Improvement in Endothelial Function by Angiotensin-converting Enzyme Inhibition in Non–Insulin-dependent Diabetes Mellitus

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OBJECTIVES

The aim of this study was to assess the effect of angiotensin-converting enzyme (ACE) inhibition with enalapril on forearm endothelial function in subjects with type II diabetes mellitus.

BACKGROUND

Endothelial function is depressed in the presence of conventional risk factors for atherosclerosis, and various therapies, such as lipid-lowering therapy in hypercholesterolemia, can improve endothelial-mediated vasodilation. ACE inhibition has improved such function in several conditions including type I diabetes, but there is no evidence for a beneficial effect in type II diabetes.

METHODS

The influence of enalapril (10 mg twice daily for 4 weeks) on endothelium-dependent and -independent vasodilator function was determined in 10 type II diabetic subjects using a double-blinded placebo-controlled crossover protocol. Forearm blood flow was measured using strain-gage plethysmography and graded intrabrachial infusion of acetylcholine (ACh), N(G)-monomethyl-L-arginine (LNMMA) and sodium nitroprusside (SNP).

RESULTS

Enalapril increased the response to the endothelium-dependent vasodilator, ACh (p < 0.02) and the vasoconstrictor response to the nitric oxide (NO) synthase inhibitor, LNMMA (p < 0.002). No difference was evident in the response to SNP.

CONCLUSIONS

In type II diabetic subjects without evidence of vascular disease, the ACE inhibitor enalapril improved stimulated and basal NO-dependent endothelial function. The study extends the spectrum of beneficial effects demonstrated to result from ACE inhibition in diabetes. (J Am Coll Cardiol 1999;33:1506–11) © 1999 by the American College of Cardiology

The importance of the endothelium in maintaining a healthy vasculature has been increasingly recognized, particularly with respect to its numerous nitric oxide (NO)-mediated functions. Impaired endothelium-dependent vasodilatation, which is largely dependent upon NO, has been found not only in the presence of overt vascular disease but also in association with conventional risk factors for vascular disease (1), and many studies have documented improvement with appropriate interventions.

In non–insulin-dependent, type II diabetic subjects, NO-related dilator endothelial function has usually (2–5), although not invariably (6), been found to be depressed, while results have been inconsistent in type I diabetics (7–10). In the latter, we recently documented that local intrabrachial administration of the angiotensin–converting enzyme (ACE) inhibitor, enalaprilat, produced an acute increase in the vasodilator response to the NO-dependent dilator acetylcholine (ACh), while the response to the endothelium-independent dilator, sodium nitroprusside (SNP), was unaltered (11). Although no improvement in endothelium-dependent flow-mediated dilatation, assessed by ultrasound, was found after oral enalapril administration to type I diabetics in a recent study (12), in our study, one month of oral enalapril produced further improvement in the endothelium-dependent response (11). Angiotensin-converting enzyme inhibitors are becoming more frequently used in both type I and type II diabetics, largely because of their beneficial effect on renal function and albuminuria (13–17). However, whether ACE inhibition improves endothelial function in type II diabetics is unclear. A substantial dose of perindopril failed to improve the forearm blood flow response to the endothelium-dependent dilator methacholine in one study (18), although postischemic hyperemic
blood flow was increased. The recent Trial on Reversing Endothelial Dysfunction (TREND) study of quinapril in patients with coronary artery disease included 9 (14%) type II diabetics in the placebo treated group, and 13 (20%) in the group treated with quinapril for six months (19). Coronary endothelial function improved in the entire group treated with quinapril, without distinction between clinical subgroups, suggesting that there was benefit in the type II diabetic patients, although this question was not specifically addressed. However, since strategies to improve vascular and endothelial function in type II diabetics are of considerable clinical importance, the question deserved further study. We therefore examined the effect of ACE inhibition with enalapril therapy of four weeks duration using a placebo-controlled crossover design. Forearm blood flow, and hence resistance vessel function, was assessed by plethysmography, with intraarterial infusion of ACh used to examine endothelium-dependent dilation, SNP to examine endothelium-independent dilation, and the competitive inhibitor of NO synthase, Nω-monomethyl-L-arginine (LNMMA), to examine resting NO-related dilated tone.

**METHODS**

**Subjects.** Ten male subjects (60 ± 3 years) with type II diabetes mellitus, without evidence of microvascular or macrovascular complications, were recruited. They underwent a screening program consisting of a medical history and examination, hematologic and biochemical profile, including measurement of blood glucose, glycated hemoglobin, serum electrolytes, urea and creatinine, uric acid, liver function and serum lipids. The following were excluded: smokers, those with renal impairment or proteinuria, hepatic impairment, gout or hyperuricemia, more than mild hypercholesterolemia or hypertension (see Table 1). Although one patient was receiving lipid-lowering therapy, none were taking vitamin supplements, ACE inhibitors or any other medication known to affect endothelial function other than that for diabetes. One subject was taking no medications, while 5 were on metformin alone, 3 on metformin and gliclazide and 1 on metformin and glipizide. Medications remained unchanged during the study. None had significant microalbuminuria on quantitative assessment (24-h excretion using nephelometric method) or significant retinopathy (full-field photography). The mean glycated hemoglobin at entry was 7.8 ± 0.6% (SE) (normal range = 4.3–6.0%), indicating moderate to good glycemic control. Mean body mass index was 28.4 ± 1.2 kg/m². The study protocol was approved by Royal Perth Hospital Ethics Committee and subjects gave written informed consent.

**Study design.** The effect of four weeks of enalapril therapy was studied using a randomized, double-blind, placebo-controlled crossover protocol. Subjects were randomized in equal numbers to receive enalapril 10 mg twice daily (Renitec; Merck, Sharp & Dohme, Australia) or a similarly packaged placebo. Forearm vascular function was studied after four weeks, following which crossover of therapy occurred with re-study four weeks later. The procedures were conducted, on average, 4 h after the study medication and, for individual subjects, at the same time of the day for the repeat study after crossover. Subjects were required to refrain from drinking alcohol or caffeine-containing beverages for 12 h before the procedure. At each visit the biochemical and hematologic parameters were repeated. There were no adverse side effects.

**Protocol.** Investigations were conducted in a quiet, climate-controlled laboratory with subjects lying supine and both forearms supported above heart level. A 20-gauge arterial cannula (Arrow, Reading, Pennsylvania) was introduced into the brachial artery of the nondominant arm under local anesthesia with <2 ml of 1% lidocaine (Astra Pharmaceuticals, Australia) to transduce pressure, for the infusion of drugs or physiologic saline and for sampling of arterial blood. Forearm blood flow (FBF; ml/100 ml forearm/min) was measured simultaneously in both arms by gallium/indium strain-gauge (SG24; Medasonics, Mountain View, California) plethysmography. Wrist cuffs, connected to a flow-regulated source of compressed air, and arm cuffs, connected to a rapid inflation device (E20; D.E. Hokanson, Bellevue, Australia), were placed on each limb. Output from the strain-gauges passed through an amplifier (SPG 16; Medasonics) and was sampled by an on-line microcomputer at 75 Hz before being displayed on a

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**Table 1. Subject Characteristics During Placebo and Enalapril Administration**

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Enalapril</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose (mmol·L⁻¹)</td>
<td>8.7 ± 1.3</td>
<td>8.4 ± 1.1</td>
</tr>
<tr>
<td>Glycosylated hemoglobin (%)</td>
<td>7.8 ± 0.6</td>
<td>7.8 ± 0.6</td>
</tr>
<tr>
<td>Plasma lipids (mmol·L⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>4.9 ± 0.2</td>
<td>4.8 ± 0.2</td>
</tr>
<tr>
<td>LDL-C</td>
<td>2.8 ± 0.5</td>
<td>2.9 ± 0.4</td>
</tr>
<tr>
<td>HDL-C</td>
<td>1.0 ± 0.1</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>2.5 ± 0.4</td>
<td>2.4 ± 0.5</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>93 ± 2</td>
<td>84 ± 2*</td>
</tr>
<tr>
<td>Resting heart rate (beats/min)</td>
<td>76 ± 2</td>
<td>73 ± 1</td>
</tr>
</tbody>
</table>

Values are mean ± SE. Arterial pressure was significantly lower after enalapril (*P < 0.001).
Table 2. Absolute FBF Responses in the Infused and Noninfused Arms After Enalapril and Placebo Administration

<table>
<thead>
<tr>
<th>Infused Arm</th>
<th>Noninfused Arm</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Placebo</strong></td>
<td><strong>Enalapril</strong></td>
</tr>
<tr>
<td><strong>Resting FBF</strong></td>
<td>3.1 ± 0.3</td>
</tr>
<tr>
<td><strong>ACh infusion</strong></td>
<td></td>
</tr>
<tr>
<td>10 μg/min</td>
<td>3.7 ± 0.5</td>
</tr>
<tr>
<td>20 μg/min</td>
<td>5.3 ± 1.0</td>
</tr>
<tr>
<td>40 μg/min</td>
<td>6.6 ± 1.2</td>
</tr>
<tr>
<td><strong>SNP infusion</strong></td>
<td></td>
</tr>
<tr>
<td>Pre-SNP baseline</td>
<td>3.1 ± 0.4</td>
</tr>
<tr>
<td><strong>LNMMMA infusion</strong></td>
<td></td>
</tr>
<tr>
<td>Pre-LNMMMA baseline</td>
<td>3.2 ± 0.5</td>
</tr>
<tr>
<td>2 μmol/min</td>
<td>2.6 ± 0.5</td>
</tr>
<tr>
<td>4 μmol/min</td>
<td>2.4 ± 0.6</td>
</tr>
<tr>
<td>8 μmol/min</td>
<td>2.2 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE in ml/100 ml forearm/min. Absolute FBF responses to ACh, SNP and LNMMMA were not significantly different after four weeks of enalapril therapy.

ACh, acetylcholine; FBF, forearm blood flow; LNMMMA, Nω-monomethyl-L-arginine; SNP, sodium nitroprusside.

monitor in real time. A software program coordinated the acquisition, storage and display of data as well as inflation and deflation of the arm cuffs, ensuring that blood flow measures were synchronized with cuff inflation during recording periods. Intraarterial pressure was measured continuously (Transpac; Abbot Laboratories, Illinois) throughout the study. Drug infusions were administered using a constant rate infusion pump (IVAC 770; IVAC Corporation, California).

Baseline measurements started at least 25 min after cannulation of the brachial artery. Blood flow measurements were taken by inflating the wrist cuffs to 220 mm Hg, to exclude the hands from the circulation, and by rapidly inflating the upper arm cuffs to 45 mm Hg for 10 out of every 15 s throughout the baseline and drug infusion periods. Output from the strain-gauges was stored, and the average of the last five flow measurements from each period was used for analysis. Between infusions, the cuffs were deflated, allowing at least 15 min for forearm blood flow to recover from the preceding infusion before further baseline measures were recorded.

All solutions were prepared aseptically from sterile stock solutions or ampules immediately before infusion into the brachial artery. ACh (Miochol; Johnson & Johnson, Australia) was infused at 10, 20 and 40 μg/min, each for 3 min, followed by SNP (David Bull Laboratories, Australia) at 2, 4 and 8 μg/min, each for 3 min and then LNMMMA (Clinalfa, Switzerland) at 2, 4 and 8 μmol/min, each for 5 min.

**Analysis.** Although the low doses of drugs infused in the study produce negligible systemic effects and showed no effect on blood pressure or heart rate, it is still desirable to exclude an alteration in overall hemodynamics as a cause of the flow changes seen in the infused forearm. Thus, FBF was measured simultaneously in both arms, although only one arm was infused, and the noninfused arm served as a control. As in earlier studies (11,20), FBF in the infused arm is described as a ratio to that in the noninfused arm. Changes in these ratios during ACh and SNP infusions are expressed as percentage changes from the baseline immediately preceding each drug administration. In addition, vascular resistance was calculated in the infused arm as the ratio of mean arterial pressure to FBF and expressed as mmHg per ml/100 ml tissue/min.

Results are expressed as means ± SE. The responses after ACE inhibition were compared with placebo responses using two-way analysis of variance with repeated measures performed on the three dose levels of ACh, SNP and LNMMMA. A p < 0.05 was considered significant.

**RESULTS**

There was no difference in blood glucose, glycated hemoglobin or serum lipids between enalapril and placebo treatment (Table 1). Blood pressure at the time of FBF measurement was lower during enalapril therapy (p < 0.001), while heart rate was not significantly altered.

Absolute FBF data recorded in the infused and noninfused limbs at baseline and during the infusion of ACh, SNP and LNMMMA at three dose levels, during placebo and enalapril administration, are presented in Table 2. The baseline FBF values preceding each drug were not different, indicating adequate washout periods between infusions. Although the responses to ACh and LNMMMA were, on average, augmented after enalapril administration, the difference from placebo was not significant. Acetylcholine...
reduced forearm vascular resistance (FVR) more, on average, after enalapril therapy than after placebo, although this did not achieve statistical significance (p = 0.054). Enalapril significantly augmented the FVR response to LNMMA (p < 0.01), while the response to SNP was unchanged.

However, as described in Methods, it is optimal to analyze the data in terms of FBF ratios, which is the ratio of flow in the infused arm to that in the noninfused arm, and to refer these to the similarly derived baseline ratios preceding each set of drug infusions. Uncorrected ratios at the three dose levels of ACh were significantly greater after enalapril than placebo administration (p < 0.02) and are presented in Table 3, while the responses to SNP and LNMMA were not different. Figures 1–3 present the percentage changes in these ratios from their baselines, in response to ACh, SNP and LNMMA. The vasodilator response to ACh was significantly augmented after enalapril therapy (p < 0.02; Fig. 1), while the response to the endothelium-independent dilator, SNP, was unchanged (Fig. 2). The NO-dependent vasoconstrictor response to LNMMA was increased (p < 0.002, Fig. 3).

**DISCUSSION**

**Principle findings and comparison with previous studies.** The results of this study indicate that enalapril therapy given orally for four weeks to type II diabetics significantly improved endothelial function. Vasodilation in response to ACh, largely mediated through NO, was increased and vasoconstriction to the NO synthase inhibitor, LNMMA,
was also augmented, the latter indicating greater resting NO-dependent dilation. These findings extend our previous observation of improvement in endothelial function, in terms of the response to ACh, in type I diabetics treated with enalapril for four weeks (11). LNMMA was not given in that study to test resting NO dilator tone. However, intrabrachial co-infusion of enalaprilat with ACh augmented the ACh response, indicating that the response is almost certainly due to a local vascular effect of ACE inhibition and not primarily to a systemic effect, for example, associated with hypotension. This contention is supported by the observation that captopril improved endothelial function in hypertensives whereas nifedipine did not (21) and, indirectly, by the observation that lisinopril improved capillary permeability and reduced urinary albumin loss more than did atenolol in hypertensive type II diabetics (17).

In contrast to the present findings, an earlier study found no beneficial effect on endothelial function, tested with methacholine, when perindopril was given for six months to type II diabetics (18). However, ACE inhibition has been reported to improve endothelial function in patients with heart failure (22,23), hypertension (21), coronary artery disease (19) and even normal subjects (24). It is therefore not surprising that it does so in diabetics but, because of the prevalence of type II diabetes and the predisposition to vascular disease, this was considered important to document.

**Mechanisms responsible for endothelial dysfunction in diabetes.** Most of our patients were being treated on hypoglycemic drugs, which may improve NO-mediated endothelial function (25). However, such drugs are commonly used in type II diabetics and it was ensured that they remained unchanged throughout the study. It was not an aim of the study to compare endothelial function in type II diabetic and normal subjects; although there are negative studies (6), there is substantial evidence that endothelial function is depressed in type II diabetics (2–5). The responsible mechanisms are unknown and the possibilities have been discussed by others (2), with emphasis on strong evidence from diabetic animal studies for inactivation of NO by oxygen-derived free radicals, such as superoxide anions, or by advanced glycosylation products. There is also evidence that insulin stimulates the production of NO through the insulin receptor and that the effector pathway has some commonality with that for glucose transport, as demonstrated in cultured human vascular endothelial cells (26). Presumably, lack of insulin production or resistance to the action of insulin in the clinical diabetic states would, therefore, depress NO production. It seems that, in vivo, either an influence of insulin on NO production, or on its inactivation or effectiveness, could explain the relationships between insulin, diabetes and NO-related vasoactivity, as recently summarized (11).

**Potential mechanisms responsible for ACE effect.** Although we have demonstrated improved basal and stimulated endothelial function in type II diabetes with ACE inhibition, this study does not elucidate mechanisms. Several possibilities exist. If greater quenching of NO by superoxide anions contributes substantially to the defect in diabetes, it would be analogous to that which is implicated in the endothelial dysfunction in hypercholesterolemia and probably in other conditions (27). The generation of such oxygen-derived free radicals may be dependent upon xanthine oxidase or upon membrane-bound vascular oxidases (27), and one possible mechanism for the beneficial effect of ACE inhibition could relate to the latter (28). Angiotensin II increases the activity of these oxidases along with the production of superoxide, an effect inhibited by selective blockade of angiotensin type I receptors (27–29). Angiotensin-converting enzyme inhibition could act through this oxidase system. Additionally, the inhibition of angiotensin II production could lead to increased activity of NO synthase, and production of NO, through an effect on protein kinase C (28). An alternate possibility is that inhibition of the breakdown of bradykinin by kininase II, which is synonymous with ACE, plays a role in the beneficial response to the enzyme inhibitor since bradykinin stimulates the endothelial production of NO (30–32). Whether the preservation of endogenous bradykinin is important is unclear, but the coronary vasodilation resulting from the intracoronary administration of bradykinin, in humans, is decreased by local infusion of LNMMA and increased by enalaprilat, indicating that the largely NO-dependent dilation induced by bradykinin is potentiated by enzyme inhibition (30).

Other interventions studied for their effect on the endothelial dysfunction of diabetes help little to elucidate mechanisms. The substantial improvement in vasodilation to intraarterial methacholine resulting from local vitamin C infusion in type II diabetics was not seen in normal subjects and tends to implicate oxidants in the defect (33). However, vitamin C also improves the endothelium-dependent posthyperemic response in the brachial artery of patients with coronary artery disease (34). Further, tetrahydrobiopterin, a cofactor for NO synthase, and an antioxidant, improves endothelial function in both experimental diabetes (35) and in hypercholesterolemia (36), as does the administration of fish oil in these clinical conditions (37,38). Yet, tetrahydrobiopterin might play a particular role in diabetes since its concentration is reduced in the diabetic rat (39) and its metabolism is dependent upon the oxidant state (35,36). In summary, a number of interventions improve endothelial function in diverse conditions associated with depressed endothelial function; the oxidant state is likely to be important in the various pathologies associated with endothelial dysfunction and to relate to the effect of ACE inhibition.

**Conclusion and implications.** Whatever the mechanism, improvement in endothelial function in type II diabetes by ACE inhibition is potentially of considerable clinical relevance in the management of this disease. It has been known for some time that ACE inhibition in this common form of
diabetes preserves renal function and reduces albuminuria (13–17). In fact, these renal manifestations and the improvement in endothelial function, demonstrated here in terms of the NO dilator response, are probably closely related. It is likely that there would be commensurate benefit in other NO-related functions such as the regulation of vascular smooth muscle proliferation, and adhesion and interaction of platelets and monocytes with vessel walls, all processes integral to the development of atherosclerosis.

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