to the base of the fourth finger of the non-dominant hand [30,31]. Images (final magnification of 200) were also obtained before and after venous congestion at the distal third forearm on the sagittal line by using a traditional pressure cuff. Capillary density was defined as the number of capillaries per square millimeter of the microscopic field and was counted by hand. The first row of the nail-fold capillaries was considered. Capillary density was determined by two independent operators and findings were averaged.

Assessment of aortic distensibility

Pulse wave velocity was measured at the carotid and femoral locations using the foot-to-foot velocity method [9]. Waveforms were obtained transcutaneously over the common carotid artery and the right femoral artery, and the time delay [transit time (t)] was measured between the feet of the two waveforms (Complior). The distance (D) covered by the waves was assimilated to the distance measured between the two recording sites (carotido-femoral distance). PWV was calculated as: PWV = D(m)/t(s); all calculations, including measurement of parameters over 5–10 cardiac cycles, were automated. We used 80% of this distance as pulse wave travelled distance (d) and calculated PWV by the formula [D(m)/t(s)] × 0.80; accordingly an increase of PWV, at least 10 m/s, was considered as macrovascular target organ damage.

In all patients, applanation tonometry was also performed using a Sphygmo-Cor device (AtCor Medical West Ryde, Australia), as described previously [10]. Briefly, the applanation probe was positioned on the radial artery (right arm), and optimal applanation was obtained using visual inspection and following built-in quality control indices. Blood pressure was measured again using an Omron 705 oscillometric device and radial waveforms were calibrated using brachial SBP and DBP measured before and after applanation (average). The central aortic waveform was calculated by the device software using the generalized transfer function [32]. Blood pressure values were derived from the curve. Augmentation index (AIx) and augmentation pressure were derived from this with the technique of pulse wave analysis [33]. The merging point of the incident and the reflected wave (the inflection point) was identified on the generated aortic pressure waveform. Augmentation pressure was the maximum systolic pressure minus pressure at the inflection point; AIx was defined as the augmentation pressure divided by pulse pressure and expressed as a percentage.

Evaluation of circulating inflammatory markers and oxidative stress

Blood samples were collected between 8 and 9 a.m. while participants were in a fasting state. After blood collection, plasma and serum were frozen in aliquots at −80°C immediately after centrifugation (4°C, 3000 r.p.m. for 10 min). Circulating levels of C-reactive protein (CRP; Bender MedSystems, Austria, Europe), pro-inflammatory cytokines interleukin (IL)-6 and IL-18, macrophage chemotactic factor-1 (MCP-1), plasminogen activator inhibitor-1 (PAI-1), soluble vascular cell adhesion molecule 1 (sVCAM-1) and soluble inter-cellular adhesion molecule 1 (sICAM-1) (Bender MedSystems, Austria, Europe) were measured in plasma by ELISA technique following the directions of the supplier company. Total antioxidant power (AOP; Oxford Biomedical Research, Michigan, USA), malonyl-dialdehyde (MDA) and lipid peroxidation (LPO) (Oxis Research, California, USA) were measured in plasma using spectrophotometric assay following the directions of the supplier company. Further details about the methods used are reported in reference [34].

Statistical analysis

The study has an 83% power to detect a 0.15 difference in wall-to-lumen ratio of retinal arterioles, considering a SD in a general population of hypertensive patients of 0.11 [20], with two-sided α-error of 5%. When the sample size calculation was performed as power to detect changes in respect to a basal value, in each of the two groups, there was an 80% power to detect changes in wall-to-lumen ratio of retinal arterioles of 0.10, with a SD in the reference population of 0.11.

All parameters were evaluated at baseline, after 4 weeks of monotherapy with lercanidipine and after 6 months of treatment with drug combinations. Results are expressed as the means ± SD. Comparison of continuous variables in the clinical study was performed by Student's paired or unpaired t test, as appropriate. The statistical significance was set at the conventional level of 5%. All variables investigated were normally distributed.

RESULTS

Demographic, haemodynamic, and humoral characteristics of the patients are summarized in Table 1. For the first 4 weeks, both groups were treated with lercanidipine alone, regardless to randomization to each of the two combination treatments. No baseline difference in age, sex, clinical SBP and DBP, BMI, fasting blood glucose, and humoral indices for cardiovascular risk was detected between patients randomized to either of the two combination treatments. No difference in the smoking habit was present between the two groups and no change in this risk factor was observed during the relatively short treatment period. No difference in the cardiovascular risk profile was observed between the two treatment groups. None of the patients had left-ventricular hypertrophy or microalbuminuria.

No change was observed in BMI and in physical activity during the treatment period.

In patients treated with the combination lercanidipine + hydrochlorothiazide, a significant increase in serum uric acid concentration was observed, compared with baseline and 4 weeks of treatment with lercanidipine alone, whereas in patients treated with the combination lercanidipine + enalapril, a slight decrease in serum uric acid concentration was observed, compared with 4 weeks of treatment with lercanidipine alone.

Clinical SBP and DBP were significantly reduced after 4 weeks of treatment with lercanidipine alone and after 6 months of treatment with the two drug combinations. No statistically significant difference was observed between drugs in the extent of the reduction of blood pressure (Table 1).

Data obtained with ABPM are reported in Table 2, and are consistent with what observed with clinic blood pressure assessment.

Evaluation or retinal arteriolar morphology

No difference in the morphology of retinal arterioles was observed between groups at baseline (Table 3, Fig. 1). After 4 weeks of treatment with lercanidipine alone, wall thickness, wall-to-lumen ratio, and wall cross-sectional area were significantly reduced, compared with baseline, whereas no difference in outer or inner diameters was observed. After 6 months of treatment with the combination lercanidipine + enalapril, a further decrease in wall thickness, wall-to-lumen ratio, and wall cross-sectional area was observed, whereas outer and inner diameter were significantly increased, compared with basal values or lercanidipine alone. On the contrary, in patients randomized to treatment with lercanidipine + hydrochlorothiazide changes of morphological parameters were no longer significant as compared with baseline (a part from a slight increase in inner and outer diameters), suggesting a worsening effect of the combination with the thiazide diuretic and a beneficial effect of the combination with the ACE inhibitor.

Evaluation of capillary density

Total capillary density was slightly, albeit not significantly, increased after 4 weeks of treatment with lercanidipine alone (Fig. 2). After 6 months of treatment with the combination lercanidipine + enalapril, the increase in capillary density compared with baseline became statistically significant (Fig. 1); this was not the case of the combination lercanidipine + hydrochlorothiazide, since no statistically significant difference was observed compared with baseline or lercanidipine alone (Fig. 2).

No change in basal capillary density (evaluated without venous congestion) was observed between groups or between time points (data not shown).

TABLE 1. Demographic data in the different groups

	Group 1 – basal (n = 10)	Group 2 – basal (n = 10)	Group 1 – 4 weeks lercanidipine alone (n = 10)	Group 2 – 4 weeks lercanidipine alone (n = 10)	Group 1 – lercanidipine+ enalapril 24 weeks (n = 10)	Group 2 – lercanidipine+ hydrochlorothiazide 24 weeks (n = 10)
Age (years)	58.1 ± 6.32	49.3 ± 11.76	–	–	–	–
Sex (M/F)	9/1	7/3	–	–	–	–
BMI (kg/m^2)	27.6 ± 3.04	26.5 ± 3.30	27.6 ± 3.04	26.5 ± 3.30	27.6 ± 3.04	26.5 ± 3.30
SBP (mmHg)	153.7 ± 9.43	158.0 ± 8.67	146.5 ± 11.00*	148.9 ± 8.52**	136.0 ± 15.34$^{**,\#}$	133.2 ± 10.05$^{***,\#\#}$
DBP (mmHg)	94.7 ± 7.15	96.6 ± 12.3	92.7 ± 5.38*	92.5 ± 6.04	84.8 ± 8.01$^{**,\#\#}$	82.2 ± 6.46$^{***,\#\#\#}$
Serum glucose (mg/dl)	92.9 ± 10.24	92.8 ± 6.07	82.2 ± 29.28	94.2 ± 8.34	94.4 ± 6.55	96.8 ± 7.97
Serum creatinine (mg/dl)	0.85 ± 0.15	0.92 ± 0.16	0.83 ± 0.17	0.91 ± 0.16	0.82 ± 0.15	0.89 ± 0.17
Triglycerides (mg/dl)	152.6 ± 121.98	142.9 ± 95.25	117.1 ± 49.91	141.0 ± 96.56	130.3 ± 81.47	150.6 ± 93.23
Total cholesterol (mg/dl)	221.5 ± 27.14	204.2 ± 36.05	202.8 ± 27.00	207.5 ± 30.29	204.7 ± 29.74	213.8 ± 35.29
LDL-cholesterol (mg/dl)	134.4 ± 20.85	120.7 ± 27.16	117.2 ± 33.18	120.3 ± 25.71	121.7 ± 27.59	128.0 ± 26.27
HDL-cholesterol (mg/dl)	55.8 ± 18.45	54.4 ± 17.44	58.8 ± 20.21	55.7 ± 16.51	56.9 ± 19.85	55.6 ± 12.99
Serum uric acid (mg/dl)	5.63 ± 1.52	4.76 ± 1.65	6.1 ± 1.16	5.8 ± 1.57	5.7 ± 1.28$^{\#}$	6.3 ± 2.02$^{*,\#}$

HDL, high-density lipoprotein; LDL, low-density lipoprotein.
Group 1: randomized to lercanidipine + enalapril.
Group 2: randomized to lercanidipine + hydrochlorothiazide.
$^{*}P < 0.05$ vs. basal.
$^{**}P < 0.01$ vs. basal.
$^{***}P < 0.001$ vs. basal.
$^{\#}P < 0.05$ vs. lercandipine alone 4 weeks.
$^{\#\#}P < 0.01$ vs. lercandipine alone 4 weeks.
$^{\#\#\#}P < 0.001$ vs. lercandipine alone 4 weeks.

TABLE 2. Twenty-four-hour blood pressure values in the different groups

	Group 1 – basal ($n=10$)	Group 2 – basal ($n=10$)	Group 1 – 4 weeks lercanidipine alone ($n=10$)	Group 2 – 4 weeks lercanidipine alone ($n=10$)	Group 1 – lercanidipine+ enalapril 24 weeks ($n=10$)	Group 2 – lercanidipine+ hydrochlorothiazide 24 weeks ($n=10$)
24-h SBP (mmHg)	139.8 ± 15.07	136.8 ± 8.78	136.7 ± 11.53*	132.7 ± 10.46*	130.2 ± 11.57***	133 ± 12.7*
24-h DBP (mmHg)	88.9 ± 9.71	86.8 ± 8.89	86.4 ± 9.16*	84.5 ± 8.78	81.7 ± 7.70***,#	82.9 ± 9.33*
24-h heart rate (beats/min)	73.2 ± 9.08	73.2 ± 6.66	73.7 ± 9.43	76.5 ± 4.99	73.2 ± 7.89	77.8 ± 6.66*
Daytime SBP (mmHg)	145.1 ± 12.7	141 ± 10.45	140.1 ± 13.49 *	136.5 ± 9.42*	133.2 ± 12.31***,#	136.7 ± 12.51*
Daytime DBP (mmHg)	92.0 ± 10.95	91.0 ± 9.38	90.1 ± 10.15	88.1 ± 7.74 *	84.8 ± 8.87***,#	86.4 ± 9.44*
Daytime heart rate (beats/min)	76.1 ± 9.01	78 ± 7.44	77.6 ± 9.71	80.4 ± 6.69	77.0 ± 8.84	83.1 ± 7.50*
Night-time SBP (mmHg)	134.3 ± 10.48	126.7 ± 8.41	129.6 ± 8.73	124.7 ± 14.17	123.9 ± 12.27 *	123.9 ± 12.75
Night-time DBP (mmHg)	82.9 ± 9.30	76.8 ± 6.88	80.0 ± 6–9	76.7 ± 11.76	74.4 ± 7.11***,##	75.1 ± 10.27
Night-time heart rate (beats/min)	68 ± 10.91	63.7 ± 7.53	65.3 ± 11.42	65.6 ± 5.56	64.4 ± 9.01	66.2 ± 7.21

Group 1: randomized to lercanidipine + enalapril.
Group 2: randomized to lercanidipine + hydrochlorothiazide.
*$P<0.05$ vs. basal.
**$P<0.01$ vs. basal.
***$P<0.001$ vs. basal.
#$P<0.05$ vs. lercanidipine alone 4 weeks.
##$P<0.01$ vs. lercanidipine alone 4 weeks.

Assessment of aortic distensibility

No change in PWV was observed between groups or between time points (Fig. 3). Central SBP was significantly reduced after 4 weeks of treatment with lercanidipine alone (Fig. 3). After 6 months of treatment with the combination lercanidipine + enalapril, a further decrease in central SBP was observed (Fig. 4). On the contrary, after 6 months of treatment with the combination lercanidipine + hydrochlorothiazide, no statistically significant difference in central SBP was observed compared with baseline or lercanidipine alone (Fig. 4). Similarly, central pulse pressure was significantly reduced after 4 weeks of treatment with lercanidipine alone (Fig. 5), and a further decrease was observed after 6 months of treatment with the combination lercanidipine + enalapril (Fig. 5). On the contrary, after 6 months of treatment with the combination lercanidipine + hydrochlorothiazide, the reduction of pulse pressure in respect to baseline, previously observed, was no longer present. No change in central DBP or AIx was observed between groups or between time points (data not shown).

Assessment of systemic oxidative stress and inflammation

A modest reduction in circulating levels of IL-18, CRP and MCP-1 was observed after treatment with lercanidipine alone; however, differences reached statistical significance only in patients who subsequently were randomized to lercanidipine + enalapril. Differences vs. basal values persisted (IL-18, MCP-1) or further improved (CRP) after treatment with lercanidipine + enalapril, but not with lercanidipine + hydrochlorothiazide (Table 4). No change was observed for the remaining markers of inflammation/oxidative stress with any treatment (Table 4).

DISCUSSION

The present study demonstrated that a short-term treatment (4 weeks) with lercanidipine alone induced a reduction in the wall-to-lumen ratio, together with an improvement of other indices of retinal arteriolar structure. There was also a reduction in central blood pressure, with no significant change in total capillary density. After 4 weeks of treatment with lercanidipine alone, the association for 24 weeks with enalapril induced a further improvement of retinal arteriole structure, a further decrease of central systolic and pulse pressure, and a significant increase in total capillary density. On the contrary, the association lercanidipine + hydrochlorothiazide did not induce any significant change in retinal arteriolar structure and in total capillary density, and did not further decrease central blood pressure.

TABLE 3. Morphological data of retinal arterioles in the different groups

	Outer diameter (mm)	Inner diameter (mm)	Wall THICKNESS (mm)	WCSA (mm^2)
Group 1 – basal ($n=10$)	90.7 ± 14.6	61.4 ± 11.6	14.7 ± 2.73	3524 ± 1179
Group 2 – basal ($n=10$)	84.5 ± 15.4	55.5 ± 10.9	16.6 ± 3.37	3304 ± 1264
Group 1 – 4 weeks lercanidipine alone ($n=10$)	84.7 ± 15.5	64.2 ± 8,93	10.2 ± 3.93*	2512 ± 1292*
Group 2– 4 weeks lercanidipine alone ($n=10$)	79.7 ± 14.3	61.6 ± 12.0	9.06 ± 3.69**	2054 ± 1068*
Group 1 – lercanidipine + enalapril 24 weeks ($n=10$)	101.5 ± 21.0#	85.4 ± 21.2**	8.02 ± 3.66**,#,oo	2353 ± 1207 ***,##,oo
Group 2 – lercanidipine+ hydrochlorothiazide 24 weeks ($n=10$)	104.5 ± 18.1#	75.5 ± 16.3*,#	14.5 ± 3.73#	4145 ± 1332

WCSA, wall cross-sectional area.
*$P<0.05$ vs. basal.
**$P<0.01$ vs. basal.
#$P<0.05$ vs. lercanidipine alone 4 weeks.
##$P<0.01$ vs. lercanidipine alone 4 weeks.
oo$P<0.01$ vs. lercanidipine + hydrochlorothiazide.

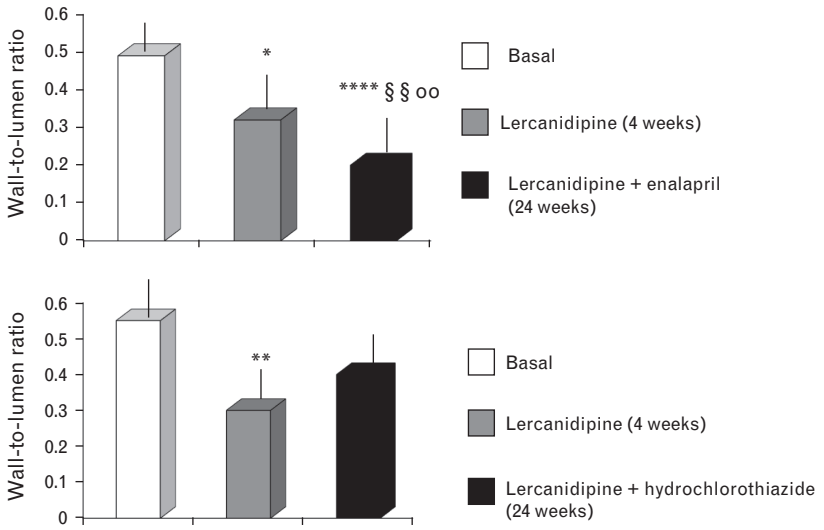

FIGURE 1 Reduction in the wall-to-lumen ratio or retinal arterioles after lercanidipine + enalapril treatment (top), but not after lercanidipine + hydrochlorothiazide treatment (bottom) in hypertensive patients ($n = 10$ per group). (*) $P < 0.05$, (**) $P < 0.01$, (***) $P < 0.001$ vs. basal; (§§) $P < 0.01$ vs. lercanidipine alone 4 weeks, (oo) $P < 0.01$ vs. lercanidipine + hydrochlorothiazide.

Haemodynamic as well as antioxidant properties of drugs might be involved in the protective effects observed on large artery properties and on structural alterations of retinal arterioles and capillaries [22,23,25].

Several classes of antihypertensive drugs have been shown to improve structural alterations in the microcirculation. Dihydropyridine calcium channel blockers and RAS blockers may decrease the media-to-lumen ratio of subcutaneous small resistance arteries [14,35], and ACE inhibitors seem to improve capillary rarefaction [36,37]. Moreover, dihydropyridine calcium channel blockers [17,18,22,23,38] and RAS blockers [19] have favourable effects on oxidative stress and inflammation, thus improving endothelial and vascular function as well as vascular structure in hypertension [39], and this might contribute to their beneficial properties even beyond their antihypertensive effect. On the contrary, thiazide diuretics may increase oxidative stress [40,41]. In addition, they may induce adverse metabolic effects, including an increase in serum uric acid [41], as observed also in the present study. Although modest, an increase in uric acid might have an adverse effect on microvascular structure [42], thus contributing to the differences between treatments observed in the present study.

An additional difference between the two therapeutic approaches was related to changes in central blood

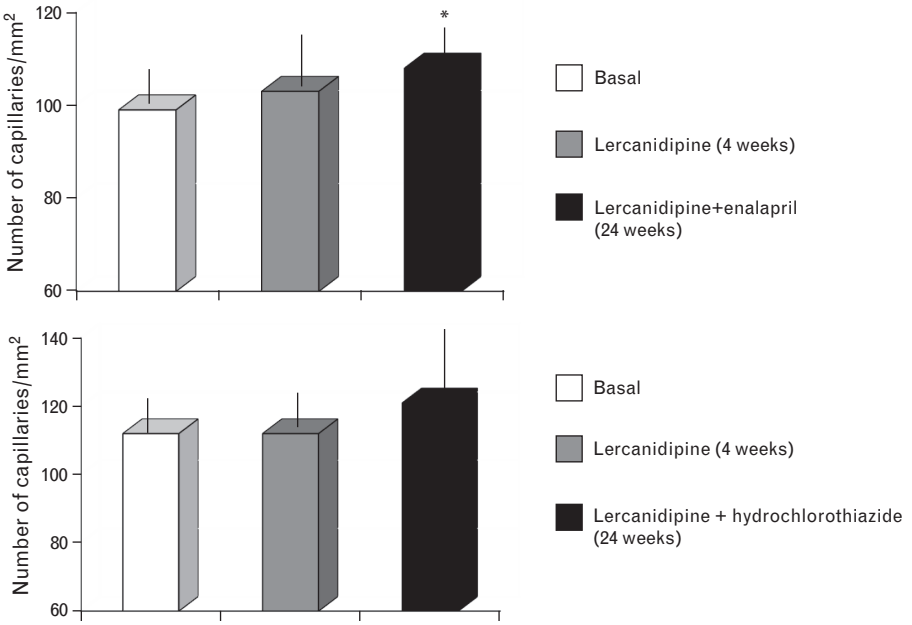

FIGURE 2 Effect of treatment with lercanidipine + enalapril (top) or lercanidipine + hydrochlorothiazide (bottom) on total capillary density ($n = 10$ per group). (*) $P < 0.05$ vs. basal.

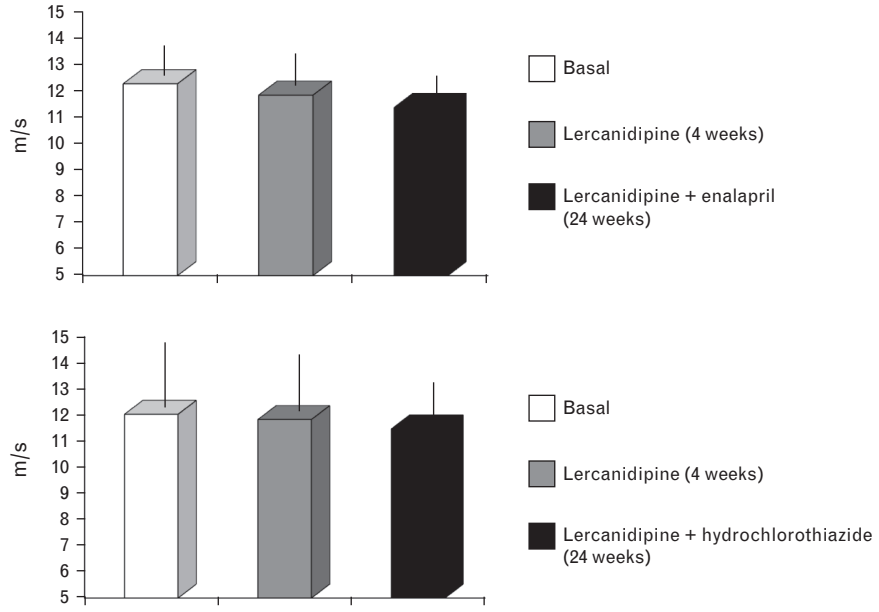

FIGURE 3 Effect of treatment with lercanidipine + enalapril (top) or lercanidipine + hydrochlorothiazide (bottom) on carotid-femoral PWV ($n = 10$ per group). PWV, pulse wave velocity.

pressure, which might reflect, at least in part, changes of large artery distensibility and of peripheral reflection sites in the microcirculation, including small resistance arteries, which increase the magnitude of wave reflections. In fact, recently, a significant relationship between changes in the microvasculature, aortic stiffness and central blood pressure has been demonstrated [10].

Mechanisms involved in vascular stiffening and small artery remodelling include an increased activity of the RAS and the consequent activation of growth factors and extracellular matrix components [43]. Hence, interventions aimed at these targets may improve both arterial stiffness and alterations in the microcirculation, thus reducing central SBP. In this regard, again, calcium channel blockers and RAS blockers seem to have some advantage over diuretics and beta blockers [43,44].

In our study, possible confounders may have been related to a different diet and/or physical activity, although

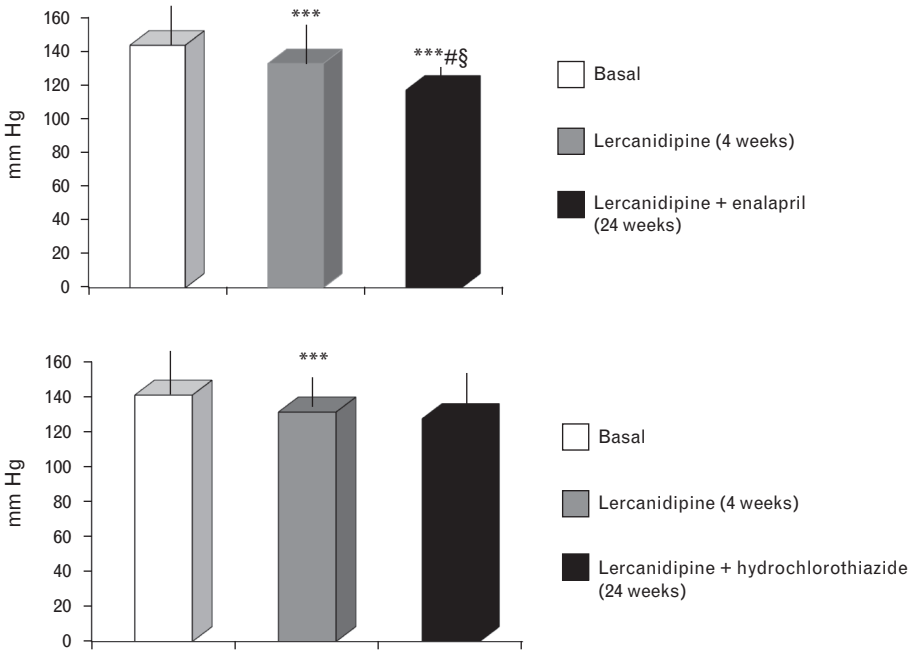

FIGURE 4 Reduction in central SBP after lercanidipine + enalapril treatment (top), but not after lercanidipine + hydrochlorothiazide treatment (bottom) in hypertensive patients ($n = 10$ per group). (***) $P < 0.001$ vs. basal, (#) $P < 0.05$ vs. lercanidipine + hydrochlorotiazide; (§) $P < 0.05$ vs. 4 weeks. ANOVA $P < 0.05$ for interaction between treatments. ANOVA, analysis of variance.

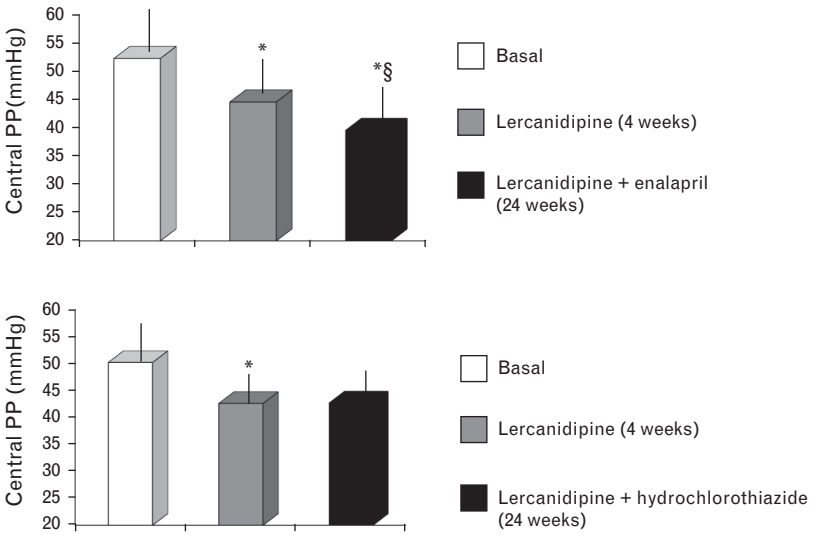

FIGURE 5 Reduction in central pulse pressure after lercanidipine + enalapril treatment (top), but not after lercanidipine + hydrochlorothiazide treatment (bottom) in hypertensive patients ($n = 10$ per group). (*) P at least <0.05 vs. basal, (§) $P < 0.05$ vs. 4 weeks.

no apparent change during treatment was recorded. It is highly improbable that diet changes might have occurred in a different way in the two treatment groups, also because the same advices concerning lifestyle changes were given to all patients.

A partially surprising factor is represented by the observation of a change in wall-to-lumen ratio of retinal arterioles after a short period of treatment with lercanidipine alone of only 4 weeks. Wall-to-lumen ratio of retinal arterioles is evaluated under in-vivo conditions; therefore a certain extent of vasoconstrictor or vasodilator tone is present, whereas under in-vitro micromyographic studies, vessels were evaluated in normalized condition, with a fixed value of transmural pressure [1]. However, in our study, there was no evidence of an increased internal diameter after treatment with lercanidipine alone; therefore we can neither affirm nor completely exclude that at least a portion of the changes observed could be due to the vasodilator effects of the drug.

Finally, persisting effects of previous antihypertensive drugs, which were discontinued for only 2 weeks, cannot be entirely discounted, and may represent a limitation of the study.

In conclusion, this study has confirmed that microvascular and macrovascular alterations may represent potential drug targets in hypertension, since they may be reliably assessed during antihypertensive treatment. In addition, alterations both in the micro and in the macrocirculation are interrelated and may influence each other. Most important, the study for the first time has

TABLE 4. Circulating indices of oxidative stress/inflammation in the different groups

	Group 1 – basal ($n = 10$)	Group 2 – basal ($n = 10$)	Group 1 – 4 weeks lercanidipine alone ($n = 10$)	Group 2 – 4 weeks lercanidipine alone ($n = 10$)	Group 1 – lercanidipine + enalapril 24 weeks ($n = 10$)	Group 2 – lercanidipine + hydrochlorothiazide 24 weeks ($n = 10$)
Total antioxidant power (μmol/l)	0.46 ± 0.091	0.42 ± 0.10	0.41 ± 0.09	0.041 ± 0.08	0.43 ± 0.050	0.41 ± 0.06
LPO (μmol/l)	2.07 ± 0.70	2.44 ± 0.46	2.12 ± 0.73	1.98 ± 0.62	3.26 ± 1.98	2.74 ± 1.86
MDA (mmol/l)	145.15 ± 54.33	108.46 ± 44.3	145.85 ± 103.1	181.26 ± 144.2	246.21 ± 229.5	102.48 ± 34.33
MCP-1 (pg/ml)	1012 ± 110	1078 ± 401	853 ± 182*	1079 ± 41	865 ± 136**,o	1204 ± 461
IL-6 (pg/ml)	11.52 ± 2.48	22.77 ± 10.98	13.01 ± 5.73	10.98 ± 2.57	11.12 ± 1.31	11.36 ± 1.19
IL-18 (pg/ml)	436.4 ± 87.81	402.4 ± 49.00	341.44 ± 106.4*	388.40 ± 62.78	400.8 ± 75.42*	423.24 ± 146.8
sICAM-1 (ng/ml)	244.12 ± 85.94	240.52 ± 58.2	211.47 ± 77.9	231.5 ± 49.4	226.02 ± 79.0	186.3 ± 48.1
sVCAM-1 (ng/ml)	887 ± 235	990 ± 246	994 ± 713	1062 ± 323	860 ± 229	1118 ± 545
TNF alpha (pg/ml)	40.46 ± 3.56	40.2 ± 3.08	40.8 ± 3.05	41.0 ± 4.00	41.6 ± 3.19	44.6 ± 9.10
PAI-1 (ng/ml)	279.37 ± 90.27	294.90 ± 40.01	247.17 ± 86.83	309.89 ± 57.38	272.23 ± 61.70	303.67 ± 54.71
CRP (ng/ml)	1076 ± 755	690 ± 457	767 ± 514*	526 ± 472	456 ± 349*,#	583 ± 543

CRP, C-reactive protein; LPO, lipid peroxidation; MDA, malonyldialdehyde; MCP-1, macrophage chemotactic factor-1; PAI-1, plasminogen activator inhibitor-1; sICAM-1, soluble intercellular adhesion molecule 1; sVCAM-1, soluble vascular cell adhesion molecule 1; TNF, tumour necrosis factor.
Group 1: randomized to lercanidipine + enalapril.
Group 2: randomized to lercanidipine + hydrochlorothiazide (HCT).
*$P < 0.05$ vs. basal.
**$P < 0.01$ vs. basal.
#$P < 0.05$ vs. lercanidipine alone 4 weeks.
o$P < 0.05$ vs. Lerca + HCT.

demonstrated that the calcium channel blocker lercanidipine in combination with the ACE inhibitor enalapril is more effective than the combination lercanidipine + hydrochlorothiazide in improving microvascular structure and decreasing central blood pressure. The regression of structural and functional alterations in both large and small arteries may have a relevant clinical impact.

ACKNOWLEDGEMENTS

The authors thank Michela Faustini for technical support.

The study was partly financed by the European Community, Sixth Framewok program 'InGeniousHyperCare'.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Mulvany MJ, Aalkjaer C. Structure and function of small arteries. *Physiol Rev* 1990; 70:921–971.
2. Schiffrin EL. Remodeling of resistance arteries in essential hypertension and effects of antihypertensive treatment. *Am J Hypertens* 2004; 17 (12 Pt 1):1192–1200.
3. Rizzoni D, Porteri E, Castellano M, Bettoni G, Muiesan ML, Muiesan P, *et al.* Vascular hypertrophy and remodeling in secondary hypertension. *Hypertension* 1996; 28:785–790.
4. Struijker Boudier HAJ. Microcirculation in hypertension. *Eur Heart J* 1999; 1 (Suppl L):L32–L37.
5. Levy BI, Ambrosio G, Pries AR, Struijker-Boudier HAJ. Microcirculation in hypertension. A new target for treatment? *Circulation* 2001; 104: 735–740.
6. Antonios TF, Singer DR, Markandu ND, Mortimer PS, MacGregor GA. Rarefaction of skin capillaries in borderline essential hypertension suggests an early structural abnormality. *Hypertension* 1999; 34 (4 Pt 1):655–658.
7. Paiardi S, Rodella LF, De Ciuceis C, Porteri E, Boari GE, Rezzani R, *et al.* Immunohistochemical evaluation of microvascular rarefaction in hypertensive humans and in spontaneously hypertensive rats. *Clin Hemorheol Microcirc* 2009; 42:259–268.
8. Safar ME, O'Rourke ME. *Handbook of hypertension, volume 23: arterial stiffness in hypertension*. Amsterdam, The Netherlands: Elsevier; 2006. p. 598.
9. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, *et al.* European Network for Noninvasive Investigation of Large Arteries. Expert consensus document on arterial stiffness: methodological issues and clinical applications. *Eur Heart J* 2006; 27:2588–2605.
10. Muiesan ML, Salvetti M, Rizzoni D, Paini A, Agabiti-Rosei C, Aggiusti C, *et al.* Pulsatile hemodynamics and microcirculation: evidence for a close relationship in hypertensive patients. *Hypertension* 2013; 61:130–136.
11. Rizzoni D, Porteri E, Boari GE, De Ciuceis C, Sleiman I, Muiesan ML, *et al.* Prognostic significance of small-artery structure in hypertension. *Circulation* 2003; 108:2230–2235.
12. Heagerty AM. Predicting hypertension complications from small artery structure. *J Hypertens* 2007; 25:939–940.
13. Laurent S, Boutouyrie P, Asmar R, Gautier I, Laloux B, Guize L, *et al.* Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. *Hypertension* 2001; 37:1236–1241.
14. Agabiti-Rosei E, Heagerty AM, Rizzoni D. Effects of antihypertensive treatment on small artery remodelling. *J Hypertens* 2009; 27:1107–1114.
15. Williams B, Lacy PS, Thom SM, Cruickshank K, Stanton A, Collier D, *et al.* CAFE Investigators; Anglo-Scandinavian Cardiac Outcomes Trial Investigators; CAFE Steering Committee and Writing Committee. Differential impact of blood pressure-lowering drugs on central aortic pressure and clinical outcomes: principal results of the Conduit Artery Function Evaluation (CAFE) study. *Circulation* 2006; 113:1213–1225.
16. Touyz RM, Schiffrin EL. Reactive oxygen species and hypertension: a complex association. *Antioxid Redox Signal* 2008; 10: 1041–1044.
17. Ganafa AA, Walton M, Eatman D, Abukhalaf IK, Bayorh MA. Amlodipine attenuates oxidative stress-induced hypertension. *Am J Hypertens* 2004; 17:743–748.
18. Taddei S, Virdis A, Ghiadoni L, Magagna A, Favilla S, Pompella A, Salvetti A. Restoration of nitric oxide availability after calcium antagonist treatment in essential hypertension. *Hypertension* 2001; 37:943–948.
19. Savoia C, Schiffrin EL. Vascular inflammation in hypertension and diabetes: molecular mechanisms and therapeutic interventions. *Clin Sci (Lond)* 2007; 112:375–384.
20. Rizzoni D, Porteri E, Duse S, De Ciuceis C, Rosei CA, La Boria E, *et al.* Relationship between media-to-lumen ratio of subcutaneous small arteries and wall-to-lumen ratio of retinal arterioles evaluated noninvasively by scanning laser Doppler flowmetry. *J Hypertens* 2012; 30:1169–1175.
21. Bang LM, Chapman TM, Goa KL. Lercanidipine: a review of its efficacy in the management of hypertension. *Drugs* 2003; 63:2449–2472.
22. Taddei S, Virdis A, Ghiadoni L, Versari D, Salvetti G, Magagna A, Salvetti A. Calcium antagonist treatment by lercanidipine prevents hyperpolarization in essential hypertension. *Hypertension* 2003; 41:950–955.
23. Incandela L, Belcaro G, Cesarone MR, De Sanctis MT, Griffin M, Cacchio M, *et al.* Oxygen-free radical decrease in hypertensive patients treated with lercanidipine. *Int Angiol* 2001; 20:136–140.
24. Borghi C, Cicero AF. Rationale for the use of a fixed-dose combination in the management of hypertension: efficacy and tolerability of lercanidipine/enalapril. *Clin Drug Investig* 2010; 30:843–854.
25. Tocci G, Palano F, Pagannone E, Chin D, Ferrucci A, Volpe M. Fixed-combination therapies in hypertension management: focus on enalapril/lercanidipine. *Expert Rev Cardiovasc Ther* 2009; 7:115–123.
26. Guidelines Committee. 2003 European Society of Hypertension–European Society of Cardiology guidelines for the management of arterial hypertension. *J Hypertens* 2003; 21:1011–1153.
27. Rizzoni D, Muiesan ML, Montani G, Zulli R, Calebich S, Agabiti-Rosei E. Relationship between initial cardiovascular structural changes and daytime and nighttime blood pressure monitoring. *Am J Hypertens* 1992; 5:180–186.
28. Harazny JM, Ritt M, Baleanu D, Ott C, Heckmann J, Schlaich MP, *et al.* Increased wall:lumen ratio of retinal arterioles in male patients with a history of a cerebrovascular event. *Hypertension* 2007; 50:623–829.
29. Ritt M, Harazny JM, Ott C, Schlaich MP, Schneider MP, Michelson G, Schmieder RE. Analysis of retinal arteriolar structure in never-treated patients with essential hypertension. *J Hypertens* 2008; 26:1427–1434.
30. De Ciuceis C, Rossini C, Porteri E, La Boria E, Corbellini C, Mittempergher F, *et al.* Circulating endothelial progenitor cells, microvascular density and fibrosis in obesity before and after bariatric surgery. *Blood Press* 2013; 22:165–172.
31. Antonios TF, Singer DR, Markandu ND, Mortimer PS, MacGregor GA. Structural skin capillary rarefaction in essential hypertension. *Hypertension* 1999; 33:998–1001.
32. Chen CH, Nevo E, Fetics B, Pak PH, Yin FC, Maughan WL, Kass DA. Estimation of central aortic pressure waveform by mathematical transformation of radial tonometry pressure. Validation of generalized transfer function. *Circulation* 1997; 95:1827–1836.
33. O'Rourke MF, Gallagher DE. Pulse wave analysis. *J Hypertens Suppl* 1996; 14:S147–S157.
34. De Ciuceis C, Porteri E, Rizzoni D, Corbellini C, La Boria E, Boari GE, *et al.* Effects of weight loss on structural and functional alterations of subcutaneous small arteries in obese patients. *Hypertension* 2011; 58:29–36.
35. Schiffrin EL, Deng LY. Structure and function of resistance arteries of hypertensive patients treated with a beta blocker or a calcium channel antagonist. *J Hypertens* 1996; 14:1237–1244; 30.
36. Debbabi H, Uzan L, Mourad JJ, Safar M, Levy BI, Tibiriçà E. Increased skin capillary density in treated essential hypertensive patients. *Am J Hypertens* 2006; 19:477–483.
37. Debbabi H, Bonnin P, Levy BI. Effects of blood pressure control with perindopril/indapamide on the microcirculation in hypertensive patients. *Am J Hypertens* 2010; 23:1136–1143.

38. Berkels R, Egink G, Marsen TA, Bartels H, Roesen R, Klaus W. Nifedipine increases endothelial nitric oxide bioavailability by anti-oxidative mechanisms. *Hypertension* 2001; 37:240–245.
39. Intengan HD, Schiffrin EL. Vascular remodeling in hypertension: roles of apoptosis, inflammation, and fibrosis. *Hypertension* 2001; 38 (3 Pt 2):581–587.
40. Zhou MS, Schulman IH, Jaimes EA, Raij L. Thiazide diuretics, endothelial function, and vascular oxidative stress. *J Hypertens* 2008; 26:494–500.
41. Neutel JM. Metabolic manifestations of low-dose diuretics. *Am J Med* 1996; 101:71S–82S.
42. Feig DI. The role of uric acid in the pathogenesis of hypertension in the young. *J Clin Hypertens (Greenwich)* 2012; 14:346–352.
43. Schiffrin EL. Vascular stiffening and arterial compliance. Implications for systolic blood pressure. *Am J Hypertens* 2004; 17 (12 Pt 2):39S–48S.
44. Safar ME, Rizzoni D, Blacher J, Muiesan ML, Agabiti-Rosei E. Macro and microvasculature in hypertension: therapeutic aspects. *J Hum Hypertens* 2008; 22:590–595.

Reviewers' Summary Evaluations

Reviewer 1

This paper is another peace of evidence of the favorable effects of antihypertensive treatment not only on arterial structure and function but also on peripheral and central blood pressure.

Reviewer 2

Strengths of this manuscripts are that the authors have demonstrated in the same study in essential hypertensive patients that a calcium channel blocker, lercanidipine, both in monotherapy and in combination with the ACE inhibitor enalapril but not with the diuretic hydrochlorothiazide, improved small artery structure using subcutaneous biospies and myography, retinal arteries examined by scanning laser Doppler flowmetry, capillary number by capillaroscopy, and at the same time showed a decrease in central blood pressure and reduction in some inflammatory markers but not in markers of oxidative stress. A surprising finding was the reduction in central systolic pressure in the absence of changes in pulse wave velocity, indicating that these parameters evaluate different vascular changes, since pulse wave velocity is the most accepted measure of large artery stiffness.

A weakness is that all these individual findings have been previously demonstrated, albeit in separate studies.

By studying all these parameters, and although the findings may not be highly original, the results add to our understanding of the effects of antihypertensive agents on the vasculature in human hypertension.

Reviewer 3

Combination therapy of hypertension with an ACE-inhibitor and a calcium-channel blocker offers the potential to lower blood pressure more quickly, obtain target blood pressure, and decrease adverse effects. In this context, De Ciuceis and co-workers compared two combination treatments (lercanidipine + enalapril vs. lercanidipine + hydrochlorothiazide) on structural alterations in retinal arterioles, on skin capillary density and on large artery distensibility. Their analysis suggests that lercanidipine in combination with enalapril is able to improve micro-vascular alterations in hypertension. Further researches are welcome to analyze if these micro-vascular changes may be used during antihypertensive treatment to predict improvement of prognosis.