Attenuation of Diabetes-Associated Mesenteric Vascular Hypertrophy With Perindopril: Morphological and Molecular Biological Studies

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Vascular disease is now the major cause of morbidity and mortality in the diabetic population. Our group explored the vascular changes associated with experimental diabetes and examined whether these changes can be ameliorated by angiotensin-converting enzyme (ACE) inhibition. The ACE inhibitor perindopril (PE) was administered to streptozotocin-induced diabetic rats for 24 weeks. At death, mesenteric vessels were perfused in vivo followed by assessment of the vascular architecture by quantitative histomorphometry. In a subgroup of animals, RNA was extracted from the mesenteric vasculature for assessment of gene expression of the prosclerotic cytokine, transforming growth factor beta 1 (TGFβ1), and the matrix protein, type IV collagen. Diabetes was associated with smooth muscle hypertrophy and extracellular matrix (ECM) accumulation. ECM accumulation, particularly collagen deposition, was observed in the medial and adventitial layers. ACE inhibition prevented mesenteric vascular hypertrophy after 24 weeks of diabetes. In addition, overexpression of TGFβ1 in the vessels of diabetic animals was prevented by PE treatment. Similarly, type IV collagen mRNA levels were increased in diabetic vessels, and this overexpression was also prevented by PE therapy. In summary, ACE inhibition attenuates many of the vascular changes observed in experimental diabetes and may have important clinical implications as a vasoprotective agent in human diabetes.

DIABETES is associated with the development of vascular complications, and these remain the major cause of morbidity and mortality in this population.[1] Our group has recently explored the changes in the vascular tree in experimental diabetes, with a particular focus on the mesenteric vasculature.[2] We documented that within 3 weeks of induction of diabetes, there was evidence of vascular hypertrophy and extracellular matrix (ECM) accumulation.[3] Furthermore, molecular biological studies have shown evidence within 1 week of induction of diabetes of increased gene expression of the prosclerotic cytokine, transforming growth factor beta 1 (TGFβ1), and the matrix protein type IV collagen in these vessels, and these changes in mRNA levels persist.[4] The present study evaluates both the morphological and molecular biological changes in vessels from animals with chronic diabetes and the effects of the angiotensin-converting enzyme (ACE) inhibitor perindopril (PE) on these parameters.

MATERIALS AND METHODS

Male Sprague-Dawley rats aged 10 weeks and weighing between 250 and 300 g were randomized to receive streptozotocin (Boehringer, Mannheim, Germany) at a dose of 50 mg/kg or citrate buffer alone (control). Diabetic animals were then randomized to receive no treatment or the ACE inhibitor PE (Servier, Courbevoie, France) at a dose of 0.6 mg/kg/d in drinking water. Only diabetic animals with plasma glucose levels greater than 15 mmol/L were included in the study. Diabetic animals were maintained on a dose of 4 U long-acting insulin (Ultralente; Novo-Nordisk, Bagsvaerd, Denmark) administered daily to promote body growth and prevent ketoacidosis. All animals had free access to rat chow and drinking water.

The following parameters were assessed every 4 weeks: body weight, systolic blood pressure (SBP) measured by tail-cuff plethysmography in conscious preheated rats,[5] and glycated hemoglobin by an automated affinity high-performance liquid chromatography method (CLC330; Primus, Kansas City, MO).[6] Mesenteric vessel histology was assessed by quantitative histomorphometry at the end of the 24-week study period as previously described.[2] In brief, animals were anesthetized and the vessels were perfused in vivo at arterial pressure via an intra-aortic cannula with saline followed by 2.5% glutaraldehyde.[7] Assessment of vascular architecture was performed in a similar manner to that previously described by Kranzhofer et al.[8] In brief, 4-μm sections were immunostained with a mouse antibody that strongly cross-reacts with human smooth muscle actin and stains the smooth muscle layer. A videoimaging system (Video Pro 32; Leading Edge, Bedford Park, South Australia, Australia) connected to a Zeiss AXIOPHOT microscope (Oberkocken, Germany) was used for histomorphometric analyses. Data were analyzed as the media to lumen ratio as previously described in other models of vascular hypertrophy.[8] All of these computerized morphometric measurements were blindly performed by one observer (Z.C.). Sections were also stained with Masson's trichrome as originally described.[9] Using this method, nuclei are stained black, smooth muscle is stained red, and collagen is stained blue.

In a separate group of animals treated identically to those just described, mesenteric vessels were removed and stripped of surrounding fat, connective tissue, and veins to yield the superior mesenteric arterial tree.[2] The vessels were weighed, snap-frozen in liquid nitrogen, and stored at −80°C for subsequent isolation of RNA. The vessels were homogenized (Ultra-Turrax; Janke and Kunkel, Staufen, Germany) and total RNA was isolated by the acid guanidinium thiocyanate-phenol-chloroform extraction method. Twenty-microgram samples were denatured and electrophoresed through 0.8% agarose formaldehyde gels. RNA integrity was verified by examination of the 28S and 18S ribosomal RNA bands of ethidium bromide–stained material under UV light. RNA was then transferred onto nylon filters (Hybond-N; Amersham, UK) by capillary action and fixed by UV irradiation. Filters were hybridized with a 985-bp cDNA probe coding for rat TGFβ1 (gift from Dr Qian, National Institutes of Health, Bethesda, MD) and a 1.8-kb cDNA probe coding for mouse α1 (IV) collagen (gift from Dr R. Timpl, Max Planck Institute, Martinsried, Germany). Probes were labeled with [α-^{32}P]dCTP (DuPont, Boston, MA) by random primed DNA synthesis (Boehringer). Hybridization procedures for both TGFβ1 and type IV collagen are as previously described. Hybridization intensity was quantified using a phosphorescent imager (Fujix BASS 3000; Fuji Photo Film, Tokyo, Japan). All results were corrected for differences in RNA loading and transfer by rehybridization with an oligonucleotide probe for 18S rRNA end-labeled with [α-^{32}P]dCTP by terminal

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Table 1. Study Characteristics

Characteristic	Group		
	C	D	DP
No. of rats	12	12	11
Body weight (g)	530 ± 13	389 ± 25*	387 ± 13*
Plasma glucose (mmol/L)	5.2 ± 0.3	23.9 ± 1.8*	21.6 ± 1.0*
Glycated hemoglobin (%)	3.8 ± 0.1	12.9 ± 0.9*	11.5 ± 0.5*
SBP (mm Hg)	132 ± 3	140 ± 3*	117 ± 4*†
Media wall to lumen ratio	1.04 ± 0.02	1.47 ± 0.05*	0.94 ± 0.04†

NOTE. Data are the mean ± SEM at week 24.
Abbreviations: C, control; D, diabetic; DP, diabetic + PE.
*$P < .01$ *v* C.
†$P < .05$ *v* D.

transferase (Boehringer). Results are expressed as the ratio of image intensity of TGFβ1 or α1 (IV) collagen to 18S relative to control vessels that were arbitrarily assigned a value of 1.

All data are shown as the mean ± SEM unless otherwise specified. Data were initially analyzed by ANOVA, with comparisons between group means made by Fisher's least-significant difference method.[10] A *P* value less than .05 was viewed as statistically significant.

RESULTS

Diabetic rats showed a reduced body weight and increased glycated hemoglobin level (Table 1). PE therapy did not affect either parameter. SBP was modestly increased in diabetic rats and was reduced by PE. The mesenteric wall to lumen ratio was increased after 24 weeks of diabetes, and this parameter was attenuated significantly by PE therapy (Table 1). Examination of trichrome-stained sections showed increased thickness of the medial layer in diabetes and associated ECM accumulation, most evident in the adventitial layer (Fig 1). These changes in medial thickness and collagen deposition in the adventitial layer were attenuated by PE treatment (Fig 1). Diabetes was associated with an increase in mRNA levels for TGFβ1 and type IV collagen (Fig 2). Gene expression of TGFβ1 tended to be reduced by PE treatment. Overexpression of type IV collagen in diabetic vessels was normalized by ACE inhibition with PE (Fig 2).

DISCUSSION

The present study confirms our previous finding that the ACE inhibitor PE attenuates mesenteric vascular hypertrophy.[2] Our previous studies focused on vascular changes after 3 weeks of experimental diabetes.[2,11] The present report explored whether the changes described after 3 weeks of diabetes persist long-term. Using quantitative histomorphometric techniques in mesenteric vessels perfused in vivo at SBP, it was evident that not only was there vascular hypertrophy, as assessed by the media to lumen ratio in diabetes, but this increase in the thickness of the medial layer was ameliorated by PE.

The mesenteric vascular bed has been a site of interest in vascular research, with several studies exploring hemodynamic changes in this vascular tree in experimental diabetes.[12,13] Our own group has reported increased levels of ACE in mesenteric vessels from rats after 3 weeks of diabetes, and this increase can be prevented by PE treatment.[2] Whether the changes in the mesenteric vascular tree in diabetes can be extrapolated to other sites such as the kidney has not been clearly determined.

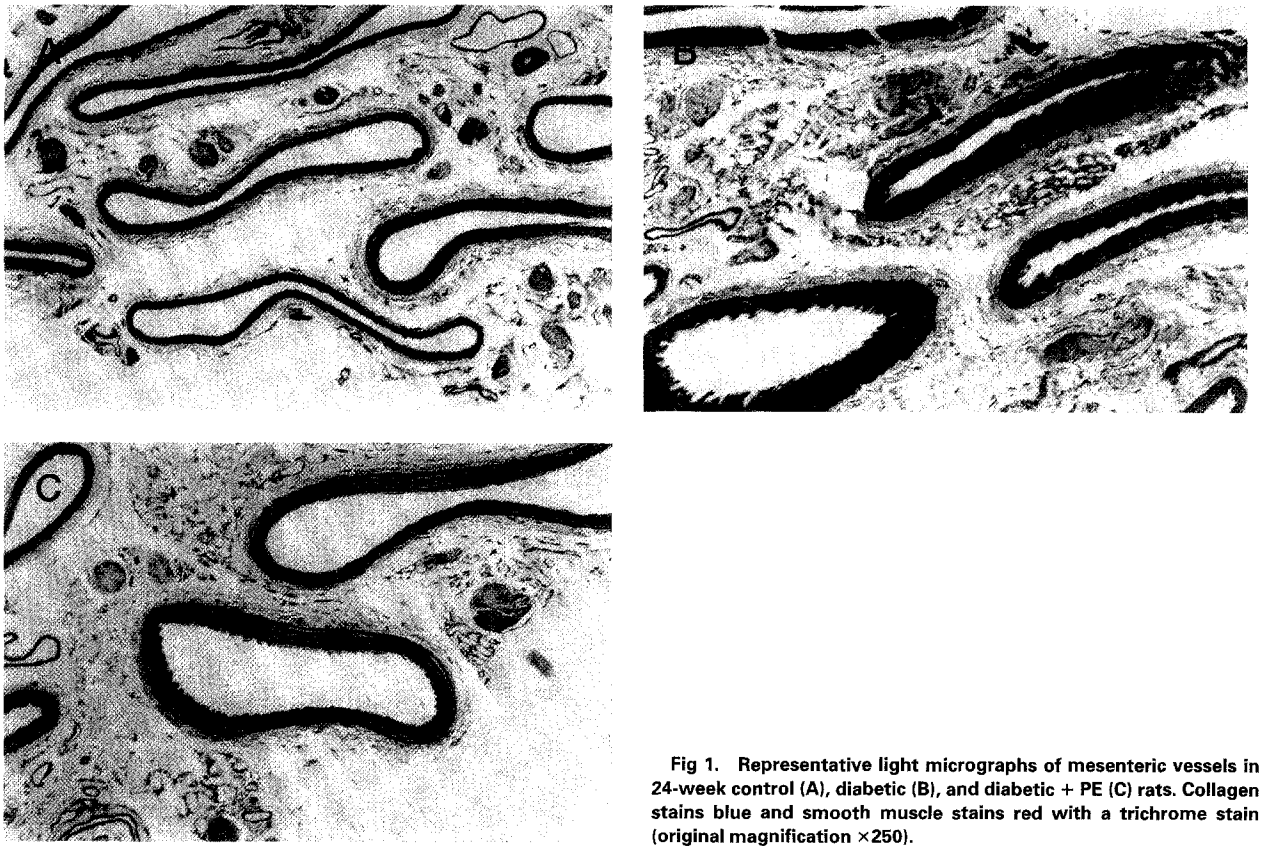

Fig 1. Representative light micrographs of mesenteric vessels in 24-week control (A), diabetic (B), and diabetic + PE (C) rats. Collagen stains blue and smooth muscle stains red with a trichrome stain (original magnification ×250).

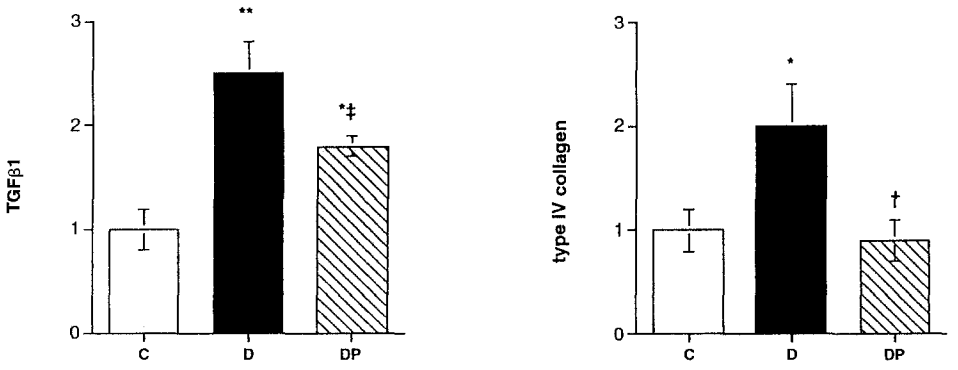

Fig 2. Quantitation of mesenteric TGFβ1 and α1 (type IV) collagen mRNA levels. Data are the mean ± SE optical density ratio of specific mRNA to 18S rRNA, relative to control animals (designated an arbitrary value of 1) at 24 weeks. *$P < .05$, **$P < .01$ *v* C; †$P < .05$, ‡$P = .08$ *v* D. C, control; D, diabetic; DP, diabetic + PE.

Anderson et al[14] have reported increases in ACE in renal vessels and glomeruli in diabetic rats using immunohistochemistry. Our own group has reported that PE prevents the increase in the wall to lumen ratio in arteries from the corticomedullary junction of the kidney in diabetic rats.[15] Therefore, it appears that ACE inhibitors exert their antitrophic effects in a range of vascular beds. In experimental renal ablation, a model of hypertension, progressive renal impairment, and vascular hypertrophy, ACE inhibition has been shown to attenuate vascular lesions not only in the mesenteric bed but also at other vascular sites.[16]

There was evidence in the present study, using Masson's trichrome stain, of widespread collagen deposition in vessels from diabetic rats. Furthermore, this accumulation of ECM was ameliorated by ACE inhibition. Subsequent studies indicated an increase in mRNA levels for the prosclerotic cytokine TGFβ1 in these vessels, as has been previously reported.[4] Furthermore, PE prevented the increase in gene expression for this cytokine, as well as the matrix protein, type IV collagen. This would be consistent with previous studies showing that angiotensin II (AII) is a powerful stimulator of TGFβ production.[17] Our own group has previously linked suppression of TGFβ1 gene expression in vessels to attenuation of diabetes-associated vascular hypertrophy by the inhibitor of advanced glycation aminoguanidine.[4] The importance of TGFβ in the genesis of diabetic complications has been suggested by several investigators, although these studies have generally focused on the kidney.[18,19] The link between TGFβ and AII in vivo has been most clearly demonstrated in nondiabetic models of renal injury. ACE inhibitors and AII receptor antagonists have been shown to be associated with a decrease in TGFβ1 mRNA levels in models of subtotal nephrectomy[20] and ureteric obstruction.[21]

In conclusion, PE has been shown to attenuate mesenteric vascular hypertrophy in both acute and chronic diabetes. It is postulated that these antitrophic effects of ACE inhibition involve suppression of TGFβ expression, a cytokine that would promote collagen deposition in the vascular wall. It remains to be determined if the antitrophic effects of PE in experimental diabetes are of relevance to man. A vasoprotective role for this agent would be of particular benefit in the diabetic population, since these individuals are at high risk of accelerated microvascular and macrovascular injury.[1]

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