EDITORIAL

The role of thymosin-β4 in kidney disease

Abstract

Therapies which modulate inflammation and fibrosis have the potential to reduce the morbidity and mortality associated with chronic kidney disease. A promising avenue may be manipulating thymosin-β4, a naturally-occurring peptide which is the major G-actin sequestering protein in mammalian cells and a regulator of inflammation and fibrosis. Thymosin-β4 is already being tested in clinical trials for heart disease and wound healing. In this editorial, we outline the evidence that thymosin-β4 may also have therapeutic benefit in chronic kidney disease.

Keywords: Ac-SDKP, fibrosis, inflammation, kidney disease, thymosin-β4

Introduction

Over the last decade an epidemic of chronic kidney disease (CKD) has occurred, linked to increased obesity, hypertension and diabetes. Current treatment strategies control known risk-factors through pharmacological inhibitors and lifestyle changes but new therapies are urgently required. Regardless of the origin, the progression to CKD is accompanied by inflammation, fibrosis, extracellular matrix accumulation and tubular atrophy. Therefore, treatments which modulate these pathophysiological processes have the potential to reduce the morbidity and mortality associated with CKD.

Thymosin-β4 is a low molecular weight naturally-occurring peptide and the major G-actin–sequestering protein in mammalian cells. Thymosin-β4 regulates actin filament assembly and disassembly in a dynamic balance with the actin-binding competitor profilin and the depolymerisation factor cofilin [1, 2]. In addition, thymosin-β4 is able to form a complex with PINCH-1 and integrin-linked kinase both of which are necessary for cell migration and survival [3]. Thymosin-β4 can also bind to stabilin-2, a membrane receptor involved in the engulfment of apoptotic cells [4]. The ability of thymosin-β4 to regulate cell movement and turnover has led to studies showing it can stimulate coronary vasculogenesis and angiogenesis [5] and regulate inflammation and fibrosis in mouse models of lung, heart and cornea injury [6-8]. Thymosin-β4 derivatives have similar properties. Thymosin-β4 sulfoxide is anti-inflammatory [9], whilst the tetrapeptide N-acetyl-seryl-aspartyl-lysyl-proline (Ac-SDKP; generated by thymosin-β4 cleavage mediated by the enzyme prolyl oligopeptidase) is able to reduce fibrosis in animal disease models [10-12].

Thymosin-β4 expression in developing, healthy adult and diseased kidneys

In the mouse kidney, thymosin-β4 transcripts can be detected from embryonic day 12 and increase throughout development until 7 days after birth [13]. In embryonic day 18 mouse kidneys thymosin-β4 is localised in the interstitium surrounding developing tubules and in differentiating glomeruli as assessed by *in-situ* hybridisation [13]. Thymosin-β4 levels are lower in the adult kidney with gene expression restricted to collecting ducts and glomeruli in 8 week old mouse kidneys [13]. Strong expression occurs in podocytes, the specialised epithelia of the glomerulus [13], a finding supported by profiling studies in adult mice identifying genes enriched in podocytes compared with the kidney cortex [14]. In contrast with these murine studies, immunohistochemisty of human fetal and adult kidneys have failed to detect thymosin-β4 protein in glomeruli [14] and further investigations are require to reconcile this discrepancy.

A proteomic study using the rat remnant kidney model provided the first evidence implicating thymosin-β4 in the progression of CKD [15]. The investigators performed unilateral nephrectomy of the right kidney and ligation of the branches of the left renal artery resulting in 5/6 renal ablation. The reduction in renal mass led to compensatory growth of the remaining nephrons resulting in glomerulosclerosis and interstitial fibrosis. Using laser capture microdissection, sclerotic and non-sclerotic glomeruli were isolated from the remnant kidney as well as normal glomeruli from the nephrectomised kidney [16]. Proteomic analysis found that thymosin-β4 expression levels were significantly increased three fold in sclerotic *versus* normal glomeruli and localised predominately to endothelial cells [16]. Subsequent studies also demonstrated increased thymosin-β4 levels in macrophages, myofibroblasts and tubular epithelia in the unilateral ureteral obstruction (UUO) model, where the ureter is ligated leading to a rapid reduction in renal blood flow and glomerular filtration rate in the obstructed kidney and subsequent inflammation and fibrosis [12]. These findings of raised thymosin-β4 in CKD provide a rationale that modulating thymosin-β4 levels could be a promising therapeutic approach for renal disease.

Exogenous thymosin-β4 and Ac-SDKP as therapeutic agents for kidney disease

The therapeutic potential of thymosin-β4 in patients is currently being assessed in several clinical trials for wound healing and cardiac repair [2]. However, to-date, there have only been two studies administering exogenous thymosin-β4 in animal models of renal disease, one in UUO and the other one in diabetic nephropathy. Administration of thymosin-β4 at a dose of 150 µg/day intraperitoneally did not alter the early phases of inflammation and fibrosis following UUO as examined five days after the surgical procedure [12]. However, by day 14 thymosin-β4 reduced fibrosis as assessed by Sirius red staining, but still had no effect on renal inflammation [12]. The authors began to explore the potential molecular mechanisms and found thymosin-β4 administration reduced plasminogen activator inhibitor-1 (PAI-1) expression and transforming growth factor-β1 signalling, both of which are important pro-fibrotic pathways in CKD. Interestingly, when thymosin-β4 was then administered to PAI-1 knock-out mice that had also undergone UUO there was no improvement in either the early or late phases of disease progression. This finding indicated that changes in PAI-1 expression could be an important mechanism underlying the actions of thymosin-β4 in the UUO model.

The second study [17] examined the therapeutic potential of thymosin-β4 in diabetic nephropathy, one of the leading causes of end-stage renal disease worldwide using a model of type 2 diabetes mellitus, *KK Cg-Ay/J* mice. Twelve week old mice were treated daily with thymosin-β4 (100 ng/10 g/day intraperitoneally) or saline for three months. Thymosin-β4 treatment improved renal function as shown by a reduction in the albumin to creatinine ratio and attenuated the renal pathological changes in *KK Cg-Ay/J* mice. However, the mechanisms that mediated these effects were not investigated; thymosin-β4 also improved hyperglycemia in *KK Cg-Ay/J* mice compared with saline controls and this may be the primary reason for the improvement in renal function and structure seen in this study.

The N-terminal tetrapeptide Ac-SDKP has been more extensively studied in CKD. Cavasin and colleagues [18] showed that decreasing basal levels of Ac-SDKP in the mouse kidney by administrating an oral prolyl oligopeptidase inhibitor (S17092, 40 mg/kg/day) increased renal fibrosis and promoted glomerulosclerosis [18]. Other investigators have demonstrated beneficial effects of exogenous Ac-SDKP in CKD animal models. In the mouse UUO model, Ac-SDKP treatment (1.6 mg/kg/day) via osmotic minipumps decreased fibrosis at both early and late time-points [12] as evidenced by significant decreases in fibronectin, collagen I, PAI-1, TGF-β1 signalling and reduced numbers of renal macrophages and myofibroblasts. Similar findings were observed in a rat UUO model using a lower dose (400 µg/kg/day) of Ac-SDKP [19]. Two weeks after UUO, Ac-SDKP reduced fibrosis in the kidney as well as decreasing the number of macrophages. This was accompanied by a reduction in the renal expression of α-smooth muscle actin, monocyte chemoattractant protein-1 (MCP-1) and TGF-β1 [19]. A set of elegant studies using prophylactic and interventional experimental protocols examined the effect of exogenous Ac-SDKP (800 µg/kg/day) or vehicle administration in rat remnant kidneys [20]. In both protocols Ac-SDKP improved albuminuria, glomerular filtration rate, macrophage infiltration, glomerulosclerosis and renal collagen content [20]. In addition, these investigators found that Ac-SDKP reversed the loss of nephrin, a molecule critical for the integrity of the glomerular filtration barrier, seen in remnant kidneys providing a potential mechanism by which Ac-SDKP may elicit its therapeutic benefit in this animal model [20]. Ac-SDKP is also renoprotective in hypertensive mice [21]. In deoxycorticosterone acetate-salt hypertensive mice, Ac-SDKP treatment (800 µg/kg/day) for three months improved glomerular matrix expansion, inflammation, fibrosis and albuminuria [21]. These effects were not due to an effect of Ac-SDKP in blood pressure which was unaltered.

Ac-SDKP has also been shown to be beneficial in experimental diabetic nephropathy [11]. Ten week old male *db/db* mice were administered either 1 mg/kg/day of Ac-SDKP or balanced salt solution and examined 8 weeks later [11]. Ac-SDKP treatment did not alter hyperglycaemia, blood pressure or peripheral erythrocyte number but prevented the pathological increase in glomerular surface area, mesangial matrix expansion, and overproduction of extracellular matrix proteins compared with *db/db* mice administered balanced salt solution [11]. Ac-SDKP therapy also reduced plasma creatinine levels, but not albumin excretion compared with *db/db* mice treated with balanced salt solution [11]. The improvement in renal histology and function in *db/db* mice following Ac-SDKP treatment was accompanied by diminished TGF-β signalling within glomeruli [11]. Ac-SDKP treatment has also been tested in a rat model of type 1 diabetes induced by streptozotocin [10]. Eight weeks after streptozotocin injection, rats were provided with Ac-SDKP at a dose of 1 mg/kg/day for two months delivered by osmotic minipump [10]. Ac-SDKP administration improved fibrosis in diabetic rats and reversed diabetes-induced loss of nephrin. Despite these molecular and structural changes, Ac-SDKP did not alter renal function with no improvement in albuminuria observed [10]. This discrepancy may be due to the fact that Ac-SDKP treatment was started too late after the induction of diabetes and further early intervention studies should be performed to address this issue.

In another study [22] Omata and colleagues examined the therapeutic potential of Ac-SDKP in a rat model of glomerulonephritis. A preventive strategy was taken and Ac-SDKP (1mg/kg/day) administered by osmotic minipump two weeks after the induction of glomerulonephritis for one month. Ac-SDKP treatment ameliorated disease progression as demonstrated by reduced proteinuria, blood urea nitrogen, plasma creatinine, glomerulosclerosis and interstitial fibrosis compared with the rats administered saline [22]. Ac-SDKP treatment diminished TGF-β signalling in the kidney shown by reduced Smad2 phosphorylation and increased Smad7 expression [22]. The renal expression of pro-inflammatory genes (intercellular adhesion molecule 1, interleukin-1β, MCP-1 and tumor necrosis factor-α) were reduced by Ac-SDKP treatment along with the accumulation of macrophages in both the glomerulus and the tubulointerstitium [22]. Interestingly, Ac-SDKP did not alter the total number of peripheral leukocytes suggesting that the main effect of Ac-SDKP was on monocyte infiltration into the kidney [22].

In conclusion, the progression of CKD involves inflammation and fibrosis; processes that can be modulated by thymosin-β4. The studies conducted so far have shown promising results for the therapeutic potential of thymosin-β4 in CKD. However more work is required to investigate the role of endogenous thymosin-β4 in the kidney, define the optimal therapeutic strategy using thymosin-β4 and its derivatives in different disease scenarios and identify the mechanisms that mediate the therapeutic effects of thymosin-β4 in CKD. These studies would strengthen the possibility of using thymosin-β4 as a novel treatment to reduce the morbidity and mortality associated with CKD.

Expert Opinion

The studies presented suggest that thymosin-β4 and its breakdown product Ac-SDKP could be a promising therapeutic avenue to reduce the morbidity and mortality associated with CKD. Further pre-clinical studies are required particularly using thymosin-β4 to determine optimal therapeutic doses and timing of treatment in different disease scenarios such as glomerulonephritis and remnant kidneys. There is also a need to compare the effectiveness of thymosin-β4 *versus* its derivatives for the treatment of kidney disease in animal models. This has been done for UUO [12] and these studies demonstrated that Ac-SDKP acts faster and reduces more disease parameters. To-date, there have been no studies examining the effect of thymosin-β4 sulfoxide in CKD.

Most of the studies to-date indicate that the benefits of thymosin-β4 and Ac-SDKP are due to reduced renal macrophage number and the attenuation of fibrosis, possibly via the modulation of TGF-β signalling. However, we do not yet understand how thymosin-β4 precisely affects peripheral macrophages and resident kidney cells. Another area of interest could be potential effects on the endothelium which plays a key contribution to the progression of CKD [23].

Despite the studies demonstrating the therapeutic potential of thymosin-β4 and Ac-SDKP in experimental kidney disease, the functional role of endogenous thymosin-β4 in the kidney is completely unknown. Given the high expression of thymosin-β4 in podocytes and the role of thymosin-β4 in regulating actin filament assembly, it could be postulated that thymosin-β4 may play a role in regulating podocyte shape which could contribute to the integrity of the glomerular filtration barrier. Studies using transgenic mice lacking thymosin-β4 in specific cell types in the kidney will provide important insights into understanding the role of endogenous thymosin-β4 in the normal and diseased kidney.

The studies described in this review have focused on using animal models to elucidate the role of thymosin-β4 and Ac-SDKP in the kidney and its relative levels in health and renal disease. Some studies have also shown that plasma Ac-SDKP is increased in CKD patients and further enhanced in those individuals also treated with angiotensin converting enzyme (ACE) inhibitors [24, 25]. These findings have been attributed to the impaired clearance of Ac-SDKP due to the decline in glomerular function in CKD [22] and the prevention of Ac-SDKP degradation which normally occurs through the actions of ACE [26]. There is currently no human data available on the circulating or kidney levels of thymosin-β4 in patients with CKD and these studies should be undertaken in the future.

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