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Podocyte foot process broadening in experimental diabetic nephropathy: amelioration with renin-angiotensin blockade

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Abstract

Aims/hypothesis. Changes in podocyte number and morphology have been implicated in the pathogenesis of proteinuria and the progression of human and experimental kidney disease. This study sought to examine podocyte foot process and slit pore architecture in experimental diabetic nephropathy and to determine whether such changes were modified with renoprotective intervention by blockade of the renin-angiotensin system.
Methods. The number of filtration slits per 100 μm of glomerular basement membrane was assessed by transmission electron microscopy and quantitated histomorphometrically in control animals and in rats with 24 weeks of streptozotocin-induced diabetes. Diabetic rats were either untreated or received the angiotensin converting enzyme inhibitor ramipril, or the angiotensin II type 1 receptor antagonist, valsartan.
Results. When compared with control animals, diabetes was associated with a decrease in the number of slit pores per unit length of glomerular basement membrane, indicative of podocyte foot process broadening. Both ramipril and valsartan attenuated these ultrastructural changes to a similar degree. These differences remained after correcting for glomerular volume as a possible confounding variable.
Conclusion/interpretation. Preservation of podocyte architecture could contribute to the renoprotective effects of renin-angiotensin system blockade in diabetic nephropathy. [Diabetologia (2001) 44: 878–882]

Keywords Podocyte, foot process, diabetic nephropathy, ramipril, valsartan.

The kidney and, in particular, the glomerulus undergo major structural changes in diabetes. Changes in mesangial matrix and glomerular basement membrane (GBM) width in diabetes have been studied extensively where the expansion of the mesangial matrix was found to relate closely to declining glomerular filtration rate [1]. The contribution of the glomerular visceral epithelial cell or podocyte to progressive renal disease has also been evaluated in diabetic and non-diabetic renal disease [2, 3]. Unlike the mesangial and endothelial cell components of the glomerulus, the podocyte is terminally differentiated and largely incapable of replication. In addition to constituting the filtration slit diaphragm, the podocytes, like pericytes elsewhere have a key role in counteracting the expansile forces of glomerular capillary pressure [4].

The most commonly identified change in podocyte structure is foot process effacement in which the interdigitating pattern of podocyte attachment to the GBM undergoes substantial simplification. Such changes are frequently identified in experimental [5–7] and human kidney diseases [8]. Agents which block the renin-angiotensin system (RAS), such as ACE inhibitors have been shown to not only reduce

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Abbreviations: GBM, Glomerular basement membrane; RAS, renin-angiotensin system; AT_1, angiotensin II type 1; SBP, systolic blood pressure; GV, glomerular volume.

Table 1. Physical and biochemical parameters at end of study.

Group	*n*	Body Weight (g)	HbA_{1c} (%)	SBP (mmHg)	AER (mg/24hr)	Kidney weight (g)	Glomerular Volume ($\times 10^6 \mu m^3$)
Control	7	650 ± 34	2.3 ± 0.1	128 ± 3	1.1	1.85 ± 0.10	0.92 ± 0.04
Diabetic	6	482 ± 14[a]	4.0 ± 0.2[a]	135 ± 4[a]	4.8[a]	2.43 ± 0.21[a]	1.35 ± 0.08[a]
Diabetic + ramipril	6	465 ± 13[a]	4.0 ± 0.3[a]	126 ± 4[b]	1.3[b]	2.53 ± 0.10[a]	1.04 ± 0.03[b]
Diabetic + valsartan	6	375 ± 17[a,b]	3.7 ± 0.3[a]	128 ± 3[b]	1.0[b]	1.77 ± 0.16[b]	0.86 ± 0.03[b]

HbA_{1c}, glycated haemoglobin; SBP, systolic blood pressure; AER, urinary albumin excretion rate in male Sprague-Dawley rats 24 weeks after induction of control vehicle or diabetes. Data are expressed as means ± SEM except AER expressed as geometric mean. [a] $p < 0.01$ vs control, [b] $p < 0.01$ vs diabetic.

intraglomerular pressure [9] but to also decrease proteinuria and the rate of decline of renal function in diabetic nephropathy [10]. These multiple effects have been attributed to a variety of mechanisms [11] although changes in the glomerular podocyte have not been specifically evaluated, despite the presence of functioning angiotensin II type 1 (AT_1) receptors on this cell type [12].

This study thus sought to examine changes in podocyte foot processes in experimental diabetes and to determine the effect of blockade of the RAS on these changes.

Materials and methods

Male Sprague-Dawley rats (225–250 g) at 8 to 10 weeks of age, were rendered diabetic with streptozotocin (50 mg/kg^{-1}; Boehringer-Mannheim, Mannheim Germany) or received citrate buffer to serve as controls. Diabetic rats were then randomized to receive one of the following regimens: (1) no treatment; (2) the ACE inhibitor ramipril 1 mg/kg in drinking water (Hoechst, Frankfurt, Germany) or (3) the AT_1 receptor antagonist valsartan 30 mg · kg^{-1} · day^{-1} by gavage (Novartis, Basel, Switzerland). Only diabetic animals with plasma glucose concentrations greater than 15 mmol/l were included in the study. Diabetic animals were maintained on a dose of 4 units of long-acting insulin (Ultralente; Novonordisk, Bagsvaerd, Denmark), administered daily to promote weight gain and prevent ketoacidosis. Rats had free access to standard rat chow and drinking water. Experimental procedures adhered to the guidelines of the National Health and Medical Research Council of Australia's Code for the Care and Use of Animals for Scientific Purposes.

After 24 weeks of diabetes, body weight was measured and systolic blood pressure (SBP) measured by tail-cuff plethysmography in conscious pre-warmed rats. Glycated haemoglobin was estimated by high performance liquid chromatography (Biorad, Richmond, Calif., USA) [13]. Rats were placed in metabolic cages (Iffa Credo, L'Arbresele, France) for 24 h to determine albuminuria by radio-immunoassay [14].

Glomerular Structure. Transmission electron microscopic morphometric analysis was carried out to accurately quantify changes in glomerular structure [15]. Renal histology was assessed by quantitative histomorphometry [16]. Animals were anaesthetised with pentobarbital sodium and the kidneys were perfused in vivo at arterial pressure via an intra-aortic cannula with saline, followed by 2.5 % glutaraldehyde. Kidney sections were then prepared for light (paraffin sections) and electron microscopic examination. The glomerular cross-sectional area (GA) was measured in 30 glomerular profiles per rat, using a videoimaging system (Video Pro 32, Leading Edge, Bedford Park, South Australia, Australia) connected to a Zeiss AXIOPHOT microscope (Oberkocken, Germany). The glomerular volume (GV) was then calculated as: $GV = (\beta/k)\,(GA)^{3/2}$, where $\beta = 1.38$ is the size-distribution coefficient and $k = 1.1$ is the shape coefficient for glomeruli idealized as a sphere [17]. For electron microscopy, twenty evenly spaced electron micrographs were obtained at a final magnification of × 18000 from one randomly chosen thin section from one randomly selected glomerulus from each rat. To determine the number of filtration slits per 100 μm of GBM, the number of filtration slits was obtained by counting the number of slit pores from each set of micrographs. The length of the GBM was calibrated and then measured using the computer-based program Sigma Scan Pro 4.0. All measurements were done in a masked fashion.

Statistical Analysis. All data are shown as means ± SEM unless otherwise specified. Because of a positively skewed distribution albuminuria was logarithmically transformed before analysis and shown as geometric means. Data were analysed by analysis of variance with comparisons between group means by Fisher's least significant difference method. A p value of less than 0.05 was considered as statistically significant.

Results

Metabolic and Biochemical Parameters. After 6 months, diabetes was associated with reduced body weight gain compared to control rats ($p < 0.01$) and was not improved with either ramipril or valsartan treatment (Table 1). Diabetes was associated with an increase in glycated haemoglobin, SBP, AER and kidney weight (Table 1; $p < 0.01$). In rats treated with either ramipril or valsartan, SBP and AER were similar to that in control animals. Glycated haemoglobin was not affected by any of the drug regimens.

Glomerular Structure. Control rats showed a thin GBM with relatively evenly spread filtration slit pores (Fig. 1A). In contrast, diabetes was associated with a diminution in the number of slit pores per 100 μm of GBM ($p < 0.01$) indicative of foot process

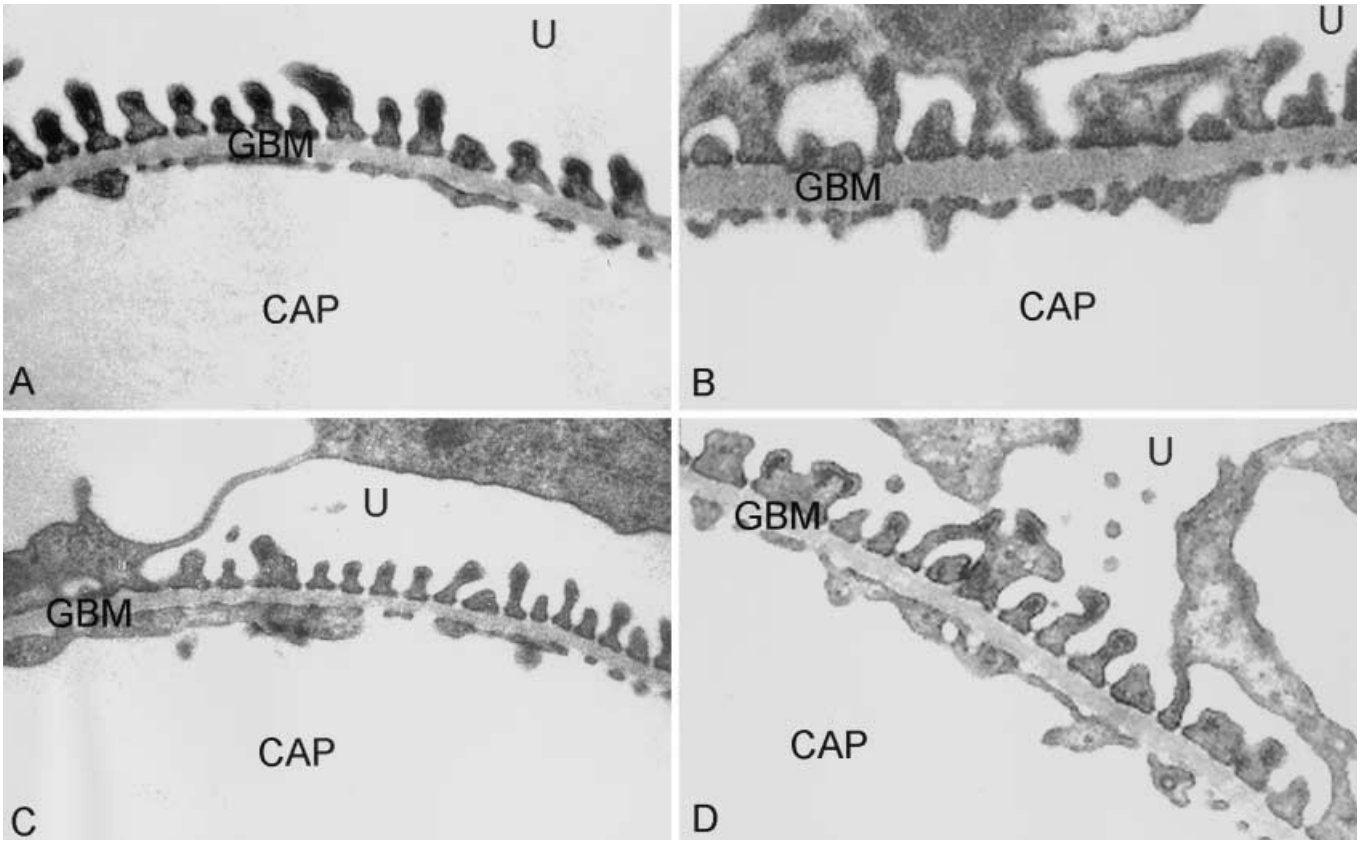

Fig. 1 A–D. Transmission electron micrographs of representative glomerular capillary loops from Sprague-Dawley rats 24 weeks after induction of control vehicle or diabetes. Control (**A**), diabetic (**B**), diabetic + ramipril (**C**) and diabetic + valsartan (**D**). GBM, glomerular basement membrane; CAP, capillary lumen; U, urinary space (Magnification × 14 200)

broadening (Fig. 2A). In addition, irregularity in podocyte foot process dimensions and spacing were also noted along with thickening of the GBM (Fig. 1B). These effects were attenuated by treatment with both ramipril (Fig. 1C), and valsartan (Fig. 1D). These quantitative changes persisted in all groups after correcting for glomerular volume (Table 1), as a possible confounding variable (Fig. 2B).

Discussion

Experimental diabetes was associated with a reduction in the number of slit pores per unit length of GBM foot indicative of foot process broadening. In both ramipril- and valsartan-treated diabetic rats slit pore number approximated that of control animals. These findings suggest that not only is podocyte foot process structure altered in experimental diabetes but that it can be attenuated by agents which interrupt the RAS.

In humans, podocyte loss develops with progressive albuminuria, such that in patients with overt nephropathy the total number of podocytes per glomerulus is reduced, without change in glomerular surface area [3]. Therefore, when compared with the glomeruli of control subjects, in diabetic nephropathy each podocyte is obliged to cover a greater surface area. The reduction in slit pore number per length of GBM as found in our study suggests that not only can podocytes not replicate but that remaining podocytes are not able to elaborate a sufficient number of foot processes to compensate for this loss. We found ramipril and valsartan treatment prevented the reduction in slit number per GBM length in diabetic animals. Whether this reflected a prevention of diabetes-associated podocyte loss or occurred as a consequence of de novo foot process elaboration cannot be determined from our study. However, the recent finding of an anti-apoptotic effect of RAS blockade in diabetes suggests that the former mechanism could be operational [18]. Podocyte number predicts progression of nephropathy in microalbuminuric Pima Indians with Type II (non-insulin-dependent) diabetes mellitus [19], suggesting that prevention of podocyte loss would be therapeutically relevant. Recent studies, however, also suggest a more direct effect of the RAS on the slit pore, showing that angiotensin II induces biochemical modifications in zonula occludens-1 that are attenuated by ACE inhibitor therapy [20]. A wide range of receptors for vasoactive hor-

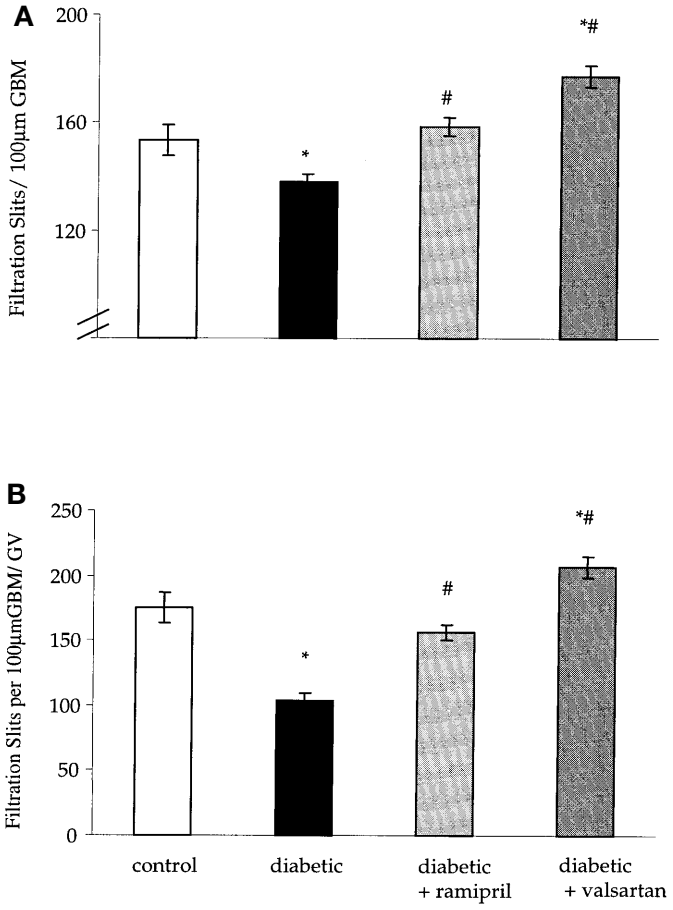

Fig. 2 A, B. The number of filtration slits per 100 μm of glomerular basement membrane (GBM) (**A**), and when the same experimental data are corrected for glomerular volume (GV) (**B**). Results are shown as means ± SEM. * $p < 0.01$ vs control. # $p < 0.01$ vs diabetic

mones and growth factors have been identified on the podocyte, including the presence of type 1 and type 2 angiotensin II receptors [12, 21] and receptors for bradykinin [22].

The relation between glomerular slit pores, proteinuria and glomerular filtration are complex. A reduction in slit pore number per unit capillary length in combination with GBM thickening, as shown in this study, would theoretically both lead to reduced ultrafiltration per capillary surface area [23]. However, these effects are counterbalanced by the increased glomerular volume and increased intraglomerular pressure such that in the presence of a normal ultrafiltration co-efficient [24, 25] glomerular hyperfiltration persists. Both ramipril and valsartan have multiple effects on these parameters such that not only did slit pore number approximate those in control animals but similar beneficial effects have also been noted in GBM thickness, glomerular volume and mesangial expansion [26]. These findings suggest that in diabetes, changes in glomerular filtration and protein permeability and the effects of RAS blockade are likely to reflect interactions between epithelial, mesangial and capillary endothelial components of the glomerulus.

We found SBP was increased in diabetic rats and normalised with ramipril therapy. Whereas hypertension is an important factor in the progression of renal disease it has been clearly established in both clinical and experimental studies that agents which block the RAS have a renoprotective effect beyond that due to blood pressure reduction alone [10, 27, 28]. In a model of experimental renal disease, ramipril, but not nifedipine preserved podocyte structure, despite equivalent blood pressure reduction [29] suggesting a specific role for the RAS in influencing podocyte structure in renal diseases.

In summary, experimental diabetes was associated with significant ($p < 0.01$) changes in podocyte foot processes and slit pore number that were attenuated by ACE inhibition and AT_1 receptor blockade. These findings suggest that such structural changes could contribute to altered glomerular function in diabetes and the therapeutic effects of blockade of the RAS.

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