6-3-1999

Mechanical strain of glomerular mesangial cells in the pathogenesis of glomerulosclerosis: Clinical implications

P Cortes
Henry Ford Health

B. L. Riser
Henry Ford Health

Jerry Yee
Henry Ford Health, JYEE1@hfhs.org

R. G. Narins
Henry Ford Health

Recommended Citation
Cortes P, Riser BL, Yee J, Narins RG. Mechanical strain of glomerular mesangial cells in the pathogenesis of glomerulosclerosis: Clinical implications. Nephrology Dialysis Transplantation 1999; 14(6):1351-1354.

Nephrol Dial Transplant (1999) 14: 1351–1354

Nephrology
Dialysis
Transplantation

Editorial Comment

Mechanical strain of glomerular mesangial cells in the pathogenesis of glomerulosclerosis: clinical implications

Pedro Cortes, Bruce L. Riser, Jerry Yee and Robert G. Narins

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

Introduction

The pre-glomerular vasodilation that characterizes a wide range of renal diseases allows delivery of a greater fraction of normal or elevated systemic pressure to glomerular capillaries. This glomerular hypertension is, at the very least, a strong contributing force to the glomerulosclerosis seen in many progressive nephropathies. How the physical force of high intraglomerular pressure is translated into the biochemical process of glomerular scar formation, i.e. extracellular matrix (ECM) accumulation, has until recently, been largely unexplored.

In the following paragraphs, citing our work and that of others, we will show that the mesangial cell stretch provoked by capillaries distended by hypertension, triggers the release of cytokines, including transforming growth factor-beta (TGF-β). The actions of these cytokines are, at least in part, responsible for net mesangial matrix synthesis. Thus, the mechanical strain of hypertension spawns the injurious accumulation of glomerular ECM which ultimately causes renal insufficiency.

Glomerular pressure: a determinant of capillary and mesangial expansion

Glomeruli in kidneys perfused and fixed *in situ*, have a larger volume than glomeruli in immersion-fixed specimens from non-perfused kidneys [1,2]. Preservation of glomerular pressure in the former setting presumably maintains expanded capillaries, implying the presence of an intrinsic pliability that allows the glomerulus to distend and contract. The characteristics of glomerular elasticity or compliance, the property that allows pressure to regulate volume, were not defined until studies in isolated microperfused glomeruli became possible [2,3]. These studies have demonstrated that the glomerulus is indeed a highly elastic structure capable of marked volume changes within 2–3 s of altering intraglomerular pressure. Further, the attainable degree of glomerular distension can be extensive. Increasing capillary pressure from zero to physiological levels expands normal glomeruli up to 30% of their basal volume [4]. However under normal conditions, because glomeruli are exposed to only small pulse pressure variations and not to the low frequency, moment-to-moment oscillations in systemic pressure, volume remains stable [5]. This tight control of intraglomerular pressure is due to the very effective autoregulation provided by the contractile activity of the afferent arteriole [6].

This autoregulatory glomerular protection is characteristically impaired in many models of progressive renal disease, including the remnant and diabetic kidney [6,7]. Continuous systemic blood pressure monitoring has shown that in addition to the increasing mean arterial pressure, subtotal nephrectomy causes marked augmentation of the moment-to-moment variations in pressure [8]. Therefore, impaired autoregulation allows for the intraglomerular transmission of systemic pressure, resulting in wide swings in glomerular volume, an alteration which is further magnified by arterial hypertension. Normal glomeruli with intact autoregulation vary their moment-to-moment volume by a mere 0.4%, while glomeruli from remnant kidneys of hypertensive animals demonstrate variations of up to 7.3% [4]. In addition, it has been shown that changes in overall volume are associated with parallel variations in all the glomerular structural components, including the mesangial areas [4].

Mechanical strain imposed on mesangial cells

Located at the centre of the glomerular lobule, mesangial cells extend cytoplasmic projections which attach to the peripheral basement membrane at the points where it deflects from its pericapillary course and at the perimesangeal areas [9]. With pressure-induced glomerular expansion, the outward displacement of these anchoring points caused by distending capillaries and mesangium results in intense mesangial cell stretch. It is therefore, anticipated that cyclic changes in

Correspondence and offprint requests to: Pedro Cortes, MD, Division of Nephrology and Hypertension, CFP-519, Henry Ford Hospital, 2799 West Grand Boulevard, Detroit, MI 48202, USA.

glomerular volume are associated with repeated episodes of mesangial cell stretch and relaxation.

The effects of cyclic stretch–relaxation of mesangial cells has been investigated in tissue culture [2,10]. These studies demonstrated that mechanical strain induces profound changes in the synthesis and catabolism of ECM components leading to their accumulation in the cell layer and in the incubation medium. Furthermore, the intensity of this metabolic change is proportional to the degree of mechanical strain imposed on the mesangial cell. Interestingly, the accumulation of ECM induced by cell stretch is markedly enhanced when the ambient glucose concentration is increased [10].

It logically follows from the above that haemodynamically induced glomerular expansion–contraction may stimulate mesangial ECM deposition and mesangial expansion with the eventual development of glomerulosclerosis [11]. In addition, the hyperglycaemia of diabetes mellitus may sensitize glomeruli to these adverse metabolic effects of mechanical strain.

Mediators of the mechanically-induced metabolic change

How mesangial cell stretch leads to the deposition of ECM is not precisely known, but recent studies have identified the participation of specific growth factors. Cyclic stretch of mesangial cells in culture is associated with the over-expression and activation of the cytokine, transforming growth factor-β1 (TGF-β1) and the upregulation of its specific receptors on mesangial cells [12,13]. This is a highly relevant finding because abundant *in vivo* and *in vitro* evidence points to the participation of this cytokine in various forms of glomerulosclerosis [14,15]. TGF-β1 neutralization experiments during cycles of stretch–relaxation of mesangial cells incubated in media with high glucose concentrations, have demonstrated that the accumulation of ECM products is mainly ascribable to this growth factor [16]. However, the metabolic effects of mechanical strain do not appear to be a result of TGF-β1 action under conditions of physiological glucose concentration, suggesting that other, as yet undefined mediators may be at play. More recently a newly identified prosclerotic cytokine, connective tissue growth factor (CTGF), has also been shown to be upregulated in stretched mesangial cells, although it remains unknown if its participation is essential for the accumulation of ECM [17]. Since the actions of TGF-β1 and CTGF appear to be intimately related, concurrently stimulating the synthesis and deposition of collagenous tissue, it is likely that they may act in concert. In relation to the relevance of mesangial cell mechanical strain in the pathogenesis of diabetic glomerulosclerosis, it is interesting that stretch-induced upregulation of TGF-β1, its receptors and CTGF are all accentuated in cultures exposed to a high glucose concentration.

The cellular sensing mechanism that translates mechanical force into biochemical changes, remains largely unknown. Possible key components of this translating system have been identified in terms of their sequence of activation. Within 30 min following cyclic stretch, mesangial cell phosphorylation of the focal adhesion kinase, $pp125^{FAK}$, is increased, suggesting that the points at which the cell anchors to its substrate and possibly the cytoskeleton, are involved in the early steps of the translating mechanism [18]. In addition, after only 5–30 min a significant stimulation of signalling cascades controlled by protein kinase C [19] and mitogen-activated protein kinases [20] is detectable. Therefore, even brief periods of glomerular distension and mesangial cell strain may effectively initiate a chain of events which, if perpetuated, alters the deposition of ECM.

Control of glomerular volume

Because restriction of glomerular distension may be pivotal in avoiding the progression of glomerular sclerosing processes, studies of the pathophysiologic factors which regulate glomerular volume have become critical. Since intraglomerular pressure is an obvious and major factor [4], it follows that perfusion pressure, the autoregulatory capacity of the afferent arteriole and the tone of the efferent arteriole, all play key roles in determining the state of glomerular distension. In this regard, it is important to note that calcium channel blockers, while diminishing systemic pressure, tend to dilate afferent arterioles [21]. The balance struck by these drugs between diminishing perfusion pressure and its oscillations and the prevalent afferent arteriolar resistance will determine whether glomerular pressures are favourably or unfavourably altered. Converting enzyme inhibitors and angiotensin II-receptor blockers may lower intraglomerular pressure by both decreasing systemic pressure and inducing efferent arteriolar vasodilatation. In addition, these agents may also inhibit the direct- or TGF-β1-mediated angiotensin II enhancement of collagen deposition [22,23]. While differences in action between these antihypertensive agents exist, settings where afferent arteriolar autoregulation is totally absent, a major factor in alleviating glomerular strain is the decrease in systemic pressure and its oscillations, regardless of how this is achieved.

A second factor determining glomerular expansion is the basal glomerular volume [4]. At any given intraglomerular pressure, larger, hypertrophied glomeruli are more distensible than smaller glomeruli. Although this effect could be the mere result of capillary lengthening, it is more likely related to increased capillary wall tension resulting from the increased radius of hypertrophied capillaries [24]. Consequently, whether induced by nephron loss or diabetes, glomerular hypertrophy magnifies the deleterious effects of altered glomerular haemodynamics. The only maneuvers known to ameliorate this hypertrophy are strict metabolic control of diabetes and protein restriction [25,26]. Whether calcium channel blockers may

also limit glomerulosclerosis by reducing glomerular hypertrophy, remains controversial [27,28].

A third factor influencing glomerular distension is the intrinsic rigidity of the glomerular scaffold, primarily provided by the composition and distribution of the collagenous components in the peripheral basement membrane and mesangium. Remnant glomeruli undergoing incipient sclerosis demonstrate a paradoxical diminution in rigidity, rendering them more compliant and prone to distension, independently of their basal size [4]. In contrast, glomerular rigidity is normal in glomeruli of diabetic animals, even after prolonged periods of the disease [10].

Finally, mesangial cell tone may also contribute to glomerular rigidity by opposing the pressure-driven expansion. However, direct assessment of this component has shown that its role is probably unimportant because in angiotensin II-perfused glomeruli, only 4% of the total glomerular rigidity could be attributed to the associated mesangial cell contraction [4].

Summary

Due to their elasticity, glomeruli will undergo excessive expansion and repetitive cycles of distension–contraction under conditions of impaired glomerular pressure autoregulation and systemic arterial hypertension. These alterations in glomerular volume are associated with mesangial cell stretch which in turn stimulates the synthesis and deposition of ECM with eventual mesangial expansion and glomerulosclerosis. Hyperactivity of growth factors with prosclerotic activity is an important component in the translation of cellular mechanical strain into the abnormal metabolism of ECM components.

Although mesangial cell mechanical strain is expected to occur in both remnant glomeruli and in glomeruli of diabetic kidneys, quantitatively different factors will determine the resultant metabolic consequences. In remnant glomeruli, the mechanical stretch is intense, being accounted for largely by the marked glomerular hypertrophy and increased glomerular compliance. In diabetic glomeruli, however, the mechanical stretch is less prominent but its effect on ECM synthesis is markedly aggravated by the presence of hyperglycaemia.

There are presently no methods clinically available to diminish the prosclerotic action of growth factors at the glomerular level. In addition, there are no effective means to specifically improve glomerular pressure autoregulation. Therefore, current therapies must be aimed at decreasing systemic arterial pressure, blocking angiotensin II action and reducing glomerular hypertrophy. While there are effective drugs for the treatment of hypertension and for angiotensin II inhibition, protein restriction is the only measure available to diminish glomerular hypertrophy. Finally, in diabetes correction of systemic and glomerular hypertension should be coupled with strict glycaemic control to correct both glomerular autoregulation and increased ECM deposition.

References

1. Miller PL, Meyer TW. Methods in laboratory investigation. Effects of tissue preparation on glomerular volume and capillary structure in the rat. *Lab Invest* 1990; 63: 862–866
2. 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: 1932–1943
3. Osgood RW, Patton M, Hanley MJ, Venkatachalam M, Reineck HJ, Stein JH. *In vitro* perfusion of the isolated dog glomerulus. *Am J Physiol* 1983; 244: F349–F354
4. Cortes P, Zhao X, Riser BL, Narins RG. Regulation of glomerular volume in normal and partially nephrectomized rats. *Am J Physiol* 1996; 270: F356–F370
5. Brenner BM, Troy JL, Daugharty TM. The dynamics of glomerular ultrafiltration in the rat. *J Clin Invest* 1971; 50: 1776–1780
6. Pelayo JC, Westcott JY. Impaired autoregulation of glomerular capillary hydrostatic pressure in the rat remnant nephron. *J Clin Invest* 1991; 88: 101–105
7. Hayashi K, Epstein M, Loutzzenhiser R, Forster H. Impaired myogenic responsiveness of the afferent arteriole in streptozotocin-induced diabetic rats: role of eicosanoid derangements. *J Am Soc Nephrol* 1992; 2: 1578–1586
8. Bidani AK, Griffin KA, Picken M, Lansky DM. Continuous telemetric blood pressure monitoring and glomerular injury in the rat remnant kidney model. *Am J Physiol* 1993; 265: F391–F398
9. Kriz W, Elger M, Lemley K, Sakai T. Structure of the glomerular mesangium: A biomechanical interpretation. *Kidney Int* 1990; 38 [Suppl 30]: S2–S9
10. 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
11. Cortes P, Riser BL, Zhao X, Narins RG. Glomerular volume expansion and mesangial cell mechanical strain: mediators of glomerular pressure injury. *Kidney Int* 1994; 45 [Suppl 45]: S11–S16
12. 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: 1915–1923
13. Riser BL, Landson-Wofford S, Sharba A *et al.* TGF-β receptor expression and binding in rat mesangial cells: modulation by glucose and cyclic mechanical strain. *Kidney Int* (in press)
14. Yamamoto T, Nakamura T, Noble NA, Ruoslahti E, Border WA. Expression of transforming growth factor β is elevated in human and experimental diabetic nephropathy. *Proc Natl Acad Sci USA* 1993; 90: 1814–1818
15. Isaka Y, Fujiwara Y, Ueda N, Kaneda Y, Kamada T, Imai E. Glomerulosclerosis induced by *in vivo* transfection of transforming growth factor-β or platelet-derived growth factor gene into the rat kidney. *J Clin Invest* 1993; 92: 2597–2601
16. 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: 827–836
17. Riser BL, DeNichilo M, Grondin JM *et al.* Connective tissue growth factor (CTGF) as a determinant of extracellular matrix (ECM) deposition in diabetic glomerulosclerosis. *J Am Soc Nephrol* 1998; 9: 640A [Abstract]
18. Hamasaki K, Mimura T, Furuya H *et al.* Stretching mesangial cells stimulates tyrosine phosphorylation of focal adhesion kinase. *Biochem Biophys Res Comm* 1995; 212: 544–549
19. Akai Y, Homma T, Burns K, Yasuda T, Bdar KF, Harris RC. Mechanical stretch/relaxation of cultured rat mesangial cells induces protooncogenes and cyclooxygenase. *Am J Physiol* 1994; 267: C482–C490
20. Ishida T, Haneda M, Isono M *et al.* Pivotal role of mitogen activated protein kinases in stretch-induced fibronectin synthesis in mesangial cells. *J Am Soc Nephrol* 1997; 8: 517A [Abstract]
21. Carmines PK, Mitchell KD, Navar LG. Effects of calcium

antagonists on renal hemodynamics and glomerular function. *Kidney Int* 1992; 41 [Suppl 36]: S43–S48
22. Wolf G, Haberstroh U, Neilson EG. Angiotensin II stimulates the proliferation and biosynthesis of type I collagen in cultured murine mesangial cells. *Am J Pathol* 1992; 140: 95–107
23. Kagami S, Border WA, Miller DE, Noble NA. Angiotensin II stimulates extracellular matrix protein synthesis through induction of transforming factor-β expression in rat glomerular mesangial cells. *J Clin Invest* 1994; 93: 2431–2437
24. Daniels B, Hostetter TH. Adverse effects of growth in the glomerular microcirculation. *Am J Physiol* 1990; 258: F1409–F1416
25. Stackhouse S, Miller PL, Park SK, Meyer TW. Reversal of glomerular hyperfiltration and renal hypertrophy by blood glucose normalization in diabetic rats. *Diabetes* 1990; 39: 989–995
26. Savin VJ, Seaton RD, Richardson WP, Duncan K, Beason-Griffin C, Ahnemann J. Dietary protein and glomerular response to subtotal nephrectomy in the rat. *J Lab Clin Med* 1989; 113: 41–49
27. Dworkin LD, Benstein JA, Parker M, Tolbert E, Feiner HD. Calcium antagonists and converting enzyme inhibitors reduce renal injury by different mechanisms. *Kidney Int* 1993; 43: 808–814
28. Irzyniec T, Mall G, Greber D, Ritz E. Beneficial effect of nifedipine and moxonidine on glomerulosclerosis in spontaneously hypertensive rats. A micromorphometric study. *Am J Hypertens* 1992; 5: 437–443

Nephrol Dial Transplant (1999) 14: 1354–1357

Aptamers: novel tools for specific intervention studies

Jürgen Floege, Tammo Ostendorf and Nebojša Janjić[1]

Division of Nephrology, Medical School, Hannover, Germany, and [1]NeXstar Pharmaceuticals, Boulder, Colorado, USA

Specific intervention studies are a crucial requirement to demonstrate the pathogenetic relevance of particular molecules and to ultimately develop novel therapeutic approaches to renal disease. Inhibition studies of cytokines or growth factors provide a good example of the various approaches that have been employed in such studies *in vivo*.

(i) Neutralizing antibodies. Generation of such antibodies is probably the easiest way to accomplish intervention studies. Problems of neutralizing antibodies relate to their immunogenicity in heterologous systems and, more importantly, to non-specific effects of immunoglobulins *per se*, which may be difficult to control.
(ii) Natural or designed antagonists (e.g. extracellular domains of receptors or receptor antagonists). An elegant, yet laborious way, which requires the expression of considerable amounts of recombinant protein.
(iii) Interference with cytokine or growth factor signalling. An attractive approach since many compounds that inhibit molecules involved in signalling are simple chemical structures that can easily be synthesized. A frequent problem of this approach, however, is non-specificity of the intervention given the convergence of many signalling pathways and/or toxic *in vivo* effects.
(iv) Targeted gene deletion. A laborious way to investigate the function of a mediator, which also suffers from the potential problem that the ontogenetic lack of a single gene might have induced compensatory mechanisms that blunt the effects of the deficiency.
(v) Antisense or ribozyme studies. While the compounds are relatively easy to synthesize, they unfortunately have to enter the cell in order to act. This renders *in vivo* studies difficult as the compounds usually have to be transfected *in vivo* using liposomes or viral vectors.
(vi) Specific interventions with aptamers.

Correspondence and offprint requests to: Jürgen Floege MD, Division of Nephrology 6840, Medizinische Hochschule, D-30623 Hannover, Germany.

What is an aptamer?

The term 'aptamer' was originally proposed by Ellington and Szostak based on the latin word *aptus*, i.e. to fit [1]. It describes the property of DNA or RNA oligonucleotides to specifically bind to other molecules outside or inside of cells. Thus, in this case biological effects of the oligonucleotide are not a function of the DNA or RNA code (as involved in transcription and translation) but rather reside in the specific three-dimensional structure of the oligonucleotide [1,2]. Aptamers are usually 20–50mers with distinct structural elements (such as stems, loops, pseudoknots, helix junctions, G-quartets etc.) that create a stable framework in which some invariant nucleotides are precisely arranged [3–5]. It is these nucleotides at specific positions in the three-dimensional structure that are central to the physicochemical interaction of the aptamer with other molecules. K_d-values for the interaction of aptamers with their targets extend from the micromolar to low picomolar range and are therefore comparable in terms of binding affinity to antibody–antigen interactions [3–5]. An impressive example of how seemingly minor changes in an oligonucleotide sequence can have dramatic effects on the specificity of an aptamer is given