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# Renal fibrosis: can we prevent it?

**T D Hewitson*, G J Becker***

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## Abstract

Fibrotic disorders are commonplace, take many forms and can be life-threatening. Fibrosis involves an excess accumulation of extracellular matrix (primarily composed of collagen) and usually results in loss of function when normal tissue is replaced with scar tissue.

No better example of this exists than that of the progressive fibrosis that accompanies of all chronic renal disease, regardless of etiology. Manifesting itself histologically as glomerulosclerosis, vascular sclerosis and tubulointerstitial fibrosis, in each case the process depends on a resident mesenchymal cell, is irreversible, and inevitably leads to end-stage renal failure.

Potentially we can ameliorate fibrosis, either indirectly by modifying the environment the kidney functions in, or more directly by interfering with activation and function of mesenchymal cells.

As we learn more about the pathogenesis of renal scarring we realise that our attempts to find anti-fibrotic therapies in the kidney is paralleled by similar efforts in other organs. Many chronic diseases progress by fibrosis, and direct anti-fibrotic agents acting on mesenchymal cells may play an important role in their management.

## Introduction

Fibrotic disorders are commonplace, take many forms and can be life-threatening. Fibrosis involves an excess accumulation of extracellular matrix (primarily composed of collagen) and usually results in loss of function when normal tissue is replaced with scar tissue.

No better example of this exists than the progressive fibrosis that accompanies all chronic renal disease. Histologically end-stage renal disease manifests itself as glomerulosclerosis, vascular sclerosis and tubulointerstitial fibrosis. In each case the process depends on a resident mesenchymal cell; represented by the mesangial cell, vascular smooth muscle cell and interstitial fibroblast respectively.

Regardless of etiology, all patients with chronic renal disease show a progressive decline in renal function with time. The process is irreversible, inevitably leading to end-stage renal failure, a condition that requires life-long dialysis or renal transplantation.

## How does fibrosis develop?

We perhaps most commonly associate scarring with an excess synthesis of matrix, usually collagen. Although matrix synthesis is of course part of the normal repair process that occurs after injury, excessive synthesis of extracellular matrix is itself hazardous, further exacerbating injury in a vicious cycle. What distinguishes healing and scarring is chronicity, with scarring perhaps simply too much of a good thing.

Keloids represent the best example of scarring which results from aberrant matrix synthesis. Not withstanding genetic factors, keloids result from the uncontrolled matrix synthesis by dermal fibroblasts. There are certainly renal parallels of this process, such as the focal scarring that accompanies a localised tissue trauma.

What does however now seem clear, is that scarring is often a multifactorial process, with aberrant matrix synthesis only part of the process. Temporal studies in experimental renal infection indicate that aberrant collagen synthesis is often transient, peaking in the first few days after infection. Histologically however, scarring as defined by increasing matrix density, continues to increase[1].

How can we account for this discrepancy? Although it has long been known that end-stage kidneys are smaller than their unscarred counterparts, it is the focal lesions found in diseases such as reflux nephropathy that provide us with a clue. The irregular surface of these kidneys (Figure 1) indicates underlying scar tissue, highlighting the fibro-contractive nature of renal scarring. In what we term the "balloon" hypothesis, fibrosis is due not only to an increase in matrix synthesis but also to the collapse of the renal parenchyma. Analogous to deflating a balloon, we are

* *Department of Nephrology, Royal Melbourne Hospital and Department of Medicine, University of Melbourne, Melbourne, Australia.*

† *Oration delivered by G J Becker.*

effectively measuring the same amount of matrix in a smaller volume.

Once again, there are good non-renal examples of this process. Wound contraction has long been recognised as an integral part of skin wound healing, with the drawing together of wound edges an important part of wound closure. The observation that a decrease in renal size parallels loss of renal function[2] provides indirect evidence of this process in the kidney. More direct evidence comes form examining the histology in experimental renal infection and scarring. Being a primary tubulointerstitial model of injury, the glomeruli are largely unaffected during fibrosis, the density of glomeruli therefore providing a measure of parenchymal collapse. Morphometric studies in this model show that the combined effect of an acute increase in collagen expression and a later collapse of the renal parenchyma account for the progressive increase in scar tissue[1].

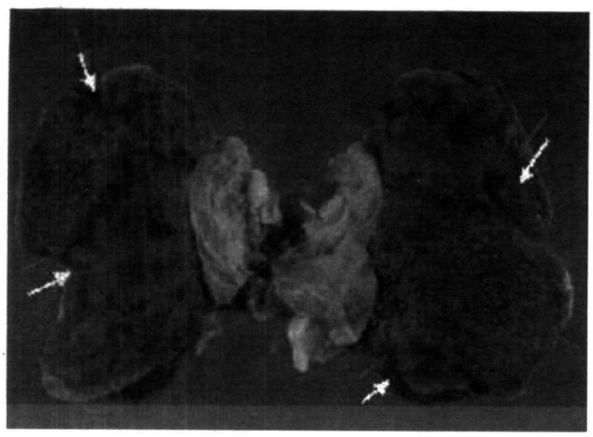

**Figure 1.** Irregular outline of the kidney surface indicates underlying fibro-contractive scars (arrows) in reflux nephropathy.

**What is the cellular basis of this process?**

Sclerosis (or fibrosis) in all three settings is associated with the activities of a mesenchyme derived cell. The interstitial fibroblast, glomerular mesangial cell and vascular smooth muscle cell are phenotypically similar, with the fibroblast and mesangial cell acquiring features of smooth muscle when activated.

In each form of renal scarring, not only is the resident mesenchymal cell the principal extracellular matrix producing cell, but it also provides the force for contraction and reorganisation of extracellular matrix, thereby increasing its density.

The renal interstitial fibroblast represents the quintessential example of this process and has been the focus of our studies of tubulointerstitial fibrosis. Recognised by its de novo synthesis of α smooth muscle actin, activated fibroblasts, so-called myofibroblasts, are a feature of all forms of progressive renal disease where their accumulation is strongly associated with disease progression[3].

In consecutive, but overlapping events, fibroblasts are activated by agonists released from adjacent injured epithelial and endothelial cells. Myofibroblasts proliferate locally, synthesise the extracellular matrix components that constitute interstitial fibrosis and contract and reorganise matrix to increase its density (Figure 2). In what is a vicious cycle, interstitial fibrosis probably both causes mechanical injury and through a reduction in vascularization, increases hypoxia. Recognised from their de novo expression of αSMA, interstitial myofibroblasts accumulate in all forms of progressive renal disease (Figure 3).

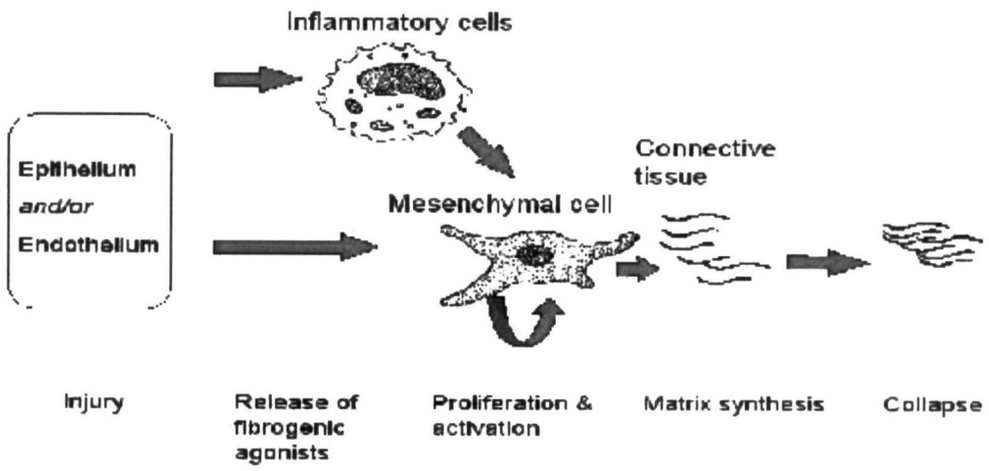


**Figure 2.** Renal mesenchymal cells are the cellular basis of both excess matrix synthesis and collapse.

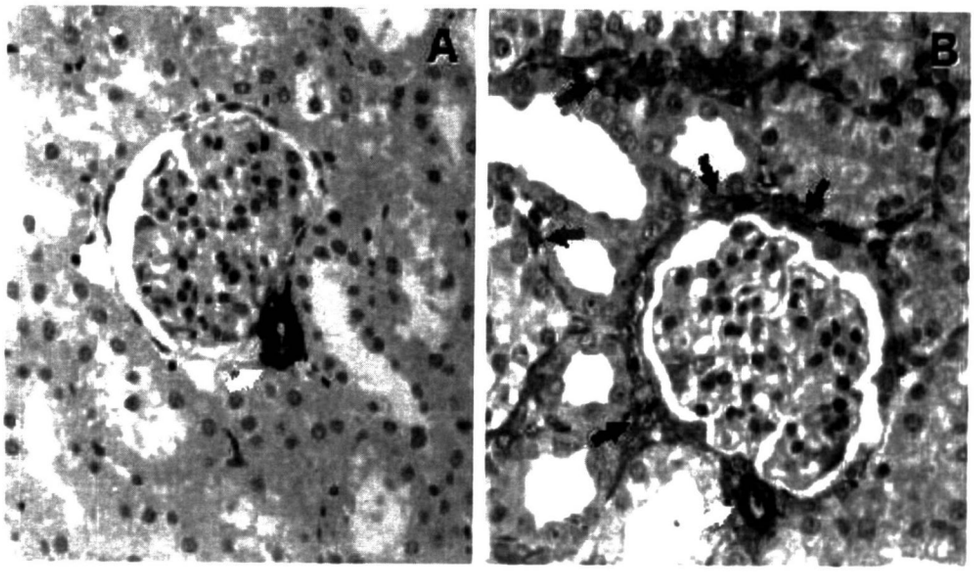


**Figure 3.** (A) In the normal rat kidney, αsmooth muscle actin expression is confined to vasculature (white arrow). (B) Aberrant interstitial expression of αsmooth muscle actin (black arrows) indicates myofibroblast recruitment in progressive fibrosis.

Regulation of interstitial fibroblasts is however complex. Differentiation of myofibroblasts, proliferation and collagen synthesis are stimulated by a variety of agents derived from stimulated tubular cells, leukocytes or from fibroblast themselves. A hierarchy exists amongst the profibrotic growth factors, with the most compelling evidence being for transforming growth factor β1 (TGFβ1) and platelet derived growth factor (PDGF). It is also likely that fibroblasts are stimulated more directly in some diseases because high glucose concentrations and angiotensin II stimulate in vitro renal cortical fibroblast proliferation and collagen production.

Most of what we know about collagen reorganisation comes from in vitro studies of collagen gel contraction and reorganisation. In a process that is directly proportional to the number of cells present, fibroblasts embedded in solidified collagen progressively contract matrix to reduce gel diameter and increase matrix density. We know that this process is dependent upon b1 integrins found on the surface of renal fibroblasts. Blocking these receptors with specific antisera is sufficient to prevent fibroblasts binding to collagen I, and in doing so abrogate gel contraction[4]. Again however, the process is complex and is due both to contraction in the surrounding matrix, in the same way as a sea anemone retracts its tentacles, and traction or migration of fibroblasts through surrounding matrix, akin to the movement of a spider through its web, pulling on the filaments.

**Can we modify behaviour of these cells?**

A variety of molecules have been shown to directly influence or interfere with the in vitro behaviour of renal profibrotic mesenchymal cells. Activities of the myofibroblast are stimulated by factors either derived from adjacent cells (inflammatory, endothelial or epithelial cells) or inherent to the uremic milieu.

Theoretically at least, we should be able to use antagonists to interfere with the pro-fibrotic activities of myofibroblasts. If we consider the functions relevant to fibrosis, interference with cell kinetics (proliferation and cell death), differentiation (αSMA expression), migration, matrix production and matrix reorganisation all have the potential to limit progressive scarring.

**Potential therapeutic strategies**

Like other groups, for practical reasons, our initial attention has focused on agents introduced for quite different indications.

Phosphodiesterase inhibitors (the most well-known of which is theophylline used in the treatment of asthma), increase the intracellular levels of cyclic nucleotides which theoretically could interfere with myofibroblast function. Pentoxifylline has now been shown to inhibit mitogenesis and collagen synthesis in renal fibroblast cultures[5,6]. Dipyridamole, another drug which increases intracellular cyclic nucleotide levels,

inhibits renal fibroblast proliferation and collagen synthesis[7], though it was originally intended as an antiplatelet agent.

So-called "statins" (HMG-CoA reductase inhibitors) introduced to lower blood cholesterol have now been shown to have variable effects on fibrogenesis. Although effects of individual statins differ, simvastatin and lovastatin have been shown to reduce *in vitro* proliferation and collagen secretion by cortical fibroblasts[8,9]. Lovastatin, at least, inhibits *in vitro* contraction of collagen gels[8,9]. At the molecular level, statins block prenylation of Ki-Ras, an important intracellular protein required for fibroblast proliferation[10]. The more specific prenylation inhibitors being developed for cancer therapy[11], are therefore likely to be valuable potential antagonists of the uncontrolled mesenchymal proliferation that accompanies fibrogenesis.

**Is there any evidence that anti-fibrotic agents work *in vivo*?**

Importantly, as most patients with chronic kidney disease are diagnosed well before they reach end-stage renal failure, agents that abrogate inflammation, alter collagen turnover and inhibit remodelling can potentially ameliorate and even reduce the fibrosis or scarring that accompanies all progressive renal disease. The similarity between the pathogenesis of tubulointerstitial fibrosis, glomerulosclerosis and vascular sclerosis suggests that treatments that affect any one of these may delay progression by ameliorating all three.

A number of the agents used *in vitro* have now been tested in experimental models of renal disease.

Studies in animal models have confirmed that statins have beneficial effects on glomerulosclerosis and vasculopathy, results which can not be attributed to cholesterol lowering[11].

Pentoxifylline has now been shown to attenuate progressive fibrosis after sub-total renal ablation[12].

Receptors for the vasoactive factors Angiotensin II (AII) and endothelin (ET) are found on mesangial cells, fibroblasts and vascular smooth muscle cells[13-15]. Administration of angiotensin converting enzyme inhibitors (ACEI), which inhibit AII production, or angiotensin type I receptor blockers, which inhibit AII action more directly, have been shown to reduce renal failure progression in experimental animals and patients with chronic renal disease[16,17]. Likewise, ET receptor antagonists reduce vascular injury, mesangial cell proliferation[18] and accumulation of new fibroblasts[19] in various experimental nephropathies.

The recognition that renal scarring is a consequence of both increased collagen synthesis and decreased breakdown has inevitably led to a search for agents which promote collagen degradation. Such strategies have been given further impetus by the clinical reality that extensive scarring has often already occurred at presentation.

Relaxin is a naturally occurring polypeptide hormone generated in increased quantities during the latter stages of gestation. Consistent with its role in softening the pubic symphysis and uterine cervix in preparation for parturition[20], antifibrotic effects have been demonstrated in experimental models of rapidly fibrosing chemically induced renal papillary necrosis[21] and renal ablation[22], data suggesting that this may relate at least in part to its *in vitro* ability to increase collagenase synthesis[23].

Pirfenidone, a pyridone compound, reduces ongoing fibrosis in both chronic anti-ethyl glomerulonephritis[24] and unilateral ureteric obstruction models of renal sclerosis[25], ostensively through a variety of mechanisms indirectly increasing collagen degradation.

Despite the function of all these cells being influenced by a myriad of factors and their interactions in a highly redundant system, perhaps surprisingly several findings now suggest that changes in even a single cytokine/growth factor can be effective in abrogating renal fibrogenesis. A variety of strategies have been used experimentally to inhibit activity of polypeptide growth factors.

$TGF\beta_1$ antisense oligonucleotides have been used successfully as an intervention in both glomerulonephritis[26] and interstitial fibrosis[27]. Anti-serum to $TGF\beta_1$ has ameliorated experimental glomerular sclerosis[28] and vascular intimal hyperplasia[29]. Pirfenidone[30] and decorin[31] have been shown to reduce experimental fibrosis, acting at the level of the latent and activated molecule respectively. Angiotensin converting enzyme inhibitors and AII receptor antagonists reduce but do not normalise aberrant $TGF\beta_1$ production[32,33], at least in experimental renal disease.

An alternative approach is to take advantage of the naturally occurring interactions between various endogenously expressed growth factors. Exogenous hepatocyte growth factor (HGF) is linked to decreased $TGF\beta_1$ expression and progression[34]. In a similar manner, bone morphogenic protein-7, a member of the TGFβ superfamily, preserves renal function and prevents interstitial fibrosis after unilateral obstruction, acting as a counterbalance to $TGF\beta_1$ activity[35].

Oligonucleotide aptamers have been developed to antagonise PDGF activity by binding with high affinity to the PDGF molecule[36]. Selective inhibitors of a PDGF specific receptor tyrosine kinase, reduce mesangial cell proliferation and matrix synthesis in mesangial proliferative glomerulonephritis[37] and attenuate, albeit to a lesser degree, interstitial fibrosis after unilateral ureteric obstruction[38].

Less is known about potential anti-fibrotic agents in human disease, although the benefits seen with indirect therapies suggest that many of these agents have the potential to improve outcomes.

Reducing blood pressure, hyperglycaemia and hyperlipidaemia slows progressive renal disease[39]. As in animal studies, the effect of angiotensin inhibition is greater than would be expected from blood pressure alone[17], making it likely that intrarenal effects on haemodynamies glomerular haemodynamics and on renal cells are both responsible.

## Anti-fibrotic agents in other organs

As the laying down of matrix is a universal evolutionary response to injury, scarring is a complication that accompanies chronic inflammation elsewhere. Understandably therefore, our attempts to find anti-fibrotic renal therapies is paralleled by similar efforts in other organs.

### *Liver fibrosis*

Liver cirrhosis is an advanced stage of fibrosis characterised by the formation of nodules of liver parenchyma separated and encapsulated by fibrotic septae. Life expectancy is directly related to degree of hepatic cirrhosis[40]. The principal matrix producing cell is the myofibroblast like hepatic stellate cell with profibrotic cytokines, toxic metabolites and certain drugs all triggering enhanced fibrogenesis.

Again, angiotensin antagonism, endothelin antagonists, pentoxifylline, HGF and TGF$\beta_1$ inhibitors have shown potential benefits in vitro and in animal models[41]. Colchicine has been used to treat alcoholic fibrosis clinically, albeit with variable results, while suppressing chronic inflammation with IL-10 limits fibrogenesis in patients with hepatitis C[41].

### *Pulmonary fibrosis*

In many respects, pulmonary fibrosis most closely resembles renal fibrosis, with little distinguishing the lung and kidney myofibroblast. As in renal disease, pulmonary scarring is the final common pathway to a number of diverse etiologies, including idiopathic pulmonary fibrosis, bleomycin and cyclophosphamide toxicity, scleroderma and environmental toxins amongst others.

Perhaps not surprisingly then, the search for therapeutic strategies in the lung closely parallels that of the kidney, with pirfenidone and relaxin having been the focus of recent clinical trials[42].

### *Cardiac fibrosis*

Injury to the heart initiates a sustained process of myocardial remodelling, involving both cardiomyocytes and nonmuscle cell. Again this process is generally accepted as a key determinant of the clinical course of heart failure, making the prevention of remodelling a therapeutic goal in treatment of patients with heart failure[43].

Angiotensin converting enzyme inhibitors[44] and aldosterone antagonists[45] have both been shown to reduce collagen synthesis after myocardial injury in humans.

## Conclusions

Many chronic diseases progress by fibrosis, and direct anti-fibrotic agents acting on mesenchymal cells may play an important role in their management.

As we learn more about the mechanisms of fibrogenesis, studies in both the kidney and elsewhere will continue to identify potential therapeutic targets. Future efforts directed at *in vivo* studies with gene deletion or over expression will determine the pathological significance of each of these mechanisms.

While much of what we have learnt about sclerosis and fibrosis has been a consequence of recognising the similarity between healing and scarring, the challenge remains to target anti-fibrotic therapies and limit inappropriate scarring without compromising the biology of healing.

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