2-5-2002

Potentiation of glucose-mediated glomerular injury by mechanical strain

Pedro Cortes
Henry Ford Health

Jerry Yee
Henry Ford Health, JYEE1@hfhs.org

Recommended Citation
Cortes P, Yee J. Potentiation of glucose-mediated glomerular injury by mechanical strain. Clinical and Experimental Pharmacology and Physiology 2002; 29(1-2):149-152.

Clinical and Experimental Pharmacology and Physiology (2002) **29**, 149–152

Experimental Biology 2001 Early Impact of Diabetic Hyperglycemia on Renal and Cardiovascular Function

POTENTIATION OF GLUCOSE-MEDIATED GLOMERULAR INJURY BY MECHANICAL STRAIN

Pedro Cortes and Jerry Yee

Division of Nephrology and Hypertension, Henry Ford Hospital, Detroit, Michigan, USA

SUMMARY

1. The glomerular injury of diabetes is characterized by the progressive accumulation of extracellular matrix in the mesangial regions, ultimately resulting in glomerulosclerosis.

2. The excessive glomerular extracellular matrix formation associated with the haemodynamic alteration of diabetes is the result of mesangial mechanical strain.

3. The increased synthesis and deposition of extracellular matrix is augmented by the presence of high glucose concentrations.

4. Both mechanical strain and high glucose share many of the mechanisms mediating their metabolic effects, including the stimulation of prosclerotic growth factors.

5. Little is known about factors that may influence the long-term effects of mechanical strain, but the preservation of the F-actin cytoskeleton is likely an important modulator of the resulting injury.

Key words: biomechanics, diabetes, glomerulosclerosis, hyperglycaemia, mechanical strain, mesangial, nephropathy.

INTRODUCTION

Numerous studies have described the glomerular injury resulting from haemodynamic stress and the adverse influence of concomitant conditions of high glucose concentration.[1] However, it has been largely unclear how the altered intraglomerular pressure in hyperglycaemic conditions translates into the accumulation of extracellular matrix and eventual glomerulosclerosis.

GLOMERULAR ELASTICITY

Although differences in glomerular volume between immersion- and perfusion-fixed renal specimens have intimated glomerular elasticity[2] (i.e. the capability to recover size after deformation), this property has been largely ignored. We have directly documented this elasticity in the isolated, microperfused glomeruli of the rat and rabbit, wherein compliance (or the ability to yield elastically when internal force is applied) can be measured.[3] Normal glomeruli demonstrate a surprisingly high compliance, increasing their volume proportionally with internal pressure up to 25% of the basal unperfused value when maximal physiological intraglomerular pressures were reached (Fig. 1). Furthermore, pressure-distended glomeruli are highly elastic, decreasing their volume to their unperfused level within 3–4 s after the removal of internal pressure. This suggests that glomeruli are capable of rapidly altering their volume with even slight and transient variations in perfusion pressure. The most important consequences of this property arise when afferent arteriolar autoregulation is impaired with the associated unrestrained pressure transmission, as in the remnant kidney and in diabetes.[4]

A systematic scrutiny of the various determinants of glomerular distention demonstrated that, in addition to intraglomerular pressure, glomerular size and the passive component of structural rigidity were the most important factors (Fig. 2). The effect of glomerular size could be related to the number and/or length of capillaries or to the capillary diameter, while the effect of structural rigidity was presumably a function of the quantity, composition and distribution of the extracellular matrix. The active component that opposes distention, which is provided by mesangial cell contraction, was studied in angiotensin II-perfused glomeruli. Surprisingly, this component only accounted for approximately 4% of the total glomerular rigidity.[3]

Interestingly, glomerular compliance may be altered in disease. Contrary to what could be expected, remnant glomeruli and glomeruli from long-term diabetic animals, both undergoing incipient sclerosis, demonstrated 59 and 14% increased compliance, respectively.[3,5] The increased compliance in diabetic glomeruli could be fully accounted for by glomerular hypertrophy. However, a second factor, related to lesser structural rigidity, was an important contributor to the marked change observed in remnant glomeruli.

MECHANICAL STRAIN AND GLOMERULOSCLEROSIS

The biological consequences of glomerular distention are illustrated by the metabolic behaviour of mesangial cells in tissue culture that are subjected to repetitive stretch as a form of mechanical strain. This mesangial strain is likely to occur *in situ* because glomerular

Correspondence: Dr Pedro Cortes, Division of Nephrology and Hypertension, CFP-5, Henry Ford Hospital, 2799 West Grand Blvd, Detroit, MI 48202, USA. Email: cortes.pedro@usa.net

Presented at the Experimental Biology Symposium Early Impact of Diabetic Hyperglycemia on Renal and Cardiovascular Function, Orlando, Florida, USA, March/April 2001.

Received 10 May 2001; accepted 5 September 2001.

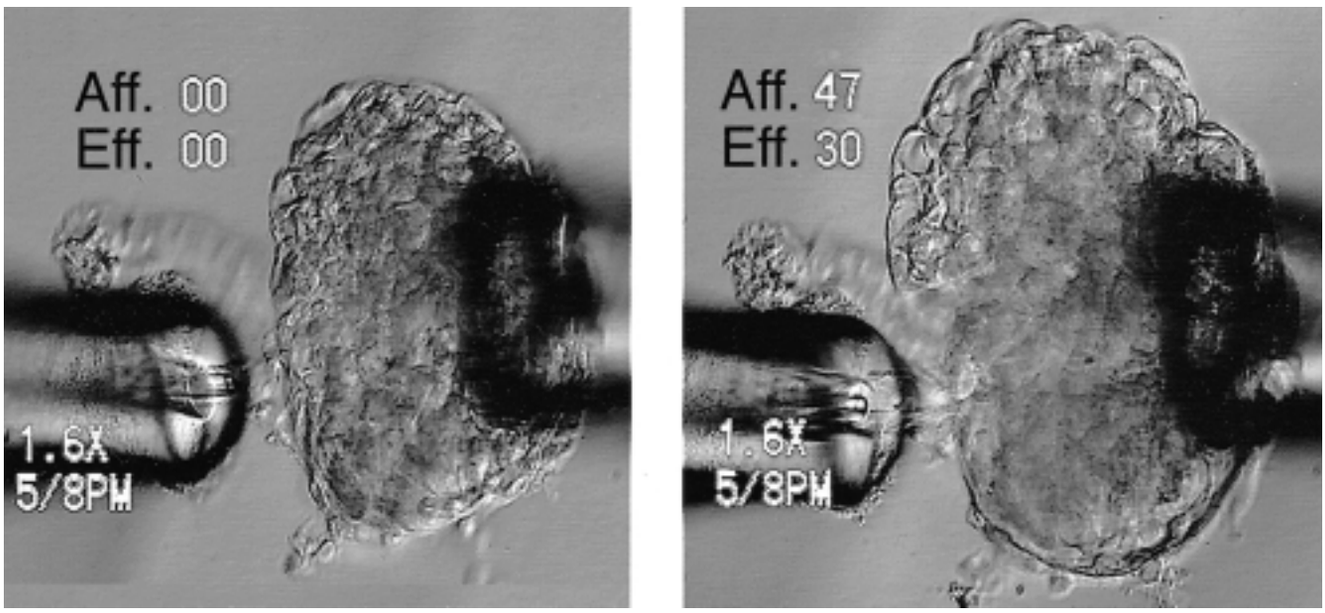

Fig. 1 Pressure-induced glomerular distention. The afferent and efferent arterioles of a freshly microdissected glomerulus were cannulated. Micropipettes for pressure monitoring were placed at the terminal and distal ends of the afferent and efferent arteriole, respectively. Glomerular volume was measured at different levels of transglomerular pressure difference induced by increasing flow rates. At pressures of 47 and 30 mmHg in the afferent and efferent arterioles, respectively, there was a 30% increase in glomerular volume over the basal unperfused value. The same glomerulus is shown before perfusion (left panel) and during perfusion at the indicated pressure (right panel).

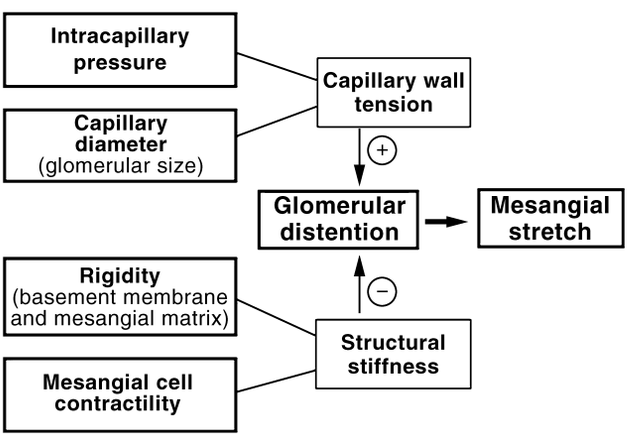

Fig. 2 Main morphological and functional determinants of glomerular distention and mesangial stretch.

distention is associated with comparable stretch of all its components, including the mesangial regions.[3] The continued cyclic stretch of mesangial cells stimulates the synthesis of extracellular matrix components, particularly collagen and fibronectin.[6,7] Furthermore, the augmented synthesis is proportional to the magnitude of the amplitude of stretch. Remarkably, the stimulation of matrix formation is greatly enhanced by an environment of high glucose concentration, likely via the stimulation of transforming growth factor (TGF)-β1 action.[8] Under these conditions, synthesis of collagen outpaces its catabolism, thus resulting in significant accumulation.

We and others[9] have shown that the stretch-stimulated formation of extracellular matrix is importantly mediated by the action of TGF-β, even under physiological glucose conditions.[10] Within 48–72 h of cyclic stretch of mesangial cells in culture, the latent and active forms of TGF-β1 accumulate in the medium. In addition, the

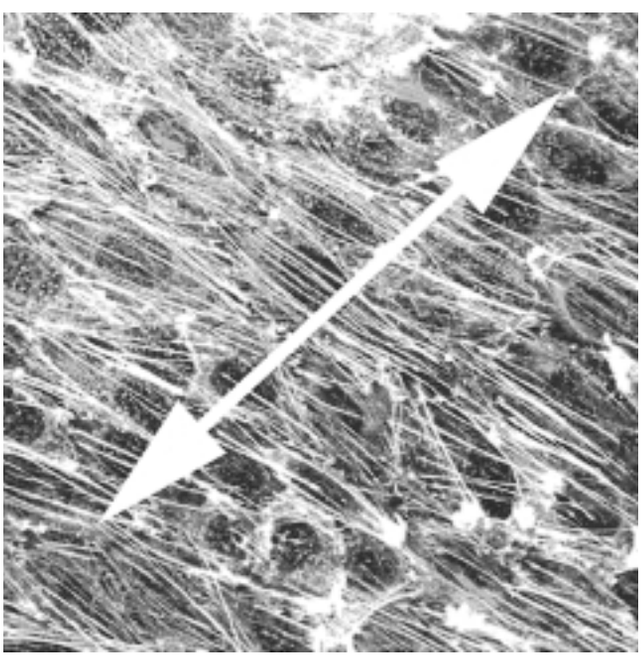

Fig. 3 Cellular and cytoskeletal alignment of mesangial cells subjected to repetitive cyclic stretch. A confluent culture of rat mesangial cells was stretched at 3 cycles/min, 25% maximum elongation, for 24 h. Cultures were fixed and the F-actin visualized by fluorescein-labelled phalloidin. The image is a *z*-axis reconstruction of 12 0.3 μm optical planes obtained by laser-confocal microscopy at a ×40 magnification. Shown are cells aligned in a perpendicular direction to the stretching force (indicated by the double arrow). F-Actin in stress fibres is also aligned in the same direction.

action of this growth factor is further enhanced because TGF-β receptors and TGF-β1-specific binding are both augmented. Finally, neutralization of TGF-β action during stretch under conditions of

high glucose concentration significantly minimizes the stimulated formation of matrix.[8]

Therefore, it is clear that the mechanical strain imposed on mesangial cells in diabetes and its metabolic sequelae are greatly accentuated by the concomitant increases in glomerular compliance and glucose concentration. However, the mechanisms that may modulate the long-term effects of mechanical strain remain unknown.

MODULATION OF MECHANICAL STRAIN

Intuitively, the transmission of the mechanical signals, or the cellular response evoked by them, should involve the cytoskeleton. The obvious candidate cytoskeletal components are those that provide mechanical stability to the cell. In the case of the glomerulus, a rich cytoskeleton of vimentin and stress fibres has been described in podocytes and mesangial cells in tissue culture.[11,12] Stress fibres are labile contractile bundles of filamentous actin (F-actin) that crisscross the cytoplasm as tense cables joining opposing focal adhesions. F-Actin is known to be essential for the maintenance of cell shape and cell migration. The intermediate filaments formed by vimentin are distributed as a non-contractile, highly elastic network of fine filaments dispersed throughout the cytoplasm that preferentially localize to the perinuclear region.

The most obvious morphological change in mesangial cells during stretch is a uniform elongation of the cell body with alignment in a direction perpendicular to the stretching force (Fig. 3). This cell reorganization is associated with stress fibre redistribution that appears as alignment along the major cell axis. Interestingly, endothelial cells subjected to fluid shear strain also realign, but in a direction that is in parallel to the flow. These observations suggest that F-actin-directed cell realignment is part of an adaptive process to minimize mechanical strain. However, although actin is rapidly depolymerized/polymerized, the alignment of stress fibres is not evident until after 12 h of continuous stretch.

The importance of F-actin realignment as a mechanism to modulate the metabolic response to mechanical strain is demonstrated by the effects of F-actin fibre disruption. Specific induction of F-actin disassembly with cytochalasin D prevents mesangial cell alignment during stretch. Interestingly, this actin disassembly is also associated with an increased accumulation of fibronectin. The increased medium content of this critical extracellular matrix component is already evident after 24 h of stretch. (Fig. 4). Finally, the cytoskeletal effects of cytochalasin D are fully reversible because its removal from the incubation medium is followed by the reappearance of an aligned system of stress fibres. In conclusion, an intact system of stress fibres capable of realignment appears to modulate the long-term metabolic effects of stretch.

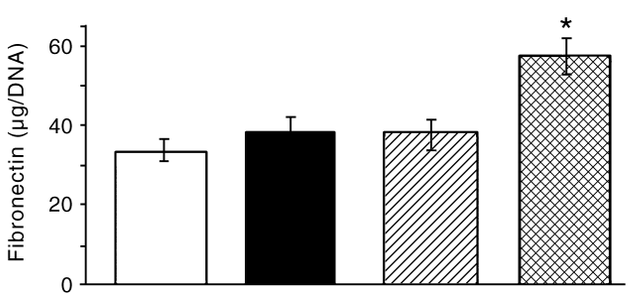

Fig. 4 Effects of stress fibre disassembly on mesangial cell formation of fibronectin. Rat mesangial cells were stretched in tissue culture as in Fig. 3 in the abscence (▨) or presence (▩) of 0.5 µmol/L cytochalasin D. Medium fibronectin and cell layer DNA were measured at the end of the stretch period. (■), static + cytochalasin D. *$P < 0.001$ compared with stretched and no cytochalasin D.

GLUCOSE-INDUCED CYTOSKELETAL ALTERATIONS

It is known that mesangial cells incubated in high glucose concentrations demonstrate disassembly of stress fibres, an alteration that may be mediated via protein kinase C activation.[13] We have reproduced and quantified this change following incubation in 30 mmol/L glucose for 12 h. Under these conditions, mesangial cells are still capable of alignment during stretch, but they contain a less abundant system of stress fibres. Thus, these observations suggest that high glucose concentrations may magnify the stretch-induced stimulation of matrix synthesis, at least in part, through its effects on actin polymerization. However, as in many other similar circumstances, the *in situ* relevance of these *in vitro* findings is to be proven.

DIABETES-INDUCED GLOMERULAR CYTOSKELETAL ALTERATIONS

We have recently studied the cytoskeletal characteristics of glomerular cells *in situ*.[14] To obtain morphological conditions closely approximating those in the perfused kidney *in vivo*, we studied glomeruli fixed by microperfusion at physiologically relevant intraglomerular pressure and glomerular distention. In normal glomeruli, stress fibres are almost exclusively present in the mesangial regions. Although podocytes demonstrate a rich system of tightly packed vimentin fibres, the only F-actin detected was localized at the base of the foot processes forming a delicate network enveloping the peripheral capillaries. In contrast, the mesangium contained abundant stress fibres in a unique distribution. Different from mesangial cells in culture, these fibres appeared as a dense system of short, undulating filaments of diverse thickness that often encircle mesangial vascular spaces. This distribution suggests that, rather than influencing overall glomerular volume, mesangial cell contraction may regulate blood flow to specific capillary areas, thus regulating single nephron glomerular filtration rate.

Mesangial stress fibres in microperfused-fixed glomeruli of rats after 9 months of streptozotocin-diabetes demonstrated important alterations.[14] After this period of diabetes, most glomeruli showed mild to moderate mesangial expansion on light microscopy. To exclude glomeruli with significant sclerosis, only well-perfused glomeruli were selected for study. In contrast with glomeruli from control animals, the mesangium of diabetic glomeruli presented either a highly disorganized system of stress fibres or a total absence of these. These findings suggest that, as shown in tissue culture, the high glucose concentration of diabetes causes mesangial stress fibre disassembly and, possibly, greater susceptibility to the injury of mechanical strain. Furthermore, although studies are lacking at early periods of diabetes when hyperfiltration is preponderant, it is hypothesized that the absence of an organized contractile mechanism in the mesangial region may explain the glomerular hyperfunction that is characteristic of the disease.

ACKNOWLEDGEMENTS

Preparation of this review and the authors' work described were supported, in part, by a grant from the Juvenile Diabetes Foundation International awarded to PC (1-200-706).

REFERENCES

1. Hostetter TH. Mechanisms of diabetic nephropathy. *Am. J. Kidney Dis.* 1994; **23**: 188–92.
2. Miller P, Meyer TW. Effects of tissue preparation on glomerular volume and capillary structure in the rat. *Lab. Invest.* 1990; **63**: 862–6.
3. Cortes P, Zhao X, Riser BL, Narins RG. Regulation of glomerular volume in normal and partially nephrectomized rats. *Am. J. Physiol.* 1996; **270**: F356–70.
4. Hayashi K, Epstein M, Loutzenhiser R, Forster H. Impaired myogenic responsiveness of the afferent arteriole in streptozotocin-induced diabetic rats: Role of eicosanoids. *J. Am. Soc. Nephrol.* 1992; **2**: 1578–86.
5. Cortes P, Zhao X, Riser BL, Narins RG. Role of glomerular mechanical strain in the pathogenesis of diabetic nephropathy. *Kidney Int.* 1997; **51**: 57–68.
6. Riser BL, Cortes P, Zhao X, Bernstein J, Dumler F, Narins RG. Intraglomerular pressure and mesangial stretching stimulate extracellular matrix formation in the rat. *J. Clin. Invest.* 1992; **90**: 1932–43.
7. Yasuda T, Kondo S, Homma T, Harris RC. Regulation of extracellular matrix by mechanical stress in rat glomerular mesangial cells. *J. Clin. Invest.* 1996; **98**: 1991–2000.
8. Riser BL, Cortes P, Yee J *et al.* Mechanical strain- and high glucose-induced alterations in mesangial cell collagen metabolism: Role of TGF-β. *J. Am. Soc. Nephrol.* 1998; **9**: 827–36.
9. Hirakata M, Kaname S, Chung U-N *et al.* Tyrosine kinase dependent expression of TGF-β induced by stretch in mesangial cells. *Kidney Int.* 1997; **51**: 1028–36.
10. Riser BL, Cortes P, Heilig C *et al.* Cyclic stretching force selectively up-regulates transforming growth factor-β isoforms in cultured rat mesangial cells. *Am. J. Pathol.* 1996; **148**: 1915–26.
11. Camussi G, Mariano F, Biancone L, Montrucchio G, Vercellone. Effect of cytokines on the cytoskeleton on resident glomerular cells. *Kidney Int.* 1993; **43** (Suppl. 39): S32–6.
12. Weinstein T, Cameron R, Katz A, Silverman M. Rat glomerular epithelial cells in culture express characteristics of parietal, not visceral, epithelium. *J. Am. Soc. Nephrol.* 1992; **3**: 1279–87.
13. Zhou X, Li C, Dlugosz J, Kapor-Drezgic J, Munk S, Whiteside S. Mesangial cell actin disassembly in high glucose mediated by protein kinase C and the polyol pathway. *Kidney Int.* 1997; **51**: 1797–808.
14. Cortes P, Mendez M, Riser BL *et al.* F-Actin fiber distribution in glomerular cells structural and functional implications. *Kidney Int.* 2000; **58**: 2452–61.