Review

Renal fibrosis in feline chronic kidney disease: Known mediators and mechanisms of injury

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A B S T R A C T

Chronic kidney disease (CKD) is a common medical condition of ageing cats. In most cases the underlying aetiology is unknown, but the most frequently reported pathological diagnosis is renal tubulointerstitial fibrosis. Renal fibrosis, characterised by extensive accumulation of extra-cellular matrix within the interstitium, is thought to be the final common pathway for all kidney diseases and is the pathological lesion best correlated with function in both humans and cats. As a convergent pathway, renal fibrosis provides an ideal target for the treatment of CKD and knowledge of the underlying fibrotic process is essential for the future development of novel therapies. There are many mediators and mechanisms of renal fibrosis reported in the literature, of which only a few have been investigated in the cat. This article reviews the process of renal fibrosis and discusses the most commonly cited mediators and mechanisms of progressive renal injury, with particular focus on the potential significance to feline CKD.

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Introduction

Chronic kidney disease (CKD) is defined as the presence of structural or functional abnormalities of one or both kidneys that have been present for an extended period of time. CKD is common in cats, one study reporting that 12% of all cats necropsied over a period of 3 years were suffering from the condition (Taugner et al., 1996). The prevalence of CKD also increases with age, with reported prevalence rates of 28% in cats over 12 years old (Bartlett et al., 2010) and 31% in cats over 15 years old (Lulich et al., 1992).

The aetiology of feline CKD is heterogeneous and includes specific disease processes which initiate renal damage or dysfunction, such as polycystic kidney disease, renal amyloidosis, renal dysplasia and renal lymphoma (Reynolds and Lefebvre, 2013). However in the majority of cats with CKD no inciting cause is identified. One study of a referral population found pathological lesions of a specific renal disease in 50% of cases (DiBartola et al., 1987), another in 33% of cases (Minkus et al., 1994). A recent study with a large population from first opinion practices identified specific renal lesions in only 16% of cats (Chakrabarti et al., 2013). The majority of cats with CKD are found to have non-specific renal lesions and the predominant morphological diagnosis in these cases is chronic tubulointerstitial inflammation and fibrosis (DiBartola et al., 1987; Chakrabarti et al., 2013).

The underlying aetiology of CKD in the majority of cats, where chronic tubulointerstitial fibrosis is present, is not understood. Various factors which may contribute to renal damage have been proposed, including diet (Hughes et al., 2002), vaccination (Lappin et al., 2006), and ageing (Lawler et al., 2006). Regardless of the aetiology, the progressive nature of renal fibrosis results in deterioration of renal function independent of the initial renal insult. Renal tubulointerstitial fibrosis is recognised as the final common pathway for all kidney diseases in human patients regardless of aetiology (Prunotto et al., 2011) and is the pathological lesion best correlated with renal function in both humans (Risdon et al., 1968; Nath, 1992) and cats (Yabuki et al., 2010; Chakrabarti et al., 2013).

Despite ongoing research there is currently no effective treatment that significantly slows the progression of renal fibrosis in humans or in cats. Therefore, much attention is directed at identifying factors which influence or drive the progression of fibrosis in order to identify potential therapeutic targets. The aim of this article is to provide a review of the renal fibrotic process and evaluate previous research published on mediators and mechanisms of renal fibrosis, with particular focus on those investigated in cats, in order to provide updated information for veterinary clinicians and pathologists.

Renal fibrosis

In most cases of tissue damage, injured cells are replaced by cells of the same type and/or fibrous tissue after the resolution of the inflammatory response. The kidney has an intrinsic capacity for

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self-repair after ischaemic or toxic insults that result in cell death and can potentially recover completely after sustaining acute injury. This repair occurs primarily through epithelial cell proliferation, and it appears that an intact basement membrane plays an important role by providing a scaffold along which regenerating epithelial cells can spread and migrate (Toback, 1992; Bonventre, 2003). Healing via focal fibrotic scarring is also seen secondary to severe parenchymal injury, for instance in pyelonephritis or infarction, and serves to maintain tissue integrity. Whilst fibrosis is a normal sequel of injury, it is thought that in CKD the normal wound healing response fails to terminate (Liu, 2006; Wynn, 2010). Although the exact mechanisms underlying this dysregulation are unclear, the result is an excessive, inexorable fibrogenic response and the expansion of the extra-cellular matrix (ECM) gradually destroys normal tissue structure (Eddy, 1996). The histopathology of renal fibrosis features excessive accumulation of ECM proteins within the tubulointerstitial space and is accompanied by loss of the renal microvasculature, infiltration of mononuclear cells, tubular atrophy and dilation (Fig. 1). The aberrant extracellular matrix is composed of normal matrix proteins and proteoglycans as well as other matrix proteins normally restricted to tubular basement membranes, such as collagen IV and laminin (Vleming et al., 1995; Eddy, 1996).

In the normal kidney, interstitial fibroblasts play a crucial role in ECM homeostasis via their dual roles in the synthesis of ECM and production of ECM degrading proteases (Strutz and Zeisberg, 2006). In fibrogenesis, these cells are believed to become activated and undergo a phenotypic change to become myofibroblasts. The induction and proliferation of myofibroblasts is probably a crucial event in the initiation and progression of renal fibrosis. Myofibroblasts possess qualities of both fibroblasts and smooth muscle cells and are considered the dominant ECM producing cells in organ fibrosis (Prunotto et al., 2011; Lebleu et al., 2013). It is not possible to differentiate these cells from fibroblasts via light microscopy and they are characterised by their expression of alpha smooth muscle actin (α-SMA) and vimentin. Both vimentin and α-SMA expression increase within the kidney in naturally occurring feline CKD and the expression of these cellular markers correlates with increasing plasma creatinine concentration and kidney fibronectin deposition (Sawashima et al., 2000; Yabuki et al., 2010).

The precise origin of myofibroblasts has historically been controversial, and several cell types as well as resident fibroblasts are

Fig. 1. Photomicrograph illustrating severe cortical interstitial fibrosis and inflammation in the kidney of a cat with CKD. Periodic acid-Schiff and haematoxylin. Reprinted with permission from *Veterinary Pathology*, SAGE publications.

known to undergo phenotypic changes to ECM producing cells in renal fibrosis, including tubular epithelial cells, circulating fibrocytes, vascular pericytes, endothelial cells and glomerular podocytes (Kim et al., 2013). The relative contribution of these cellular types to the myofibroblast pool is the focus of ongoing investigation. One recent comprehensive analysis examining the relative contribution of various cell types to the myofibroblast lineage, using mice with experimentally induced obstructive renal disease, found that 50% of the population arose from proliferation of resident fibroblasts whilst the remainder differentiated from bone marrow derived cells (35%), endothelial cells (10%) and epithelial cells (5%) (Lebleu et al., 2013).

Myofibroblast induction, proliferation and ECM production are regulated by a number of local and circulating factors. These include paracrine or autocrine growth factors, direct interaction with leucocytes/macrophages, and environmental stimuli such as hypoxia and hyperglycaemia (Qi et al., 2006). Recent studies have also emphasised the importance of renal tubular epithelial cell damage and altered epithelial–mesenchymal cell signalling in the activation of renal fibroblasts towards the myofibroblast type in CKD (Prunotto et al., 2012; Moll et al., 2013).

Pro-fibrotic mediators

Transforming growth factor beta (TGF-β)

The cytokine TGF-β is potentially the most important pro-fibrotic mediator responsible for myofibroblast activation, appearing to be a convergent pathway that integrates the effects of many other fibrogenic factors (Liu, 2006; Farris and Colvin, 2012). TGF-β1 is the most abundant isoform and is synthesised by all cell types of the kidney, with epithelial cells demonstrated to be the main source in experimentally induced disease (Fukuda et al., 2001; Wu et al., 2013). TGF-β is secreted as a latent precursor and must undergo proteolytic cleavage in order for the active form of the cytokine to be liberated, which may be initiated directly by a variety of molecules including plasmin and reactive oxygen species (Annes et al., 2003). Once activated, TGF-β exerts its subsequent effects by binding to the TGF-β receptor II and regulating the transcription of target genes (Wang et al., 2005). Production of TGF-β has been demonstrated to be induced by a variety of stimuli, including the renin–angiotensin–aldosterone system (Wolf, 2006), hypoxia (Orphanides et al., 1997), proteinuria (Eddy and Giachelli, 1995), increased single nephron glomerular filtration rate (Rohatgi and Flores, 2010) and oxidative stress (Shin et al., 2008).

TGF-β is upregulated in nearly all mammalian chronic kidney diseases, and evidence from rodent models supports the hypothesis that TGF-β signalling is central to the induction of kidney disease. Mice overexpressing TGF-β develop interstitial fibrosis (Koesters et al., 2010) and downstream disruption of the TGF-β signalling pathway has been demonstrated to ameliorate renal fibrosis (Sato et al., 2003). The profibrotic effects of TGF-β include inducing the formation of myofibroblasts from various cell types in vitro, including fibroblasts (Serini et al., 1998), pericytes (Wu et al., 2013) and potentially also endothelial cells (He et al., 2013), although the relative significance of these findings in vivo is controversial. It directly stimulates transcription of ECM genes in many renal cells, including tubular, endothelial and mesangial cells (Border and Noble, 1993) and also decreases matrix degradation (Edwards et al., 1987; Krag et al., 2005). TGF-β1 signalling in tubular epithelial cells alone, independent of myofibroblasts, is sufficient to cause tubular injury, apoptosis and accumulation of inflammatory cells (Gentle et al., 2013). As well as direct effects, TGF-β also acts to stimulate cell proliferation and ECM accumulation through downstream activation of the pro-fibrotic cytokine connective tissue growth factor (CTGF) (Grotendorst, 1997).

TFG-β (normalised to urinary creatinine) has been shown to be present in increased concentrations in the urine of cats with
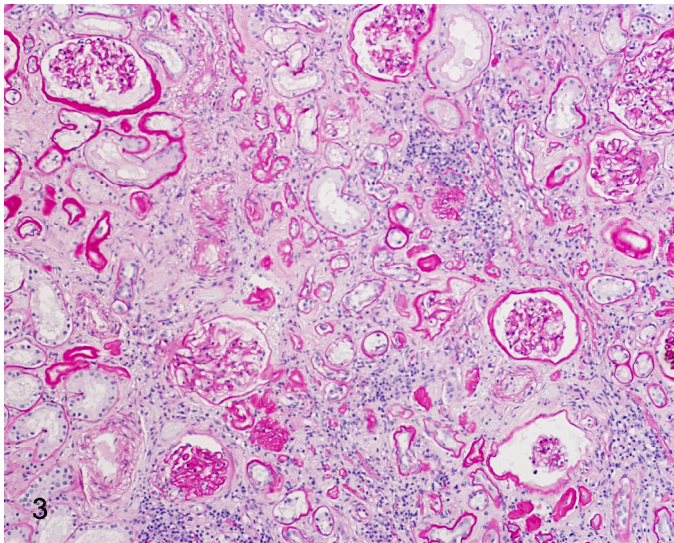
naturally occurring CKD. One study found a significant increase in urine TGF-β1:urine creatinine ratio (TGF-β1:UCrR) in a group of 23 cats with CKD in comparison to a group of 13 healthy controls, whilst documenting no difference in serum TGF-β1 concentrations (Arata et al., 2005). Another study comparing 18 healthy cats to 26 with CKD also demonstrated an increased TGFβ1:UCrR in cats with CKD, and a positive correlation between TGF-β1:UCrR and serum creatinine (Habenicht et al., 2013). These preliminary results suggest urinary TGF-β1 may be useful as a biomarker of renal injury in CKD and a potential tool for assessing response to therapeutic interventions. However, a major disadvantage of feline studies to date is the lack of concomitant histopathology with which to correlate these findings. Furthermore, the relative value of the different forms of TGF-β1 measured in feline urine and/or serum as biomarkers of active renal pathology warrants further study.

Components of the renin–angiotensin–aldosterone system

The renin–angiotensin–aldosterone system (RAAS) is an endocrine pathway that plays an integral role in the homeostatic control of arterial pressure, tissue perfusion and extracellular volume (Atlas, 2007) (Fig. 2). The RAAS is upregulated early in CKD (Siragy and Carey, 2010) and plasma renin, aldosterone and angiotensins I and II have been demonstrated to be increased in the circulation of cats with experimentally induced CKD (Watanabe and Mishina, 2007). Both systemic and intrarenal RAAS exist, and assessment of the systemic RAAS alone does not necessarily indicate the state of the RAAS system in the kidney. Angiotensin II in particular may be present in much higher concentrations within the kidney than in the circulation (Kobori et al., 2007).

The RAAS is a mediator of progressive renal injury via increasing glomerular pressure and the subsequent filtration of plasma protein, but its components also have direct fibroproliferative effects. Angiotensin II, the primary active product of the RAAS, is believed to be the most important via upregulation of TGF-β signalling and pro-inflammatory gene transcription (Ruster and Wolf, 2011). As well as TGF-β, angiotensin II also has the potential to induce a variety of other pro-inflammatory and pro-fibrotic mediators including CTGF and endothelin 1 (Yokoi et al., 2001; Wolf et al., 2002). Aldosterone and renin are also implicated as effectors of fibrogenesis by increasing TGF-β expression and production respectively (Sun et al., 2000; Juknevicius et al., 2004; Huang et al., 2006). Aldosterone is also believed to have TGF-β independent effects via stimulating production of CTGF (Han et al., 2006).

Suppression of the RAAS via inhibition of angiotensin converting enzyme (ACE) has been demonstrated to ameliorate or halt renal fibrosis in several models of CKD (Ishidoya et al., 1996; Garber et al., 1998; Mizuno et al., 1998), although conflicting reports exist (Turan et al., 2003). Administration of ACE inhibitors to human patients with a range of kidney diseases delays progression of CKD, independent of their anti-hypertensive action (Maschio et al., 1996), but this effect has not yet been demonstrated in cats. In clinical trials of ACE inhibitors in cats with CKD, those receiving benazepril showed a significant reduction in proteinuria, but to date, no significant survival benefit (King et al, 2006; Mizutani et al., 2006; Watanabe and Mishina, 2007). One study investigating the relationship between the intra-renal expression of renin and angiotensin II and renal histopathology in cats found no relationship between their expression and tubulointerstitial fibrosis, although there were only 13 cats enrolled in this study (Mitani et al., 2013). The same study documented a positive correlation between interstitial angiotensin II expression and fibrosis in dogs, and thus it is possible that the intrarenal RAAS is not as significant a mediator of interstitial fibrosis in cats as it appears to be in other species.

Transglutaminase 2

Transglutaminase 2 (TG-2) is an ubiquitously expressed member of the transglutaminase family of calcium dependent cross-linking enzymes with important enzymatic and non-enzymatic functions in the extracellular matrix (Belkin, 2011). The transamidase activity of TG-2 plays a role in extracellular matrix stabilisation by cross-linking extracellular matrix proteins (Shweke et al., 2008; Iismaa et al., 2009). This leads to increased ECM deposition and resistance to ECM breakdown via proteolytic enzymes (Johnson et al., 1999). In addition to its direct enzymatic effects TG-2 is involved in cross-linking TGF-β1 to the ECM (Annes et al., 2003) and may contribute to tissue fibrosis via activation of TGF-β1 (Shweke et al., 2008), as well as stimulating pro-inflammatory signalling (Kim et al., 2010).

TG-2 has been linked to a variety of fibrotic conditions affecting the liver, lung, heart and kidney (Iismaa et al., 2009). Experimental administration of TG-2 inhibitors to rodent models of kidney disease decreases the severity of fibrosis, implicating TG-2 as an active player in the progression of fibrosis in CKD (Johnson et al., 2007; Huang et al., 2009). A strong association between TG-2 expression and renal fibrosis has been observed in rodent models (Johnson et al., 1999), humans (Johnson, 2003) and recently also
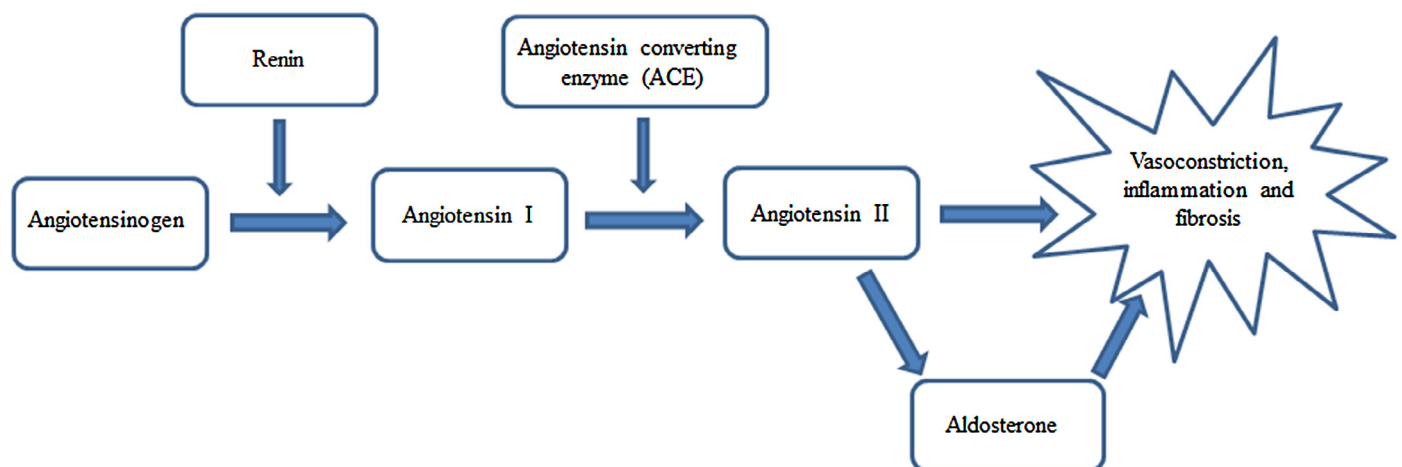
Fig. 2. The renin–angiotensin–aldosterone system.

in cats (Sánchez-Lara et al., 2013). In addition to histopathological changes, extracellular TG-2 activity was also positively correlated with plasma creatinine, urea and phosphate in cats in this study. This dual correlation of TG-2 with histopathological evidence of fibrosis and biochemical markers of disease severity suggests it may be an ideal target for further investigation and a potential future therapeutic target.

Endothelin 1

Endothelin 1 (ET-1) is a peptide produced in the heart, kidney, central nervous system and posterior pituitary gland with varied physiological roles throughout the body. Within the kidney it is released in response to activation of the RAAS (Kohno et al., 1992), hypoxia (Hughes et al., 1996) and other pro-fibrotic mediators such as TGF-β (Schnermann et al., 1996). ET-1 acts as a potent vasoconstrictor but also has a considerable pro-fibrotic effect. Overexpression of ET-1 results in development of interstitial fibrosis in transgenic mice (Hocher et al., 1997) and increased renal ET-1 expression has been demonstrated in human renal disease (Duan et al., 1999; Nakamura et al., 2000) as well as experimental models of kidney disease (Lebel et al., 2006). Pharmacological antagonism of the ET-1 receptor is known to attenuate fibrosis in rats with experimentally induced kidney disease (Benigni et al., 1993; Ebihara et al., 1997).

An increase in ET-1 mRNA expression in biopsied renal tissue has been reported in one study of cats affected by renal failure (Uchide and Saida, 2006). Conversely, in one recent analysis, plasma and urinary ET-1 concentrations were not associated with plasma creatinine concentration and tubular ET-1 expression was not associated with fibrosis (Chakrabarti, 2012). Total ET-1 expression may however be unrepresentative of this peptide's true role in CKD. ET receptors have been found to be upregulated in experimentally induced acute kidney injury (Roubert et al., 1994), as well as in human patients with polycystic kidney disease (Ong et al., 2003), and it is possible that it is an increased sensitivity to ET-1 that leads to pathological effects.

Factors involved in the progression of renal fibrosis

The mechanisms behind continued and progressive fibrotic change in CKD are not completely understood. It is thought that the intra-renal environment in CKD is significantly pro-fibrotic, leading to continued production of pro-inflammatory and pro-fibrotic cytokines and the perpetuation of the wound healing response rather than its resolution. The factors with the most influence on maintaining this state and which have undergone the most investigation to date are believed to be proteinuria, chronic inflammation, hypoxia, ageing and hyperphosphataemia.

Proteinuria

The classical 'hyperfiltration theory' postulates that as single nephron glomerular filtration rate increases in proportion to the amount of renal tissue lost, plasma protein filtration increases and subsequent glomerular and tubular damage occurs, triggering further fibrosis and creating a vicious cycle (Hostetter et al., 1981). It is believed that excess protein within the glomerular filtrate may have intrinsic renal toxicity due to increased uptake by tubular cells and accumulation of abnormal amounts of protein within the interstitium. Protein overload has been associated with proximal tubular cell apoptosis in experimentally induced renal disease (Tejera et al., 2004) and proximal tubular cells subjected to protein overload upregulate genes encoding for pro-inflammatory and pro-fibrotic mediators in vitro and in vivo (Eddy and Giachelli, 1995; Zoja et al., 1999; Nakajima et al., 2002). These factors cause increased infiltration of leukocytes in the protein-overloaded areas and chronic inflammation results (Abbate et al., 1998). Proteinuria has been suggested to be associated with increased ET-1 (Vlachojannis et al., 2002), TGF-β (Eddy and Giachelli, 1995) and ACE expression (Largo et al., 1999). Tubular protein overload is also believed to cause epithelial to mesenchymal transition of proximal tubular cells to myofibroblasts, thus contributing directly to the increase in ECM producing cells that occurs in CKD (although this is of debateable significance) (Abbate et al., 2002).

Proteinuria is an established negative prognostic factor in feline CKD. Urine protein to creatinine ratio (UPC) is correlated with plasma creatinine concentration and independently associated with shorter survival times (Syme et al., 2006). In one study evaluating predictors of the development of azotaemia in cats, proteinuria at presentation was significantly associated with the development of azotaemia and alongside creatinine was the only significant predictor in a multivariable analysis (Jepson et al., 2009). UPC has also been shown to be a significant and independent risk factor for a >25% increase in plasma creatinine concentration within 12 months of diagnosis of CKD (Chakrabarti et al., 2012a) and to be positively correlated with post-mortem interstitial fibrosis score (Chakrabarti et al., 2013). Despite these associations, it is not yet possible to infer a causal role for proteinuria in feline CKD and renal fibrosis as these findings may just reflect decreased tubular uptake capacity in diseased kidneys. It is also possible that glomerular disease, which proteinuria is characteristically associated with, represents a more progressive form of CKD and is itself linked with increased fibrosis.

Inflammation

Tubulointerstitial infiltration of lymphocytes and macrophages is an early feature of CKD and may be induced by proteinuria, reactive oxygen species generation and upregulation of angiotensin II (Rodriguez-Iturbe et al., 2001). Regardless of the initial cause, chronic renal inflammation is believed to play a critical role in the pathophysiology of CKD and the perpetuation of renal fibrosis (Rodriguez-Iturbe and Garcia Garcia, 2010). Depletion of inflammatory cells, such as macrophages and T-cells, has been demonstrated to ameliorate progression of tubulointerstitial fibrosis in rodent models of kidney disease (Ko et al., 2008) (Tapmeier et al., 2010). A variety of agents which interfere with the inflammatory process have also demonstrated the ability to prevent inflammatory cell infiltration and progression of renal fibrosis in experimental models, including the immunosuppressive drug mycophenolate mofetil (Romero et al., 1999), chemokine receptor CCR1 antagonists (Vielhauer et al., 2004), and inhibitors of the pro-inflammatory NF-κB pathway (Tamada et al., 2003).

Chronic inflammation sustains the production of pro-fibrotic growth factors and chemokines from activated epithelial and endothelial cells, as well as infiltrating leukocytes. Macrophages have been hypothesised to promote fibrogenesis via TGF-β signalling (Ko et al., 2008) but have also been demonstrated to switch from a pro-inflammatory to a suppressive phenotype after initial kidney damage (Lee et al., 2011), and their true role in renal injury and disease is likely to be complex. T-cells are believed to modulate myofibroblast activation, either via direct effects on resident fibroblasts or via inducing macrophages/tubular cells to produce profibrotic cytokines/growth factors (Nikolic-Paterson, 2010). Inflammatory cells are also able to differentiate into myofibroblasts and may contribute directly to ECM accumulation via this pathway (Jabs et al., 2005).

In cats, tubulointerstitial fibrosis is associated with infiltration of lymphocytes and plasma cells (DiBartola et al., 1987) and one recent study found a moderate correlation between the degree of inflammation and tubulointersitial fibrosis in feline CKD (Chakrabarti et al., 2013). The chemokine IL-8, a neutrophil chemotactic factor

which is released by leukocytes and endothelial cells in response to inflammation, has also been shown to be present in increased concentrations in the urine of cats with CKD (Habenicht et al., 2013).

Hypoxia

Chronic hypoxia of the renal tubulointerstitium has been hypothesised to be the final common pathway by which CKD progresses to end stage renal disease (Mimura and Nangaku, 2010). This is supported by evidence from experimental models of CKD where tubular hypoxia has been demonstrated to precede histopathological evidence of kidney damage, suggesting a causal relationship (Manotham et al., 2004).

Although the kidney receives 25% of cardiac output, far in excess of metabolic requirements, it is intrinsically susceptible to hypoxia due to the arteriovenous shunting of oxygen which results in the extraction of no more than 10% of the oxygen delivered by the renal artery (Evans et al., 2008). The renal cortex is perfused at a substantially greater rate than the outer and inner medulla, but tubular epithelial cells are especially vulnerable to hypoxic injury due to their high metabolic rate and reliance on aerobic metabolism (Epstein, 1997). In CKD, a series of factors lead to the development of a hypoxic state. The oxygen consumption of remnant nephrons within the diseased kidney is up to three times the oxygen requirement of normal nephrons due to the up-regulated metabolic activity of tubular cells (Harris et al., 1988). The capability of the cardiovascular system to meet this demand is hindered by the loss of peritubular capillaries associated with tubulointerstitial fibrosis (Choi et al., 2000), RAAS mediated vasoconstriction (Nangaku and Fujita, 2008) and the increased diffusion distance between capillaries and tubular cells created by the expansion of the ECM, resulting in tissue hypoxia.

Hypoxia acts to drive fibrogenesis through multiple pathways. Tubular cells exposed to mild hypoxia have been demonstrated to undergo transition into myofibroblasts (Xie et al., 2001; Zell et al., 2013) and exposure to severe hypoxia can result in apoptosis (Khan et al., 1999). Hypoxia also has direct effects on renal fibroblasts, resulting in increased matrix production, decreased matrix turnover and production of pro-fibrotic mediators (Norman et al., 2000). Hypoxia may also act as a stimulus for the inflammatory response via the recruitment of inflammatory cells (Kong et al., 2004).

In cats high urinary vascular endothelial growth factor (VEG-F), a marker of hypoxia within tissues, has been associated with shorter survival and progression of azotaemia, suggesting renal hypoxia is associated with progression in feline CKD (Chakrabarti et al., 2012b). However another recent study found that urinary VEG-F was significantly lower in cats with CKD than normal cats, so the significance of this marker and the role of hypoxia in feline CKD remains undetermined (Habenicht et al., 2013).

Ageing

The ageing kidney is characterised by a number of progressive morphological changes, including loss of renal mass, glomerulosclerosis and interstitial fibrosis (Pannarale et al., 2010). The amount of ECM present within the renal interstitium increases significantly with age (Abrass et al., 1995; Thomas et al., 1998), although this change differs substantially from the interstitial fibrosis of CKD with respect to the ECM composition. In cats, there is evidence that tubulointerstitial inflammation begins early in life and that these histopathological changes increase significantly with age (Lawler et al., 2006). The mechanisms behind age-related fibrogenesis are multifactorial and include increased inflammatory cell infiltration, increased myofibroblast activation, increased susceptibility to apoptosis and adverse haemodynamic changes in aged kidneys (Thomas et al., 1998). The expression of several pro-regenerative

growth factors, including epidermal growth factor (EGF) (Chou et al., 1997) and insulin like growth factor (IGF-1) (Chou et al., 1997), also decreases with age whilst there is an increase in pro-fibrotic TGF-β signalling (Ding et al., 2001).

The ageing process compromises the ability of the kidney to heal after acute or chronic injury, with various alterations contributing towards perpetuation of the chronic inflammatory response and subsequent fibrosis rather than resolution of the reparative phase (Pannarale et al., 2010). Studies utilising experimental models of renal injury have revealed an age dependent decline in the proliferative potential of tubular epithelial cells (Schmitt et al., 2008) believed to be associated with cellular senescence, the phenomenon where cells lose the ability to divide or replicate and thus cannot contribute to active repair. Cellular senescence is associated with shorter length of telomeres, the structures which protect the chromosome ends from degradation (Bodnar et al., 1998), and is characterised by morphological changes and altered cellular function. Markers of cellular senescence have been associated with renal fibrosis in experimental rodent models of renal disease as well as in humans (Melk et al., 2009; Braun et al., 2012) and, together with shortened telomere length, have also recently been linked with feline CKD (Quimby et al., 2013).

Hyperphosphataemia

Whilst elevated plasma phosphate may be a consequence of progressive loss of renal function, phosphate has been demonstrated to be associated with progression of renal disease independent of GFR in human studies (Zoccali et al., 2011), suggesting that phosphate might be causally implicated in renal disease progression.

It has long been recognised that increased plasma phosphate is associated with pathological changes within the kidney, with publications dating back to 1935 noting dramatic histological changes, including necrosis of the convoluted tubules, calcification and an increase in connective tissue, in normal animals fed high phosphate diets (Mackay and Oliver, 1935; Craig, 1959; Haut et al., 1980). Feeding of a phosphate restricted diet to cats with experimentally induced renal disease has been demonstrated to prevent histological damage in comparison with cats fed a high phosphate diet, the kidneys of which developed calcification, fibrosis and mononuclear cell infiltration (Ross et al., 1982). Initially there was controversy regarding these findings, as diets low in phosphate are also intrinsically associated with protein restriction (Laouari et al., 1982). Subsequent studies have supported the fact that phosphate restriction reduces renal injury in experimental models of disease independent of protein intake (Lumlertgul et al., 1986; Finco et al., 1992; Koizumi et al., 2002), whereas the benefit of protein restriction on the progression of CKD is controversial.

The mechanism for the detrimental effect of hyperphosphataemia on kidney tissue is unknown. The most common explanation for the deleterious effects of phosphate in these models is phosphate-induced calcification of the renal parenchyma resulting in an inflammatory and fibrotic response. There is evidence from human CKD and experimental models that hyperphosphataemia is associated with nephrocalcinosis and subsequent functional deterioration (Gimenez et al., 1987; Cozzolino et al., 2002). However, in the only published work relating kidney lesions to clinicopathologic variables in cats with naturally occurring CKD, plasma phosphate was associated with interstitial fibrosis but was not associated with tubular mineralisation (Chakrabarti et al., 2013). This suggests that the association between phosphate and progressive kidney fibrosis in cats may not involve parenchymal mineralisation but occurs via another mechanism.

Several alternative mechanisms have been proposed. In patients with CKD and other syndromes which cause elevated plasma phosphate, hyperphosphataemia is associated with vascular

Table 1
Target reference ranges for plasma phosphate in cats with CKD.

IRIS classification	Target phosphate (mmol/L)
Stage 2	0.81–1.45
Stage 3	0.81–1.61
Stage 4	0.81–1.94

calcification and stiffness (Kendrick and Chonchol, 2011). Hyperphosphataemia may also interfere with the function of, and even directly damage, the microvascular endothelium within the kidney (Kang et al., 2002; Di Marco et al., 2008; Shuto et al., 2009). These negative haemodynamic consequences may lead to ischaemia and hypoxia, and hence progressive fibrotic change. Phosphate may have direct effects on epithelial and mesenchymal cells within the kidney, leading to a pro-fibrotic state. Increased extracellular phosphate has wide ranging effects on cellular physiology (Camalier et al., 2013) and has been associated with apoptosis (Ohnishi and Razzaque, 2010), cellular senescence (Takemura et al., 2011) and oxidative stress (Kuro-o, 2010). Increasing concentrations of extracellular phosphate have also been demonstrated to increase the production of ECM and pro-fibrotic molecules by fibroblasts (Beck et al., 2000; Chen et al., 2012). Alternatively, phosphate may act indirectly via effects on the RAAS. Increased plasma phosphate may result in increased ACE expression within the kidney (Eraranta et al., 2012), and high plasma phosphate was associated with attenuation of the renoprotective effects of ACE inhibition in one human epidemiological study (Zoccali et al., 2011).

Conclusions

Halting or slowing the insidious progression of renal fibrosis is an ideal treatment target for preventing deterioration of CKD to end stage kidney disease, as it is the final convergent process of a variety of renal pathologies. Several of the mechanisms reviewed in this article are already targeted in the treatment recommendations for CKD in cats published by the International Renal Interest Society.[1] Cats diagnosed with stage 2 CKD and above with urine protein to creatinine ratio persistently higher than 0.4 should be treated with anti-proteinuric measures, which may include dietary protein restriction and pharmacological ACE or angiotensin II inhibition. The IRIS guidelines also include target reference ranges for plasma phosphate for cats in difference stages of CKD and a reduction of phosphate intake via dietary restriction, often in combination with a dietary phosphate binder, is recommended to maintain plasma phosphate within these ranges (Table 1). The future promises further therapeutics targeting the progression of renal fibrosis. A number of inhibitors of the TGF-β signalling system have been trialled experimentally and there is ongoing research into pharmacological manipulation of the kidney's response to hypoxia. It is hoped that further elucidation of the role played by these potential mediators of injury will lead to novel therapeutic targets, and better clinical outcomes, in the treatment of feline CKD.

Conflict of interest statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

[1] See IRIS Guidelines: http://www.iris-kidney.com/guidelines/ (accessed 9 April 2014).

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