Effects of a Long-Term Treatment With Aliskiren or Ramipril on Structural Alterations of Subcutaneous Small-Resistance Arteries of Diabetic Hypertensive Patients

Carolina De Ciuceis,* Carmine Savoia,* Emanuele Arrabito, Enzo Porteri, Monica Mazza, Claudia Rossini, Sarah Duse, Francesco Semeraro, Claudia Agabiti Rosei, Alessandro Alonzo, Lidia Sada, Elisa La Boria, Annamaria Sarkar, Beatrice Petroboni, Paolo Mercantini, Massimo Volpe, Damiano Rizzoni, Enrico Agabiti Rosei

Abstract—Structural alterations of subcutaneous small-resistance arteries are associated with a worse clinical prognosis in hypertension and non–insulin-dependent diabetes mellitus. The effects of the direct renin inhibitor aliskiren on microvascular structure were never previously evaluated. Therefore, we investigated the effects of aliskiren in comparison with those of an extensively used angiotensin-converting enzyme inhibitor, ramipril, on peripheral subcutaneous small-resistance artery morphology, retinal arteriolar structure, and capillary density in a population of patients with non–insulin-dependent diabetes mellitus. Sixteen patients with mild essential hypertension and with a previous diagnosis of non–insulin-dependent diabetes mellitus were included in the study. Patients were then randomized to 1 of the 2 active treatments (aliskiren 150 mg once daily, n=9; or ramipril 5 mg once daily, n=7). Each patient underwent a biopsy of the subcutaneous fat from the gluteal region, an evaluation of retinal artery morphology (scanning laser Doppler flowmetry), and capillary density (capillaroscopy), at baseline and after 1 year of treatment. Subcutaneous small arteries were dissected and mounted on a pressurized micromyograph, and the media-to-lumen ratio was evaluated. A similar office blood pressure–lowering effect and a similar reduction of the wall-to-lumen ratio of retinal arterioles were observed with the 2 drugs. Aliskiren significantly reduced media-to-lumen ratio of subcutaneous small-resistance arteries, whereas ramipril–induced reduction of media to lumen ratio was not statistically significant. No relevant effect on capillary density was observed. In conclusion, treatment with aliskiren or ramipril was associated with a correction of microvascular structural alterations in patients with non–insulin-dependent diabetes mellitus. (Hypertension. 2014;64:717-724.) ● Online Data Supplement

Key Words: aliskiren ★ antihypertensive agents ★ capillaries ★ microvessels ★ non–insulin-dependent diabetes mellitus ★ ramipril

During the past 30 years, the in vitro examination of small arteries has been developed, and this has allowed a more comprehensive understanding of vascular remodeling in cardiovascular diseases. In particular, direct investigation of small-resistance arteries harvested from human subcutaneous and omental fat tissue has been possible using wire or pressure myography.1–4 The majority of available data from patients with essential hypertension indicate that the resistance vessels demonstrate an increased media thickness, a slightly reduced lumen diameter, and a decreased external diameter with a subsequent increased media-to-lumen ratio.1–3 However, no significant change in the total amount of tissue within the vascular wall was observed, because the medial cross-sectional area was consistently found to be unchanged.5,6 In essential hypertension, therefore, the observed increase in the media-to-lumen ratio can be ascribed to a process of eutrophic inward remodeling, that is, a rearrangement of the constituents of the vascular wall around a smaller vessel lumen without any cell growth.5 However, in patients with secondary hypertension (renovascular or, to a lesser extent, primary aldosteronism), we observed the development of hypertrophic remodeling, because of an increase in vascular smooth muscle cell volume.6,8 Also, in patients with non–insulin-dependent diabetes mellitus (NIDDM), a clear increase of media cross-sectional area in subcutaneous small-resistance arteries was observed, despite the presence or not of increased blood pressure values,
thus suggesting the development, also in these patients, of medial hypertrophy.9

An increased media-to-lumen ratio of subcutaneous small-resistance arteries is a potent predictor of cardiovascular events in patients with increased blood pressure values,10–12 part of them being also diabetics.10,11 Antihypertensive treatment is able to regress structural alterations in subcutaneous small-resistance arteries of essential hypertensive patients13; however, despite similar blood pressure–lowering effects, angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptors blockers, and calcium channel blockers proved to be more effective than diuretics and β-blockers in terms of reduction in the media-to-lumen ratio.13 The extent of the reduction in the media-to-lumen ratio of subcutaneous small-resistance arteries was recently demonstrated to be an independent predictor of cardiovascular events in essential hypertension.14 However, in patients with diabetes mellitus, it seems more difficult to obtain a complete regression of small-resistance artery structural alterations, despite prolonged treatment.15–18 A possible explanation is related to the presence, in patients with diabetes mellitus, of hypertrophic remodeling of small arteries, which represents a less mechanically efficient mechanism of adaptation to high blood pressure values, possibly bringing also an even worse prognosis.19,20 Aliskiren is the first orally active direct renin inhibitor recently approved for the treatment of hypertension.21,22 Aliskiren’s inhibitory effect on angiotensin I generation, through renin blockade, is highly specific and long-lasting.22 Because compensatory increases in plasma renin levels that lead to adjustments in angiotensin production and conversion represent limitations for existing renin–angiotensin–aldosterone system inhibitors, it was proposed that direct renin inhibitors, such as aliskiren, could provide a more effective blockade of the system, with possible favorable consequence in terms of regression of target organ damage in hypertension.22,23 Preliminary data in animal models suggest that aliskiren may induce an improvement of inward remodeling and endothelial function in mesenteric small arteries24 from hypertensive rats with high plasma renin. For all these reasons, we considered worthwhile to investigate the effects of aliskiren in comparison with those of an extensively used ACE inhibitor, ramipril, on microvascular morphology in a population of patients with NIDDM.

**Patients and Methods**

The study protocol was approved by the Ethics Committee of Sant’Andrea Hospital, Faculty of Medicine and Psychology, University of Rome, and by the Ethics Committee of the University of Brescia Medical School. Each participant provided informed consent. The procedures followed were in accordance with institutional guidelines.

Sixteen patients, aged between 30 and 70 years, with mild essential hypertension (sitting diastolic blood pressure between 90 and 99 mm Hg or sitting systolic blood pressure between 140 and 159 mm Hg at the end of a 3-week placebo run-in period)25 and with a previous diagnosis of NIDDM, with or without ongoing oral hypoglycemic therapy, were enrolled in the study. Nine of them had never received antihypertensive medication. In 7 patients, previous antihypertensive therapy was withdrawn ≤2 weeks before enrollment. Characteristics of previous antihypertensive therapy were similar in the 2 groups (Table 1). Patients previously treated with ACE inhibitors and angiotensin receptor blockers, as well as patients with secondary forms of hypertension or with any disease that could have interfered with the study protocol, were excluded. NIDDM was assessed according to the Guidelines of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus.26 Additional exclusion criteria were a systolic blood pressure >160 mm Hg, a diastolic blood pressure >120 mm Hg, and a creatinine clearance <60 mL/min. End-organ damage was assessed by measuring left ventricular hypertrophy by electrocardiography (Sokolow–Lyon and Cornell criteria) and creatinine clearance by Cockcroft–Gault and Modification of Diet in Renal Disease formula.

Patients were randomly assigned to 1 of the 2 active treatments (aliskiren 150 mg once daily, n=9; or ramipril 5 mg once daily, n=8). After 2 weeks of active treatment, if blood pressure was >130/85 mm Hg, the dose of aliskiren or ramipril was doubled (300 mg once daily and 10 mg once daily, respectively). If blood pressure was still uncontrolled after 6 weeks of active treatment, open-label hydrochlorothiazide 12.5 mg once daily was added. After 10 weeks of treatment, if blood pressure was still uncontrolled, the dosage of diuretic was raised to 25 mg once daily. Patients were then re-evaluated at 6 and 12 months after enrollment. Venous blood samples were obtained with the participants in the supine position, after a wash-out period of ≤2 weeks, for standard hematology and serum biochemistry tests (including triglycerides and total cholesterol), at baseline and 12 months after enrollment. Blood pressure was measured using a standard sphygmomanometer. The study was competitive, randomized, and single-blind.

Micromyography gluteal subcutaneous biopsy (3 cm long, 0.5 cm wide, 1.5 cm deep) was obtained under local anesthesia (2% lidocaine) at baseline and at the end of the study (12 months).23,5,13,16,18 Small arteries (≈100–280 μm of average diameter in relaxed conditions; 2 mm long) were dissected from the subcutaneous fat of the biopsy samples and mounted on a pressurized myograph immediately after biopsy. Experiments were performed as described previously,15,18 In brief, total time for dissection and preparation was ≤45 minutes. Vessels were equilibrated and relaxed for ≤30 minutes in physiological saline solution. The following structural parameters were measured: wall thickness, media thickness, intima thickness, internal diameter, media/lumen ratio, and media cross-sectional area. The average values obtained from 2 vessels in each experiment were considered.

**Evaluation of Retinal Arteriolar Morphology**

All patients underwent an evaluation of the retinal arteriolar morphology at baseline and at the end of the study (12 months). Wall-to-lumen ratio of retinal arterioles was assessed using scanning laser Doppler flowmetry at 670 nm (Heidelberg Retina Flowmeter; Heidelberg Engineering, Heidelberg, Germany), an established method to investigate retinal perfusion.27–29 Details about this method are reported in the online-only Data Supplement.

**Evaluation of Capillary Density**

Skin capillary density was assessed by capillaroscopy before and after venous congestion, as described elsewhere.30–32 Details about this method are reported in the online-only Data Supplement.

**Evaluation of Mechanical Properties and Vascular Collagen Content of Subcutaneous Small Arteries**

Collagen content within the vascular wall was assessed by confocal microscopy, whereas mechanical properties (stiffness, distensibility) were evaluated as stress–strain relationships as previously reported.13,33 The methods used are reported in the online-only Data Supplement (expanded methods).

**Statistical Analysis**

All data are expressed as mean±SD, unless otherwise stated. Student paired and unpaired t tests were used to evaluate differences between and among groups. The study had a 82% power to detect a difference of 0.01 in the media–to–lumen ratio of subcutaneous small arteries within groups and had a 70% power to detect a between-groups difference of 0.02 at 5% of significance level.
### Table 1. Demographic and Hemodynamic Data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Aliskiren (n=9)</th>
<th>Ramipril (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>54±6</td>
<td>60±11</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>8/1</td>
<td>5/2</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>102.6±15.4</td>
<td>95.9±16.2</td>
</tr>
<tr>
<td>After 12 mo</td>
<td>99.8±14.5*</td>
<td>94.1±14.0</td>
</tr>
<tr>
<td>Height, cm</td>
<td>171±5</td>
<td>171±8</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>34.8±3.84</td>
<td>32.8±4.61</td>
</tr>
<tr>
<td>Known duration of diabetes mellitus, y</td>
<td>5.3±8.4</td>
<td>4.3±5.1</td>
</tr>
<tr>
<td>Known duration of hypertension, y</td>
<td>2.2±0.5</td>
<td>3.25±2.5</td>
</tr>
<tr>
<td>No. of patients who never received antihypertensive treatment (before randomization)</td>
<td>6 (67%)</td>
<td>3 (43%)</td>
</tr>
<tr>
<td>No. of antihypertensive medication per patient</td>
<td>1,0</td>
<td>0.6</td>
</tr>
<tr>
<td>No. of patients on CCBs</td>
<td>2 (22%)</td>
<td>1 (14%)</td>
</tr>
<tr>
<td>No. of patients on β-blockers</td>
<td>0</td>
<td>2 (29%)</td>
</tr>
<tr>
<td>No. of patients on α-blockers</td>
<td>2 (22%)</td>
<td>1 (14%)</td>
</tr>
<tr>
<td>No of patients on diuretics</td>
<td>1 (11%)</td>
<td>3 (43%)</td>
</tr>
<tr>
<td>Number of patients on oral hypoglycemic therapy</td>
<td>8/9 (89%)</td>
<td>6/7 (86%)</td>
</tr>
<tr>
<td>Number of patients on lipid-lowering agents</td>
<td>5/9 (56%)</td>
<td>3/7 (43%)</td>
</tr>
<tr>
<td>Fasting glucose, mg/dL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>138.9±25.4</td>
<td>137.6±16.9</td>
</tr>
<tr>
<td>After 12 mo of treatment</td>
<td>135.9±49.8</td>
<td>142.3±33.7</td>
</tr>
<tr>
<td>Hemoglobin A1c, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>7.4±1.2</td>
<td>7.9±1.2</td>
</tr>
<tr>
<td>After 12 mo of treatment</td>
<td>7.2±1.6</td>
<td>6.9±0.6</td>
</tr>
<tr>
<td>Serum creatinine, mg/dL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>0.77±0.13</td>
<td>0.78±0.08</td>
</tr>
<tr>
<td>After 12 mo of treatment</td>
<td>0.80±0.13</td>
<td>0.82±0.13</td>
</tr>
<tr>
<td>Creatinine clearance, Cockcroft–Gault/MDRD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>166/111</td>
<td>135/101</td>
</tr>
<tr>
<td>After 12 mo of treatment</td>
<td>156/105</td>
<td>129/97</td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>202±52.7</td>
<td>207±34.1</td>
</tr>
<tr>
<td>After 12 mo of treatment</td>
<td>181±44.4</td>
<td>212±21.7</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>166±75.8</td>
<td>191±31.8</td>
</tr>
<tr>
<td>After 12 mo of treatment</td>
<td>162±50.6</td>
<td>184±84.0</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>153±8.9</td>
<td>151±10.6</td>
</tr>
<tr>
<td>After 6 mo of treatment</td>
<td>126±7.4 †</td>
<td>125±5.0 †</td>
</tr>
<tr>
<td>After 12 mo of treatment</td>
<td>128±7.3 †</td>
<td>121±12.1 †</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>94.2±7.17</td>
<td>84.7±12.22</td>
</tr>
<tr>
<td>After 6 mo of treatment</td>
<td>82.2±5.56‡</td>
<td>78.9±3.98</td>
</tr>
<tr>
<td>After 12 mo of treatment</td>
<td>81.4±6.31‡</td>
<td>78.6±7.48</td>
</tr>
<tr>
<td>Mean blood pressure, mm Hg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>114±6.39</td>
<td>107±10.41</td>
</tr>
<tr>
<td>After 6 mo of treatment</td>
<td>96.7±5.51 †</td>
<td>94.1±3.77*</td>
</tr>
<tr>
<td>After 12 mo of treatment</td>
<td>97.0±5.68 †</td>
<td>92.6±8.86*</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD. CCB indicates calcium channel blocker; and MDRD, Modification of Diet in Renal Disease.

*P<0.05, †P<0.001, ‡P<0.01 vs basal.
Results

Demographic Data

Sixteen hypertensive patients with NIDDM were randomized, 9 to treatment based on aliskiren and 7 to treatment based on ramipril. In 5 patients in the aliskiren group and in 5 in the ramipril group, hydrochlorothiazide was added (12.5 or 25 mg) to further reduce blood pressure.

The 2 groups were well balanced at baseline (Table 1). Known duration of previous antihypertensive treatment was similar in the 2 groups. Average body mass index was in the grade 1 obesity. Fasting glucose or glicosilate hemoglobin were above the threshold of optimal metabolic control despite an adequate oral antidiabetic therapy, and no significant changes were observed during the treatment period. Systolic blood pressure was significantly and equally reduced by both treatments, whereas diastolic blood pressure was significantly reduced only in patients receiving aliskiren (Table 1); 89% of patients in the aliskiren group and 86% in the ramipril group had a final blood pressure <140/90 mm Hg, whereas 67% and 86%, respectively, had a final blood pressure of <130/85 mm Hg. No signs of renal impairment were present in any patient (Table 1). No left ventricular hypertrophy was detected by using electrocardiographic criteria of Sokolow-Lion or Cornell at baseline or after treatment for 1 year with aliskiren or ramipril.

Morphology of Subcutaneous Small Arteries

Media-to-lumen ratio was significantly reduced by aliskiren treatment (Table 2, Figure 1), whereas ramipril-induced reduction in the media-to-lumen ratio did not reach statistical significance (Table 2, Figure 1). Neither aliskiren nor ramipril had any effect on internal diameter (Table 2).

Evaluation or Retinal Arteriolar Morphology

The wall-to-lumen ratio of retinal arterioles was significantly reduced by both aliskiren and ramipril (Table 2). Internal diameter was significantly increased in patients treated with ramipril. The increase in internal diameter did not reach statistical significance in patients treated with aliskiren (Table 2). No other statistically significant differences were observed in other morphological parameters. Representative images of retinal arterioles for each group before and after treatment are reported in Figure 2.

Evaluation of Capillary Density

Basal and total capillary density in different districts (nailfold, dorsum of the fourth finger and forearm) at baseline and at the end of the study is reported in Table 3. No differences between groups were observed at baseline for basal or total capillary density. No difference versus baseline values was observed in either group after 1 year of treatment.

Evaluation of Mechanical Properties and Vascular Collagen Content of Subcutaneous Small Arteries

No significant differences were observed in stress–strain relationship before and after treatment with either aliskiren or ramipril (see the online-only Data Supplement).

Collagen content in the tunica media of subcutaneous small-resistance arteries was similar in both groups of treatment and before and after treatment (see the online-only Data Supplement).

Discussion

This is the first prospective study aimed to compare the effect of 1 year treatment with a direct renin inhibitor and an ACE inhibitor on microvascular structure in hypertensive patients with NIDDM using reliable and well-assessed techniques. The main result of our study is that aliskiren and ramipril proved to be equally effective in correcting the arteriolar remodeling particularly in the retina, although aliskiren might have some modest advantages compared with ramipril in terms of reduction in media-to-lumen ratio of subcutaneous small-resistance arteries. No difference was observed between drugs or time points in internal diameter as well as in wall or media thickness of subcutaneous small arteries. However, the only structural parameter that is independent from the vessel dimensions, and therefore free from possible sampling bias, is the media-to-lumen ratio as previously demonstrated.5
Aliskiren or Ramipril and Small Artery Structure

De Ciucel et al

Alterations of vascular structure in peripheral microvessels, particularly the increased media-to-lumen ratio, seem related to an impaired coronary vasodilator capacity in patients with mild to moderate hypertension. Structural alterations in the subcutaneous vascular district may, therefore, be representative of similar alterations in coronary microcirculation, contributing to a reduced coronary flow reserve. Importantly, media-to-lumen ratio of subcutaneous small-resistance arteries has been demonstrated as an independent predictor of major cardiovascular events in different populations of hypertensive patients, including also patients with diabetes mellitus. In particular, the remodeling of resistance arteries, particularly the hypertrophic remodeling, characterized by increased media-to-lumen ratio and cross-sectional area, is commonly observed in patients with diabetes mellitus and is thought to be associated with poor prognosis.

As previously mentioned, it is difficult to obtain a complete regression of small-resistance artery structural alterations in patients with NIDDM, despite the use of combination therapy and consistent blood pressure reductions. It was proposed that reduction of blood pressure values represents a necessary but not sufficient condition for obtaining a regression of microvascular alterations, because drugs with similar hemodynamic effects have disparate effects on small artery morphology. Even the presence of a dissociation of blood pressure and resistance artery structure was postulated, with potential clinical implications. In this study, the hemodynamic effects of aliskiren and ramipril were similar, and both treatments induced similar pressure control in hypertensive and diabetic patients.

Although changes in diastolic blood pressure were not statistically significant in the ramipril group, values observed during treatment were similar in the 2 groups (mean difference 3 mmHg). In this regard, it was proposed that vasodilation, not hypotension, improves resistance vessel design during treatment of essential hypertension; therefore, as previously mentioned, the extent of blood pressure reduction during treatment may have a limited role.

Target blood pressure values in our study were established according to guidelines available when the study was planned (<130/85 mmHg). More recently, it was acknowledged that the scientific evidence supporting a reduction of blood pressure values <130/85 mmHg in patients with NIDDM is rather weak, and therefore the general advice is to reduce blood pressure <140/85 mmHg.

The reduction in the media-to-lumen ratio of subcutaneous small-resistance arteries in hypertensive and diabetic patients after 1 year of treatment with aliskiren and ramipril suggests that a correction of microvascular remodeling may occur during therapy with renin–angiotensin system blockers, particularly with aliskiren. In both normotensive and hypertensive patients with NIDDM, microvascular remodeling is characterized by hypertrophy of the tunica media, although no statistically significant reduction of media cross-sectional area was observed in our patients after treatment with both drugs. This can be explained in part by the fact that plasma glucose levels of the population enrolled in our study remained above the threshold of optimal metabolic control despite an adequate oral antidiabetic therapy. Metabolic control is an important factor in modulating microvascular remodeling in patients with NIDDM. It has been reported that in patients with diabetes mellitus with poor metabolic control, small peripheral resistance arteries presented hypertrophic growth in response to elevated blood pressure, whereas metabolic improvements enabled eutrophic remodeling to occur in response to an increase in blood pressure. In any case, a full regression of eutrophic remodeling is rarely seen in patients with diabetes mellitus, despite treatment with effective drugs, such as ACE inhibitors or angiotensin receptor blockers, is able to induce a reduction of the media-to-lumen ratio of subcutaneous small arteries; therefore, in our study, we did not expect to see major changes in media cross-sectional area values.

Mechanisms involved in the observed improvement in microvascular remodeling could be ascribed to the blockade of the deleterious effects of the renin–angiotensin–aldosterone system, including a proinflammatory action, or to the restoration of myogenic response whose impairment is possibly involved in the development of hypertrophic remodeling. Recently, it has been shown that aliskiren induced favorable
effects similar to that induced by ACE inhibition in improving vascular remodeling in hypertensive rats with high plasma renin levels, independently of blood pressure control. This was associated with the reduction of reactive oxygen species production and the improvement of nitric oxide bioavailability, both of which contribute to the improvement of vascular inflammation and remodeling.24

Intervention studies with specific drugs have demonstrated an improvement or even an almost complete normalization of the structure of subcutaneous small-resistance arteries with drugs that block the renin–angiotensin system, namely ACE inhibitors (cilazapril, perindopril, lisinopril) or angiotensin II receptor blockers (losartan, irbesartan, candesartan, valsartan).13,35 None of the studies that used a micromyographic approach specifically evaluated the effects of ramipril. We decided to use ramipril as a competitor for aliskiren for 2 main reasons: ramipril is the most widely used ACE inhibitor in our country, and it was previously demonstrated to be highly effective in terms of cardiovascular protection, both in hypertensive patients42 and in patients with NIDDM.43

It is not clear whether a reduction in blood pressure is a main determinant of the effects on microvascular structure, because a large amount of data have shown that drugs with similar blood pressure–lowering effects have disparate effects on vascular structure.13

Our study provides evidence that aliskiren might be at least as protective as ramipril in reducing subcutaneous structural alterations and particularly the media-to-lumen ratio, which is known to be inversely related to coronary flow reserve34 and to predict future cardiovascular events.10 Indeed, because of its peculiar mechanisms of action, aliskiren may provide a more effective blockade of the renin–angiotensin system, with possible greater effects on target-organ damage in hypertension.22,23

Scientific interest focused of the therapeutic potential of direct renin inhibitors has dramatically increased. It was initially suggested that a combined treatment with aliskiren and an ACE inhibitor, or an angiotensin receptor blocker, could offer major benefits in patients with elevated cardiovascular risk. However, recently, concerns have raised about the clinical use of aliskiren in patients with NIDDM, especially when associated with ACE inhibitors, by the results of at least a couple of studies,44 suggesting that the addition of aliskiren to standard therapy with renin–angiotensin system blockers might be even harmful.46 Also in nondiabetic patients, there are concerns about the association of aliskiren with ACE inhibitors or angiotensin receptor blockers.44 In most of these studies, a greater incidence of side effects, such as renal failure, hyperkalaemia, and hypotension, was observed. For all these reasons, the association with aliskiren and a blocker of the renin–angiotensin system is presently contraindicated, especially in patients with diabetes mellitus.47 The ongoing Aliskiren Trial to Minimize Outcomes in Patients with Heart Failure48 is comparing separately the effects of aliskiren, ramipril, and their combination in patients with heart failure, and the results are expected in 2014.

**Study Limitations**

Because of the relatively small number of patients enrolled in the study or because of large measurement variability, type 2 errors have occurred in our study, in particular as the absence of statistical significance of differences in media-to-lumen ratio of subcutaneous small arteries or diastolic blood pressure between basal and treatment values in the ramipril group is concerned. This could also be true for differences in capillary density, which is a partially surprising finding, because previous studies in hypertensive patients have demonstrated beneficial effects of blockers of the renin–angiotensin system.49,50 It is, however, possible that patients with diabetes mellitus could be, in some instance, resistant to the drug effects in terms of inhibition of microvascular growth.

In the present study, we have seen modest differences in the effects of the 2 drugs investigated in 2 vascular districts, they being equally effective in the retina, whereas the absolute change of media-to-lumen ratio of subcutaneous small-resistance arteries was −9% with aliskiren and −4.8% with ramipril. Although the small number of patients evaluated could have affected the results obtained, previous evidence suggests that structural alterations in small-resistance arteries occur simultaneously in several vascular districts33,34; therefore, their regression should theoretically be similar in different vascular beds.

**Perspectives**

Treatment with aliskiren or ramipril is associated with a correction of microvascular structural alterations in patients with NIDDM. Aliskiren seems to have some advantages compared with ramipril in terms of reduction of the media-to-lumen ratio of subcutaneous small-resistance arteries. This finding may have important clinical consequences, because it was recently demonstrated a prognostic role of changes of microvascular structure, as evaluated by the media-to-lumen ratio of subcutaneous small-resistance arteries during antihypertensive treatment,14 independently of the

**Table 3. Data of Skin Capillary Density at the Level of Nailfold, Dorsum of the Fourth Finger and Forearm**

<table>
<thead>
<tr>
<th>Group of Patients</th>
<th>Nailfold BCD</th>
<th>Nailfold TCD</th>
<th>Dorsum BCD</th>
<th>Dorsum TCD</th>
<th>Forearm BCD</th>
<th>Forearm TCD</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients basal (n=10)</td>
<td>7.2±1.3</td>
<td>7.4±1.4</td>
<td>80.8±21.4</td>
<td>98.6±13.9</td>
<td>78.4±20.6</td>
<td>101.9±22.2</td>
</tr>
<tr>
<td>All patients 1 y (n=10)</td>
<td>8.0±1.8</td>
<td>8±1.8</td>
<td>91.8±20.3</td>
<td>106.5±20.27</td>
<td>83.3±8.6</td>
<td>97±11.6</td>
</tr>
<tr>
<td>Ramipril basal (n=5)</td>
<td>7.6±1.5</td>
<td>7.8±1.6</td>
<td>87.8±20.0</td>
<td>135.0±13.8</td>
<td>79.2±28.2</td>
<td>102.8±20.9</td>
</tr>
<tr>
<td>Aliskiren basal (n=5)</td>
<td>6.8±1.1</td>
<td>7.0±1.2</td>
<td>73.8±22.5</td>
<td>96.8±15.6</td>
<td>77.6±12.6</td>
<td>101.0±26.0</td>
</tr>
<tr>
<td>Ramipril 1 y (n=5)</td>
<td>8.6±1.9</td>
<td>8.6±1.9</td>
<td>95.8±16.63</td>
<td>107.0±12.4</td>
<td>85.6±11.3</td>
<td>97.0±11.9</td>
</tr>
<tr>
<td>Aliskiren 1 y (n=5)</td>
<td>7.0±1.2</td>
<td>7.0±1.2</td>
<td>87.8±24.6</td>
<td>106.0±27.8</td>
<td>81.0±5.0</td>
<td>97.0±12.7</td>
</tr>
</tbody>
</table>

Results are expressed as mean±SD. BCD indicates basal capillary density; and TCD, total capillary density.
extent of blood pressure reduction, substantially supporting the idea that microvascular structure might be considered an intermediate end point in the evaluation of the benefits of antihypertensive treatment.

Disclosures

None.

References

What Is New?

- This is the first prospective study aimed to compare the effect of 1-year treatment with a direct renin inhibitor and an angiotensin-converting enzyme inhibitor on microvascular structure in hypertensive patients with non–insulin-dependent diabetes mellitus using reliable and well-assessed techniques. The study demonstrated that aliskiren seems to have some advantages compared with ramipril in terms of reduction of the media-to-lumen ratio of subcutaneous small-resistance arteries.

What Is Relevant?

- Our findings may have important clinical consequences, because it was recently demonstrated a prognostic role of changes of microvascular structure, as evaluated by the media-to-lumen ratio of subcutaneous small-resistance arteries during antihypertensive treatment, independently of the extent of blood pressure reduction, substantially supporting the idea that microvascular structure might be considered an intermediate end point in the evaluation of the benefits of antihypertensive treatment.

Summary

Treatment with aliskiren or ramipril was associated with a correction of microvascular structural alterations in patients with non–insulin-dependent diabetes mellitus. Aliskiren seems to have some advantages compared with ramipril in terms of reduction of the media-to-lumen ratio of subcutaneous small-resistance arteries.
Effects of a Long-Term Treatment With Aliskiren or Ramipril on Structural Alterations of Subcutaneous Small-Resistance Arteries of Diabetic Hypertensive Patients

Carolina De Ciuceis, Carmine Savoia, Emanuele Arrabito, Enzo Porteri, Monica Mazza, Claudia Rossini, Sarah Duse, Francesco Semeraro, Claudia Agabiti Rosei, Alessandro Alonzo, Lidia Sada, Elisa La Boria, Annamaria Sarkar, Beatrice Petroboni, Paolo Mercantini, Massimo Volpe, Damiano Rizzoni and Enrico Agabiti Rosei

Hypertension. 2014;64:717-724; originally published online June 30, 2014;
doi: 10.1161/HYPERTENSIONAHA.114.03380

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2014 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/64/4/717

Data Supplement (unedited) at:
http://hyper.ahajournals.org/content/suppl/2014/06/30/HYPERTENSIONAHA.114.03380.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/
EFFECTS OF A LONG-TERM TREATMENT WITH ALISKIREN OR RAMIPRIL ON STRUCTURAL ALTERATIONS OF SUBCUTANEOUS SMALL RESISTANCE ARTERIES OF DIABETIC HYPERTENSIVE PATIENTS

Online supplement

Carolina De Ciuceis\textsuperscript{1}, Carmine Savoia\textsuperscript{2}, Emanuele Arrabito\textsuperscript{2}, Enzo Porteri\textsuperscript{1}, Monica Mazza\textsuperscript{1}, Claudia Rossini\textsuperscript{1}, Sarah Duse\textsuperscript{3}, Francesco Semeraro\textsuperscript{3}, Claudia Agabiti Rosei\textsuperscript{1}, Alessandro Alonzo\textsuperscript{2}, Lidia Sada\textsuperscript{2}, Elisa La Borla\textsuperscript{1}, Anna Maria Sarkar\textsuperscript{1}, Beatrice Petroboni\textsuperscript{1}, Paolo Mercantini\textsuperscript{4}, Massimo Volpe\textsuperscript{2}, Damiano Rizzoni\textsuperscript{1}, Enrico Agabiti Rosei\textsuperscript{1}.

\textsuperscript{1}Clinica Medica, Department of Clinical and Experimental Sciences, University of Brescia, Italy;
\textsuperscript{2}Division of Cardiology, Department of Clinical and Molecular Medicine, Sant'Andrea Hospital, Sapienza University of Rome, Rome, Italy;
\textsuperscript{3}Chair of Ophthalmology, University of Brescia, Italy;
\textsuperscript{4}Surgical Department of Clinical Sciences, Biomedical Technologies and Translational Medicine, Sant'Andrea Hospital, Sapienza University of Rome, Italy.

Carmine Savoia and Carolina De Ciuceis equally contributed to this paper.

PATIENTS AND METHODS

**Evaluation of retinal arteriolar morphology**

Wall to lumen ratio of retinal arterioles was assessed using Scanning Laser Doppler Flowmeter at 670 nm (Heidelberg Retina Flowmeter, Heidelberg Engineering, Heidelberg, Germany) (1-3). Briefly, an arteriole (size between 80 and 140 $\mu$m) of the superficial retinal layer in a retinal sample of 2.56 X 0.64 X 0.30 mm was scanned within 2 seconds, at a resolution of 256 points x 64 lines x 128 lines. Measurements were performed in the juxtapapillary area of the right eye, 2 to 3 mm temporal superior to the optic nerve; the mean from 3 measurements was taken (1,2). Only
arterioles that could 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 minutes of rest, at room temperature and daylight conditions between 8 AM and 2 PM, but before lunch. Analyses of diameters were performed offline with automatic full-field perfusion imaging analysis program (Nirox Optoelectronics, Brescia, Italy). Outer arteriole diameter (AD) was measured in reflection images, and lumen diameter (LD) was measured in perfusion images (1-3). Wall to lumen ratio was calculated using the formula (AD-LD)/LD (1-3).

**Evaluation of capillary density**

Skin capillary density was assessed by capillaroscopy before and after venous congestion, as described elsewhere (4-6). After a period of rest in sitting position in a quiet and temperature controlled room (21-22°C), capillaries from nailfold 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 x) were obtained by video-microscopy (Videocap 3.0 D1 200, DS Medica, Milano, 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 at to 60 mmHg for 2 minutes a miniature blood pressure cuff applied to the base of the fourth finger of the non-dominant hand (5,6). Images 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. Only the first row of the nailfold capillaries was considered. Capillary density was determined by two independent operators and findings were averaged.
Evaluation of mechanical properties of subcutaneous small arteries

Small resistance arteries were mounted on a pressure myograph and perfused with Ca\(^{2+}\)-free PSS containing 10 mmol/L EGTA for 30 minutes to eliminate myogenic tone. Intraluminal pressure was increased stepwise 3 to 140 mm Hg. Internal diameter and media thickness were measured at each step in order to evaluate vascular mechanic (7). Mechanic parameters were calculated as previously reported (8,9). Media cross sectional area was calculated as \((\pi/4)\times(D_c^2-D_i^2)\), where \(D_c\) and \(D_i\) are external and internal diameters, respectively. Circumferential strain (\(\varepsilon\)) is obtained as \((D-D_o)/D_o\), where \(D\) is the internal diameter for a given intraluminal pressure, and \(D_o\) is the original diameter at 3 mmHg (baseline diameter). Circumferential stress (\(\sigma\)) is \((PD)/(2M)\), where \(P\) is the intraluminal pressure (dyne/cm\(^2\)), and \(D\) and \(M\) are lumen diameter and media thickness, respectively. Elastic modulus was assessed by fitting stress-strain data to \(\sigma = \sigma_o e^{\beta \varepsilon}\), where \(\sigma_o\) was stress at \(D_o\) and \(\beta\) is a constant related to the rate of increase of the stress-strain curve.

Vascular collagen content evaluation by confocal microscopy

Subcutaneous small resistance arteries were fixed for 30 min at 60 mmHg of intraluminal pressure with a solution containing 3.5\% formaldehyde and 0.75\% glutaraldehyde in 50 mmol/l phosphate buffered saline (PBS), pH 7.4 (7,10). Vessels were then incubated with 0.5\% BSA/PBS (5 minutes at 42\(^\circ\)C), treated with hyaluronidase (1 mg/ml), and incubated with antibody to collagen type I/III (1:20, overnight at 4\(^\circ\) C) (Calbiochem). A secondary antibody (Alexa Fluor 647 anti-rabbit IgG, Molecular Probes) was applied for 30 minutes at 37\(^\circ\)C. For the final 30 minutes of incubation, rhodamine/phalloidin (Sigma, 10 \(\mu\)mol/L, Molecular Probes) was added to stain \(\alpha\)-actin. Arteries were mounted in 1:1 glycerol/PBS (pH 7.4) on glass coverslips and studied by confocal immunofluorescence microscopy with a Zeiss LSM 510 system and a stack of sliced images was obtained. The amount of collagen I/III present in the vessels were quantified by imaging (Zeiss...
LSM Examiner Software), and expressed as a percentage of collagen/total surface area (% pixel/surface area).

RESULTS

Vascular mechanics

Increasing intraluminal pressure to 140 mmHg decreased the media/lumen ratio of relaxed subcutaneous small resistance arteries from vessels of patients before and after treatment with either aliskiren or ramipril to similar degrees without altering media cross-sectional area (data not shown). No significant difference was observed in passive distensibility, as expressed by strain curve (Figure S1 a) as well as in media stress (Figure S1 b) after treatment with both drugs compared to baseline. Consequently, no statistically significant difference was observed in the stress-strain curve of small resistance arteries in patients treated with aliskiren or ramipril compared with baseline (Figure S2 a). Indeed, when plotted against intraluminal pressure or stress, no difference were observed in incremental elastic modulus in patients before and after treatment with aliskiren or ramipril (Figure S2 b).

Collagen content in subcutaneous small resistance arteries

No significant difference in vascular collagen content, expressed as % of total surface area, was observed in patients treated with aliskiren or ramipril for one year compared to baseline (Figure S3).

References


**Figure S1**

Mechanic properties of subcutaneous small resistance arteries. Passive distensibility (a) and media stress (b) at different intraluminal pressures in relaxed subcutaneous small resistance arteries from diabetic hypertensive patients before and after treatment with either aliskiren or ramipril. Results are expressed as mean±SEM. Full triangle: diabetic hypertensive patients before treatment with aliskiren; empty triangle: diabetic hypertensive patients after treatment with aliskiren. Full square: diabetic hypertensive patients before treatment with ramipril; empty square: diabetic hypertensive patients after treatment with ramipril.
Figure S2

Stress–strain (a) and elastic modulus-stress relationship (b) in relaxed subcutaneous small resistance arteries from diabetic hypertensive patients before and after treatment with either aliskiren or ramipril. Results are expressed as mean±SEM. Full triangle: diabetic hypertensive patients before treatment with aliskiren; empty triangle: diabetic hypertensive patients after treatment with aliskiren. Full square: diabetic hypertensive patients before treatment with ramipril; empty square: diabetic hypertensive patients after treatment with ramipril.
Figure S3

Representative images of collagen content of subcutaneous small resistance arteries from diabetic hypertensive patients before (upper panel) and after (lower panel) treatment with either aliskiren (left) or Ramipril (right).