Effects of Angiotensin Type-1 Receptor Antagonism on Small Artery Function in Patients With Type 2 Diabetes Mellitus

Rayaz A. Malik, Ian J. Schofield, Ashley Izzard, Clare Austin, Georgina Bermann, Anthony M. Heagerty

Abstract—Endothelial dysfunction has been demonstrated to occur in small arteries from patients with type 2 diabetes and hypertension. The effects of angiotensin II receptor blockade on vessel function were examined using pressure myography in a randomized 12-week double-blind placebo-controlled parallel group study using candesartan cilextil. The maximal vascular response to acetylcholine (Ach) was impaired at baseline and improved with candesartan. This improvement was primarily caused by an effect in the nitric oxide component of Ach-mediated dilatation. The degree of endothelial dysfunction directly correlated with serum low-density lipoprotein cholesterol levels. Sodium nitroprusside-induced endothelium-independent dilatation was reduced in diabetic patients and intervention with candesartan lead to an improvement in EC50 with no change in maximal response. Vasoconstriction to norepinephrine was normal and did not change with intervention, but responses to angiotensin II were reduced after candesartan in diabetic patients. These results demonstrate that even brief treatment with angiotensin II receptor blockade is associated with a significant improvement in resistance vessel endothelial function. (Hypertension. 2005;45:264-269.)

Key Words: angiotensin II ■ clinical trials ■ diabetes mellitus ■ endothelium ■ nitric oxide ■ arterioles

In patients with type 2 diabetes (type 2DM), both macrovascular and microvascular diseases are a cause of considerable morbidity and mortality.1,2 Accelerated vascular disease in type 2DM is associated with hyperglycemia, hypertension, and dyslipidemia.3 Intensive glycemic control reduces the risk of predominantly microvascular and to a lesser extent macrovascular complications in both type 1 diabetes and type 2DM.4,5 Treatment with angiotensin-converting enzyme inhibitors6 and angiotensin II receptor blockers7 in patients with type 2DM significantly improves both macrovascular and microvascular end points, including nephropathy,8–10 retinopathy,11 and neuropathy.12 Cholesterol lowering may produce a greater clinical impact in diabetic compared with nondiabetic patients with coronary heart disease.13 Small arteries of patients with type 2DM demonstrate both functional and structural alterations that include deficient endothelium-dependent relaxation to acetylcholine (Ach) and bradykinin and hypertrophic remodeling.14 Recently, we have demonstrated endothelial dysfunction and a highly significant loss of myogenic responsiveness leading to resistance vessel hypertrophy in patients with type 2DM.15 Also, Rizzoni et al have shown that structural alterations in the resistance vessels are significantly associated with the occurrence of future cardiovascular events.16

The severity of endothelial dysfunction reported in type 2DM is related to the degree of dyslipidemia.15 Although the detailed mechanism remains undetermined, enhanced oxidative stress appears to be crucial for the perturbation in endothelial function brought about by hypercholesterolemia.17 Angiotensin II type-1 (AT1) receptor activation is a predominant source of free radical release in the vascular wall.18,19 Studies in vitro and in humans have shown that hypercholesterolemia induces AT1 receptor overexpression and increased oxidative stress.20,21 Accordingly, it was decided to examine whether blocking the AT1 receptor would have favorable effects in small arteries from patients with type 2DM in a short-term treatment trial using the AT1 receptor antagonist candesartan cilextil. In particular, we wished to examine whether improvement occurred in small artery function in normotensive and hypertensive patients with type 2DM.

Methods

A randomized 12-week double-blind, placebo-controlled, parallel group study was performed in 32 patients with type 2DM with (placebo, n=7; candesartan, n=7) and without (placebo, n=9; candesartan, n=9) hypertension. All subjects gave full informed written consent before taking part in the study, which was approved by the local research ethics committee. To standardize treatment, patients with type 2DM continued their therapy for maintaining...
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semi-automatic machine (OMRON 705 CP; White Medical, Rugby, was quantified using 3 timed overnight urine collections. Blood group and in 1 patient in the candesartan group. Microalbuminuria profile (total cholesterol and lipid subfraction), and glycosylated antecubital vein for assessment of renal function, glucose, and lipid weeks, which was then increased to 16 mg daily in all patients. On patients were randomized to placebo or candesartan 8 mg daily for 4 weeks before the study. The presence of hypertension and type 2DM was in accord with internationally defined guidelines.22 Patients were randomized to placebo or candesartan 8 mg daily for 4 weeks, which was then increased to 16 mg daily in all patients. On the day of study, venous blood samples were drawn from an antecubital vein for assessment of renal function, glucose, and lipid profile (total cholesterol and lipid subfraction), and glycosylated hemoglobin. Atorvastatin was prescribed in 1 patient in the placebo group and in 1 patient in the candesartan group. Microalbuminuria was quantified using 3 timed overnight urine collections. Blood pressure was measured sitting after 15 minutes of rest using a semi-automatic machine (OMRON 705 CP; White Medical, Rugby, UK) with the mean of 3 readings recorded.

Pressure Arteriography
A single subcutaneous gluteal fat biopsy sample was obtained from each subject at baseline and after 12 weeks of intervention; 3 to 5 mL of 1% lignocaine was infiltrated, allowing tissue (2×1.5×1.5 cm) to be harvested and placed immediately in ice-cold physiological saline solution.23 Small arteries 65 μm to 230 μm were isolated and carefully cleaned under a dissecting microscope. Vessels were transferred to an arteriograph bath chamber (Living Systems Instruments, Burlington, Vt),24 cannulated,24,25 and examined as described previously.15

Pharmacological Assessment
To assess viability, vessels were challenged serially with 60 mmol KPSS until a steady vasoconstriction >50% was reproducible. Each vessel was stimulated with the cumulative addition (3 to 5 minutes per concentration) of norepinephrine, angiotensin II, Ach after preconstriction with 10−3 norepinephrine, Ach after 30 minutes of incubation with 5×10−5 M L-Ar-monomethyl-arginine (L-NNMA), and sodium nitroprusside (SNP) as described in detail previously.15 The lumen diameter (μm) of the vessels was measured and the contractile or dilatory responses were calculated as the percentage change in diameter normalized to maximum response to that agonist. EC50 values (concentration to give 50% maximum contractile or dilatory response) used the percentage changes normalized to maximum response to the agonist.

Statistical Analysis
All data are presented as mean±SEM. One-way ANOVA with Bonferroni correction for multiple comparisons were used to evalu-
to 77.0 ± 2.9; hypertensive: systolic, 155.4 ± 5.8 to 148.9 ± 8.7; diastolic, 94.8 ± 2.9 to 86.9 ± 5.6) patients with no significant difference for either systolic (P = 0.247) or diastolic (P = 0.717) blood pressure between candesartan and placebo in patients with type 2DM. There was no significant linear relation between age and myography variables. All data are presented at baseline comparing the arteries from diabetic patients with and without hypertension with vessels from control subjects. The effects of intervention were assessed by determining the change in the candesartan group compared with the change in the placebo group. The data are presented without the placebo curves but these are available in electronic form.

Vasoconstrictor Function

With norepinephrine, the maximum contractile response was not significantly different between control subjects and hypertensive and normotensive type 2DM subjects (Figure 1a). Treatment with candesartan did not influence maximum response but improved sensitivity (EC50) (P = 0.012) to norepinephrine in normotensive subjects (Figure 1b) but had no effect in hypertensive (Figure 1c) type 2DM subjects.

The contractile response and sensitivity (EC50) to angiotensin II did not differ in small arteries from control subjects compared with normotensive and hypertensive type 2DM subjects (Figure 2a). Treatment with candesartan had no significant effect on either maximum response or EC50 in normotensive (Figure 2b) and hypertensive (Figure 2c) type 2DM subjects. The overall contraction was significantly reduced in normotensive type 2DM with candesartan subjects. The baseline-adjusted responses for change from baseline with angiotensin II were reduced by 23% in normotensive type 2DM and by 71% in hypertensive type 2DM.

Vasodilator Function

There was a significant reduction in the maximal relaxation response to Ach in small arteries from both normotensive (56.3 ± 20.8%; P < 0.001) and hypertensive type 2DM subjects (49.0 ± 20.3%; P < 0.001) when compared with control subjects (87.2 ± 16.0%) (Figure 3a). Treatment with candesartan significantly improved overall relaxation to acetylcholine in small arteries from normotensive (P < 0.001) (Figure 3b) and hypertensive (P < 0.05) (Figure 3c) type 2DM subjects, with an additional improvement in EC50 in hypertensive type 2DM subjects (P < 0.001) (Figure 3c). There was a significant negative correlation between serum LDL cholesterol and maximum relaxation to acetylcholine in vessels from normotensive (r = -0.83; P < 0.01) (Figure 4a) and hypertensive (Figure 4b) type 2DM subjects (r = -0.71; P = 0.03).

Small arteries incubated with l-NMMA and then challenged again with Ach showed a 27% reduction in maximum

![Figure 1](image1.png)

**Figure 1.** Constriction of vessels comparing control subjects with normotensive and hypertensive type 2 diabetes (type 2DM) (A) and response assessed by change in EC50, maximal relaxation, and overall relaxation to candesartan in normotensive (B) and hypertensive (C) type 2 DM subjects exposed to increasing concentrations of norepinephrine.

![Figure 2](image2.png)

**Figure 2.** Constriction of vessels comparing control subjects with normotensive and hypertensive type 2DM (A) and response assessed by change in EC50, maximal relaxation, and overall relaxation to candesartan in normotensive (B) and hypertensive (C) type 2 DM subjects exposed to increasing concentrations of angiotensin II.
relaxation. However, vessels from hypertensive and normotensive type 2DM subjects showed 7.2% and 5.4% reduction in maximum response (Figure 5a). Small but significant improvements were observed with candesartan after L-NMMA in response to Ach for overall relaxation in normotensive subjects ($P<0.002$), (Figure 5b) and for maximal response ($P<0.05$) and EC50 ($P<0.001$) in hypertensive type 2 DM subjects (Figure 5c).

The maximal response to SNP was reduced in normotensive diabetic patients ($46.6\pm18.7\%$), reaching significance in hypertensive type 2DM ($39\pm16.7\%; P<0.04$) compared with control subjects ($57\pm15.3\%$) (Figure 6a). Intervention with candesartan improved EC50 in both normotensive ($P<0.05$) (Figure 6b) and hypertensive ($P<0.05$) (Figure 6c) type 2 DM subjects with no effect on maximal response or overall relaxation.

**Discussion**

This study confirms the results of previous work demonstrating a significant alteration in the function of small arteries from normotensive and hypertensive patients with type 2DM.\(^{14,15}\) Now we have shown that these functional abnormalities can be ameliorated in part using an angiotensin type 1 receptor antagonist, thereby possibly providing some explanation toward the clinical benefit observed in preventing progression of diabetic nephropathy,\(^{8-10}\) retinopathy,\(^{11}\) and neuropathy.\(^{12}\)

Treatment for 3 months with candesartan cilexitil produced small decreases in blood pressure, but the study was not designed to show significant changes in this parameter because the cohorts were recruited to examine effects on arterial function. The sensitivity and reactivity to Ach were impaired in small vessels from patients with type 2DM. These findings are consistent with previous reports in both type 1 diabetes\(^{25}\) and type 2DM.\(^{14,15}\) One mechanism by which Ach effects vascular dilatation is by the release of nitric oxide from the endothelium. The studies with L-NMMA confirm that the main abnormality underlying deranged dilator function in small arteries from type 2DM is deficiency of functionally active nitric oxide. However, the small but significant improvement after L-NMMA suggests that nitric oxide-independent relaxation is also improved. Our studies suggest that the nitric oxide-dependent abnormality is related to circulating levels of LDL cholesterol that have been linked to abnormalities of endothelial function in subjects with normal and raised cholesterol concentrations.\(^{26-28}\) To establish causality, further work is required. Although it is recognized that aging progressively impairs endothelial function in humans,\(^{29}\) and that our diabetic patients were older than control subjects, careful analyses have discounted age as a
major factor in this and in our previous work. It is recognized that hypercholesterolemia induces increased AT1 receptor expression and oxidative stress, and AT1 receptor activation is a powerful source of free radical release in the vascular wall. Recently, it has been demonstrated that LDL induces the expression of AT1 receptor upregulation and hypercholesterolemic rabbits display enhanced vascular expression of AT1 receptors, representing increased activity of angiotensin II. Therefore, our finding that AT1 antagonism with candesartan cilexetil improves endothelial function by as much as 30% would support the role of the renin-angiotensin system in provoking endothelial dysfunction in these patients secondary to slightly raised LDL cholesterol levels. Angiotensin receptor antagonists not only enhance both endothelium-dependent and endothelium-independent vascular vasodilation capacity in patients with essential hypertension but also have been recently reported to improve endothelial dysfunction in hypercholesterolemic patients. The mechanism implicated must involve improvement in the bioavailability of nitric oxide, and blocking AT1 receptors would achieve this with free radical generation being ameliorated. Our data on the maximal contractile response to angiotensin II confirm that effective blockade of the resistance vessel AT1 receptors is achieved with candesartan, particularly in hypertensive type 2 DM patients. In this context, it has been recently shown that C-reactive protein, which is an important inflammatory mediator as well as an acute phase reactant, upregulates AT1 receptors in the vasculature, an effect attenuated by losartan.

The implications for patients with type 2 DM are that treatment strategies that lower lipid levels (even in the normal range at presentation) improve mortality and morbidity, and it is known that endothelial function can be restored to normal in small arteries of patients with hypercholesterolemia. The reduced dilation to sodium nitroprusside could represent a reduction in smooth muscle sensitivity to nitric oxide, because this was improved with intervention with candesartan, whereas the maximal response remained unchanged. Candesartan improved both sensitivity and maximal response to Ach in type 2 DM, supporting the idea of a greater benefit of candesartan on endothelial dysfunction.

It is attractive to suggest that the combination of lipid-lowering drugs with angiotensin II receptor antagonism might provide a combination that would be associated with synergistic effects on mortality and morbidity in diabetes.

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Figure 5. Dilation of vessels comparing control subjects with normotensive and hypertensive type 2 DM (A) and response assessed by change in EC50, maximal relaxation, and overall relaxation to candesartan in normotensive (B) and hypertensive (C) type 2 DM subjects exposed to increasing concentrations of Ach after pre-incubation of preconstricted small arteries with L-NMMA (5 × 10^{-5} m).

Figure 6. Dilation of vessels comparing control subjects with normotensive and hypertensive type 2 DM (A) and response assessed by change in EC50, maximal relaxation, and overall relaxation to candesartan in normotensive (B) and hypertensive (C) type 2 DM subjects exposed to increasing concentrations of sodium nitroprusside after preconstriction with norepinephrine.
References


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