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Short communication

Temocapril, an angiotensin converting enzyme inhibitor, protects against diabetes-induced endothelial dysfunction

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Abstract

The effect of chronic treatment with the angiotensin converting enzyme inhibitor, temocapril, on prevention of endothelial dysfunction was evaluated in an experimental model of diabetes mellitus. Endothelium-dependent relaxation to acetylcholine was impaired while endothelium-independent relaxation to nitroglycerin was unaltered in diabetic aortic ring segments. Treatment of diabetic animals with temocapril prevented the impaired endothelium-dependent relaxation without altering responses to nitroglycerin. Acetylcholine-induced relaxation was largely due to nitric oxide (NO)-mediated relaxation; however, a small but significant portion of relaxation in aortic rings from temocapril-treated diabetic rats was resistant to inhibition by the nitric oxide synthase (NOS) inhibitor, L-nitroarginine.

Keywords: Endothelium; Nitric oxide (NO); Diabetes mellitus; Angiotensin

1. Introduction

Studies conducted from a variety of laboratories confirm impaired endothelium-dependent relaxation in experimental diabetes (Pieper and Gross, 1991; Cohen, 1993; Fortes et al., 1994; Kamata et al., 1995; Poston and Taylor, 1995; Pieper, 1998). A variety of different factors have been described to contribute acutely to defective endothelium-dependent relaxation which may vary by species and the type of artery evaluated (refer to the review articles listed above). In contrast, less investigation has been targeted at chronic factors in vivo, which might contribute to the etiology of diabetes-induced endothelial dysfunction. Of the therapeutic modalities tested, the interventions tested were primarily aldose reductase inhibitors or agents which scavenge or interfere with reactive oxygen production. Angiotensin converting enzyme inhibitors are one class of therapeutic agents that have received scant evaluation on benefits specific to the endothelium in experimental diabetic models. Information in this area is potentially important because of the significant frequency of use of angiotensin converting enzyme inhibitors among human diabetic patients.

The atherogenic rabbit is one experimental model which exhibits impaired endothelium-dependent relaxation. Ramipril, an angiotensin converting enzyme inhibitor, has been shown to prevent impaired endothelium-dependent relaxation in this atherosclerotic model (Finta et al., 1993). Experiments from this same laboratory revealed that ramipril failed to correct the defective endothelium-dependent relaxation to acetylcholine in diabetic rat aorta (Murray et al., 1994). Since that initial report, another laboratory using fosinipril revealed that inhibitor intervention significantly improved endothelium-dependent relaxation in diabetic rat mesenteric arteries (Olbrich et al., 1996). Because of this contradictory data and the sparse information of intervention with an angiotensin converting inhibitor in experimental diabetic models, we evaluated the efficacy of a relatively newer angiotensin converting enzyme inhibitor, temocapril (Furuta et al., 1993), to improve endothelial function in an experimental model of diabetes mellitus.

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2. Materials and methods

Male Sasco, Sprague–Dawley rats (at 10–11 weeks of age) were anesthetized with an intraperitoneal injection of 60 mg/kg sodium pentobarbital followed by tail-vein injection of 55 mg/kg streptozotocin to induce diabetes mellitus. Hyperglycemia was verified after 48 h from a drop of tail blood using an ExacTech glucometer and test strips (Medisense, Cambridge, MA, USA). After verification of glycemic status, a subgroup of diabetic animals received 0.1 mg/ml of temocapril (Sankyo, Tokyo, Japan) in drinking water. This concentration was previously shown to be effective in a rat model of cardiovascular remodeling (Takemoto et al., 1997).

At the end of 8-week period with or without treatment with temocapril, nonfasted animals were anesthetized with 65 mg/kg pentobarbital. Nonfasting conditions were used to avoid fasting-induced changes in antioxidant levels (Lammi-Keefe et al., 1984; Wohaieb and Godin, 1987), which might complicate evaluation of endothelial function due alterations in diabetes-induced reactive oxygen production (Pieper et al., 1992, 1996). Descending thoracic aorta was removed and cleaned of fat and connective tissue. Aortae were sectioned into 3-mm rings and mounted in tissue baths containing Krebs–bicarbonate buffer. Rings were stretched to optimal resting tension of 2.0 g. Isometric tension was recorded using a Gould TA6000 recorder (Valley View, OH, USA) and Radnoti force-displacement transducers and amplifiers (Monrovia, CA, USA).

After 1 h or equilibration, rings were contracted with increasing concentrations of norepinephrine, washed and equilibrated. Rings were then contracted to a submaximal concentration of norepinephrine followed by addition of cumulative concentrations of acetylcholine (endothelium-dependent vasodilator) or nitroglycerin (endothelium-independent vasodilator). Some rings were pretreated for 15 min with 100 μM L-nitroarginine in order to verify NO-mediated vasodilation. To eliminate any possible vasodilator interactions, only one vasodilator was used in any given ring segment.

Data were calculated as means ± S.E.M. and analyzed by repeated-measures analysis of variance followed by Student–Neuman–Keuls test for multiple mean comparisons or paired *t*- or unpaired *t*-test for comparison of two group means, where appropriate. Statistical significance was established to be comparisons with $P < 0.05$.

3. Results

Blood glucose at 48 h after injection of streptozotocin was significantly ($P < 0.001$) increased over baseline. Blood glucose was similar between untreated diabetic rats ($n = 9$; 362 ± 22 mg/dl) and diabetic rats selected for treatment with temocapril ($n = 9$; 365 ± 14 mg/dl) but was significantly increased compared to control rats ($n = 11$; 73 ± 4 mg/dl). Glucose values did not vary widely throughout the study as a consequence of treatment with temocapril (not shown). At the conclusion of the study, blood glucose was significantly increased in both untreated diabetic (391 ± 14 mg/dl) and temocapril-treated diabetic (396 ± 20 mg dl) rats compared to control rats (68 ± 4 mg/dl).

Maximal contraction and sensitivity to norepinephrine was not significantly altered by diabetes (not shown). Treatment with temocapril reduced maximum contraction to norepinephrine (1.34 ± 0.15 g) compared to untreated control (1.70 ± 0.17 g) or untreated diabetic groups (1.77 ± 0.14 g), but this did not reach statistical significance ($P < 0.18$).

Rings were precontracted with a submaximal concentration of norepinephrine to produce equipotent contraction (i.e. $62 \pm 1\%$, $62 \pm 1\%$ and $56 \pm 5\%$ of maximum for control, diabetic and temocapril-diabetic groups, respectively). Acetylcholine-stimulated relaxation was impaired in diabetic rings compared with control rings (Fig. 1). Oral treatment with temocapril prevented the impaired relaxation to acetylcholine. Treatment ex vivo with L-nitroarginine completely inhibited relaxation to acetylcholine in untreated controls and nearly completely inhibited relaxation in the untreated diabetic group ($9 \pm 1\%$ residual relaxation). In the temocapril-treated diabetic group, there was an increase in the L-nitorarginine-resistant component of relaxation to acetylcholine ($16 \pm 3\%$ residual relaxation; $P < 0.05$ vs. untreated diabetic group). Rings relaxed completely to nitroglycerin in all three experimental groups (not shown). Also the pD_2 or sensitivity was unaltered by

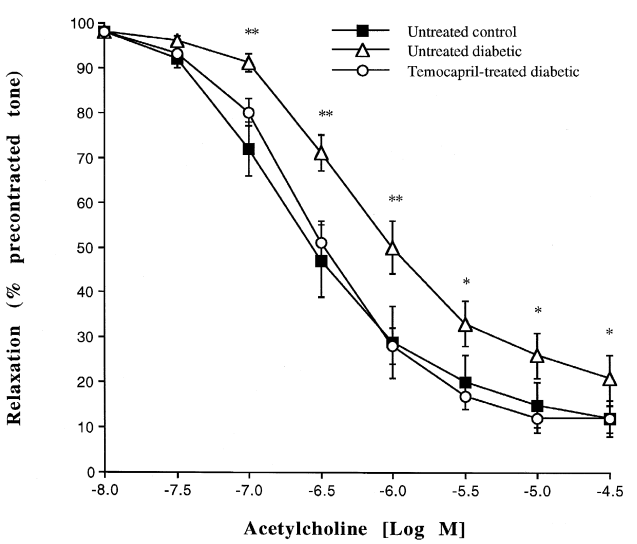

Fig. 1. Effects of chronic treatment with temocapril on endothelium-dependent relaxation to acetylcholine in diabetic rat aorta. Results are the mean ± S.E.M., $n = 11$ for the control group and $n = 9$ for each diabetic group. Analysis of variance revealed $P < 0.01$ for control or temocapril-diabetic groups vs. untreated diabetic group. For convenience, individual P values are listed between the diabetic groups (with vs. without treatment) at each concentration of acetylcholine. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. temocapril-diabetic group.

diabetes or by treatment with temocapril (i.e., untreated control: 7.0 ± 0.2; untreated diabetic: 6.8 ± 0.2; temocapril-treated diabetic: 7.3 ± 0.3).

4. Discussion

Long-term oral treatment with temocapril prevented diabetes-induced endothelial dysfunction. This improved function by temocapril cannot be accounted for by a change in the sensitivity of activation of vascular smooth muscle guanylate cyclase because the responses to nitroglycerin were not altered by drug treatment. These findings using temocapril are similar to that previously shown in our laboratory using drug interventions aimed at scavenging or preventing reactive oxygen formation (Pieper et al., 1996; Pieper and Siebeneich, 1997, 1998). Most or all of the relaxation to acetylcholine in untreated control and untreated diabetic rings was eliminated upon incubation with the NOS inhibitor, L-nitroarginine, suggesting that relaxation in this model is almost exclusively due to NO-mediated relaxation. The acetylcholine-induced relaxation in the temocapril-treated diabetic group was also largely due to NO; however, there was a modest but significant increase in an L-nitroarginine-resistant component suggesting that temocapril treatment may also enhance a NOS-independent vasoactive factor.

Our studies conducted in conductance arteries agree with previous findings in mesenteric resistance arteries and strengthen the hypothesis that angiotensin converting enzyme inhibitor therapy may benefit the endothelium in diabetes mellitus. The fact that treatment with another angiotensin converting enzyme inhibitor, enalapril, was shown to improve endothelium-dependent relaxation in insulin-dependent diabetic patients (O'Driscoll et al., 1997) suggests that angiotensin converting enzyme inhibitors may be useful protective agents in diabetes-induced vascular disease.

Our results using temocapril agree with other reports showing that treatment with other angiotensin converting enzyme inhibitors did not alter glycemic control (Murray et al., 1994; Olbrich et al., 1996). Therefore, reductions in blood glucose cannot be argued to contribute to the beneficial actions of temocapril on improving endothelial function. We hypothesize that the benefits arise from two potential pathways. The first is decreased in vivo degradation of bradykinin, thereby, increasing NO production. This would tend to offset the decreased NO bioactivity observed in diabetes mellitus. The second is by inhibiting the formation of angiotensin II. Since elevated levels of angiotensin converting enzyme have been reported in experimental diabetes (Goyal et al., 1998), inhibition of angiotensin converting enzyme activity by temocapril may lead to reduced formation of angiotensin II in diabetes. This might impact by reducing the angiotensin-induced upregulation of superoxide anion radicals by NADH/NADH oxidase shown in other vascular model systems (Ushio-Fukai et al., 1996).

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