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Tβ4–Ac-SDKP pathway: Any relevance for the cardiovascular system?

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Abstract

The last 20 years witnessed the emergence of the thymosin β4 (Tβ4)–*N*-acetyl-seryl-aspartyl-lysyl-proline (Ac-SDKP) pathway as a new source of future therapeutic tools to treat cardiovascular and renal diseases. In this review article, we attempted to shed light on the numerous experimental findings pertaining to the many promising cardiovascular therapeutic avenues for Tβ4 and (or) its N-terminal derivative, Ac-SDKP. Specifically, Ac-SDKP is endogenously produced from the 43-amino acid Tβ4 by 2 successive enzymes, meprin and prolyl oligopeptidase. We also discussed the possible mechanisms involved in the Tβ4–Ac-SDKP-associated cardiovascular biological effects. In infarcted myocardium, Tβ4 and Ac-SDKP facilitate cardiac repair after infarction by promoting endothelial cell migration and myocyte survival. Additionally, Tβ4 and Ac-SDKP have antifibrotic and anti-inflammatory properties in the

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Conflict of interest

The authors declare that there is no conflict of interest associated with this work.

arteries, heart, lungs, and kidneys, and stimulate both in vitro and in vivo angiogenesis. The effects of Tβ4 can be mediated directly through a putative receptor (Ku80) or via its enzymatically released N-terminal derivative Ac-SDKP. Despite the localization and characterization of Ac-SDKP binding sites in myocardium, more studies are needed to fully identify and clone Ac-SDKP receptors. It remains promising that Ac-SDKP or its degradation-resistant analogs could serve as new therapeutic tools to treat cardiac, vascular, and renal injury and dysfunction to be used alone or in combination with the already established pharmacotherapy for cardiovascular diseases.

Résumé:

Au cours des 20 dernières années, nous avons assisté à l'émergence de la voie de signalisation de la thymosine β4 (Tβ4)– *N*-acétyl-séryl-aspartyl-lysyl-proline (Ac-SDKP) comme nouvelle source d'outils thérapeutiques futurs pour le traitement de maladies cardiovasculaires et rénales. Dans cet article de synthèse, nous avons tenté de mettre en lumière les nombreux résultats expérimentaux quant aux nombreuses avenues thérapeutiques cardiovasculaires prometteuses pour le Tβ4 ou l'Ac-SDKP, son dérivé N-terminal. Spécifiquement, l'Ac-SDKP est un produit endogène obtenu à partir de Tβ4 de 43 acides aminés par 2 enzymes successives : la méprine et la prolyl oligopeptidase. Nous avons aussi discuté d'éventuels modes d'action pouvant jouer un rôle dans les effets biologiques cardiovasculaires associés au Tβ4–Ac-SDKP. Dans le myocarde infarci, le Tβ4 et l'Ac-SDKP facilitent la réparation du cœur après l'infarctus en favorisant la migration des cellules endothéliales et la survie des myocytes. En outre, le Tβ4 et l'Ac-SDKP ont des propriétés anti-fibrotiques et anti-inflammatoires dans les artères, le cœur, les poumons et les reins, et stimulent l'angiogenèse tant in vitro qu'in vivo. Les effets du Tβ4 peuvent être médiés directement par l'intermédiaire d'un récepteur putatif (Ku80) ou de l'Ac-SDKP, son dérivé N-terminal, libéré de manière enzymatique. En dépit de la localisation et de la caractérisation des sites de liaison de l'Ac-SDKP dans le myocarde, d'autres études seraient nécessaires pour caractériser entièrement et cloner les récepteurs de l'Ac-SDKP. Il demeure prometteur que l'Ac-SDKP ou ses analogues résistants à la dégradation puissent servir de nouveaux outils thérapeutiques contre les lésions et le dysfonctionnement du cœur, des vaisseaux et des reins utilisés seuls ou en association avec des agents pharmacothérapeutiques déjà établis contre les maladies cardiovasculaires. [Traduit par la Rédaction]

Keywords

Ac-SDKP; thymosin beta 4; cardiovascular; renal; angiotensin-converting enzyme

Mots-clés

Ac-SDKP; thymosine bêta 4; cardiovasculaire; rénal; enzyme de conversion de l'angiotensine

General aspects of thymosin β4 (Tβ4)–*N*-acetylseryl-aspartyl-lysyl-proline (Ac-SDKP)

Tβ4 is an endogenous 43-amino acid peptide, first isolated in the thymus and subsequently found in the blood circulation, urine, and various organs, including the heart and kidneys

(Mora et al. 1997). Tβ4 was best known for its G-actin sequestering protein, and thus preventing actin polymerization and ensuring the availability of an optimal amount of actin monomer for rapid filament elongation (F-actin formation) when it is needed for specific cell activity (Cavasin 2006). However, it became evident that Tβ4 has numerous biological functions, including stimulation of cell migration, angiogenesis, cell survival, tissue regeneration, and inhibition of inflammation (Crockford et al. 2010). Tβ4 is the precursor of Ac-SDKP because it contains the Ac-SDKP sequence in its NH_2-terminal (Hannappel 2010). Our group has shown previously that Ac-SDKP is released from Tβ4 by the peptidases present in kidney homogenates, and specific inhibitors of prolyl oligopeptidase (POP) block this release (Cavasin et al. 2004). However, POP has a structural characteristic that prevents the enzyme from hydrolyzing peptides containing more than 30 amino acids (Polgár 2002), meaning that larger peptides and proteins are resistant to POP hydrolysis. Therefore, prior to Ac-SDKP release via POP cleavage, Tβ4 must undergo hydrolysis by a newly described peptidase, meprin α (Kumar et al. 2016).

Tβ4 has several biological functions that have been reported in numerous studies. In permanently ligated mouse and ischemia–reperfusion pig models, Tβ4 stimulated myocardial cell migration, promoted angiogenesis and survival of cardiomyocytes, and decreased inflammation, thus improving cardiac function (Hinkel et al. 2008). We have also reported that T4, at a dose that is unable to generate optimal circulating Ac-SDKP concentrations (Rhaleb et al. 2001*b*), prevents cardiac rupture and improves cardiac function post-myocardial infarction (MI) via its anti-inflammatory, proangiogenic, and anti-apoptotic actions in a murine model of acute MI (Peng et al. 2014). Comparable results are obtained when Ac-SDKP was used instead of Tβ4 (Peng et al. 2019, in press). The MI model in rodents shares both clinical and pathological features of post-MI with changes found in human hearts, including cardiac rupture and dysfunction (Bock-Marquette et al. 2004). Thus, Tβ4 could be used as an alternative therapy in preventing cardiac rupture and restoring cardiac function in patients with MI.

Synthesis and degradation

Tβ4 is a naturally occurring peptide consisting of 43 base pairs of amino acids and generates the N-terminal tetrapeptide AcSDKP (Kumar et al. 2016; Ma and Fogo 2009). Ac-SDKP is widely found in mammalian organs, plasma, urine, and mononuclear cells (Mora et al. 1997; Roth et al. 1999). Like Tβ4, Ac-SDKP has anti-inflammatory and antifibrotic properties. For example, they have been shown to reduce tissue invasion by detrimental inflammatory cells, and collagen deposition in the hypertension, diabetes, renal, and cardiovascular diseases (Cavasin 2006). Until recently, how Ac-SDKP was liberated from Tβ4 was unknown. A peptidase database revealed to us that meprin-α metalloprotease could hydrolyze Tβ4 first by releasing NH_2-terminal intermediate peptides that are less than 30 amino acids. Then POP hydrolyzes the intermediate peptides to release Ac-SDKP (Kumar et al. 2016; Ma and Fogo 2009). Indeed, actinonin, a specific inhibitor for meprin-α, blocked both in vivo and in vitro generation of Tβ4 fragments, including the N-terminal sequence Ac-SDKP. Thus, both meprin-α metalloprotease and POP are needed for Ac-SDKP to be released from Tβ4 (Fig. 1).

Angiotensin-converting enzyme (ACE) is an essential enzyme of the renin–angiotensin system (RAS). Renin first produces angiotensin (Ang) I from angiotensinogen, and then ACE cleaves Ang I releasing the endogenous agonist Ang II (Carretero et al. 2009). The ACE protein is a single cell surface zinc metallopeptidase chain, composed of 2 separate and independent catalytic domains. Each domain contains the zinc-binding site HEMGH. These domains, called N- and C-domains, have a high conservation of sequence and exon structure, which suggest that they originated from a gene duplication event during the course of evolution (Bernstein et al. 2011). The C-domain of ACE plays the key role in the conversion of Ang I to Ang II, whereas the N-domain is responsible for the degradation of other peptides such as the antifibrotic peptide Ac-SDKP (Bernstein et al. 2011). Selective inhibition of the ACE N-domain by the phosphinic peptide RXP 407 (Junot et al. 2001) or specific gene deletion of the ACE N-domain has resulted in significant and substantial increases of circulating concentrations of Ac-SDKP without diminishing the pressor effects of Ang I (Bernstein et al. 2011; Fuchs et al. 2004). Therefore, one cannot exclude the participation of endogenous Ac-SDKP in the anti-inflammatory and antifibrotic beneficial effects of ACE inhibitor (ACEI) in the cardiovascular system and kidneys by increasing circulating and tissue levels of endogenous Ac-SDKP (Romero et al. 2017). To further document the role of Ac-SDKP in the beneficial effects of ACE inhibition, we used a model of mineralocorticoidsalt- or Ang-II-induced hypertension in rats treated with the ACEI either alone or combined with a blocking monoclonal antibody (mAb) to Ac-SDKP (Peng et al. 2005, 2007). In these studies, we reported that mAb-anti-Ac-SDKP was able to block the antifibrotic and anti-inflammatory effects of ACEI captopril without affecting blood pressure or cardiac hypertrophy, letting us to conclude that those beneficial effects of ACE inhibition are in part due to protection of endogenous Ac-SDKP from hydrolysis. Others have used POP inhibitor to demonstrate the key role played by Ac-SDKP in the protective effects of ACEIs; indeed, Li et al. have shown that inactivation of POP by selective inhibitor S-17092 reduced the formation of Ac-SDKP and blocked the protective effect mediated by the N-terminal catalytic site of ACE in a model of bleomycininduced lung fibrosis via Ac-SDKP (Li et al. 2010). Similarly, Zuo et al. found that the renal protective effects of Tβ4 could be almost fully opposed by treatment of animals under the unilateral ureter obstruction-induced nephritis with a POP inhibitor, indicating again the important role played by Ac-SDKP in the Tβ4 effects (Zuo et al. 2013).

Tβ4 and fragment biology: endogenous regulation in health and during disease (Fig. 2)

A study using short hairpin (sh) RNA knockdown of Tβ4 specifically in an Nkx2.5-Cre expression domain resulted in significant cardiac developmental defects. Tβ4 is pivotal in restoring pluripotency and triggering differentiation of fibroblasts, smooth muscle cells, and endothelial cells (Banerjee et al. 2012). Tβ4 was also found to be a regulator of heart valve formation in zebrafish embryos (Shin et al. 2014). Additionally, a recent study demonstrated that mice with global or cardiac-specific Tβ4 knockout did not exhibit any significant embryonic or adult cardiovascular phenotype; had no deleterious effects on angiogenesis; and did not have any noticeable histological defects, cardiac function, or age-related decline in cardiovascular function (Banerjee et al. 2012; Smart et al. 2007). However, Tβ4

deficiency does result in exacerbated renal and cardiac injury and dysfunction in Ang-II-induced hypertension. Whether this effect is due to lack of Tβ4 per se or deficiency in production of Ac-SDKP is awaiting further investigations (Kumar et al. 2018).

Ac-SDKP has protective effects in hypertensive kidney disease models of glomerulosclerosis. Our laboratory has shown that AcSDKP prevents increased collagen deposition and cell proliferation in the heart and kidney in aldosterone-salt hypertensive rats (Peng et al. 2001). Moreover, endogenous Tβ4 is upregulated in the kidney ischemia–reperfusion model (Liao et al. 2010). We found that Ac-SDKP greatly attenuates albuminuria and renal fibrosis; it improves renal function in rats with 5/6th nephrectomy (Liao et al. 2010). Our laboratory further investigated the roles of Tβ4 and Ac-SDKP in modulating early versus late tubulointerstitial fibrosis. We demonstrated that Tβ4 is upregulated in kidneys after obstructive injury, along with occasional tubular cells and increased numbers of infiltrating macrophages, fibroblast proliferation, and myofibroblasts differentiation. We also showed that exogenous administration of Tβ4 plus POP inhibitor, which prevents the metabolism of Tβ4 to Ac-SDKP, exacerbated both early and late interstitial fibrosis in mice, indicating a crucial role by Ac-SDKP in the protective effects of Tβ4 (Cavasin et al. 2004, 2007). In contrast, Ac-SDKP treatment decreased both early and late fibrosis with less total collagen and fibronectin deposition, decreased myofibroblasts and monocyte/macrophages, and suppressed profibrotic factors, plasminogen activator inhibitor-1 (PAI-1) and transforming growth factor (TGF)-β1 (Zuo et al. 2013).

In the normal physiologic state of the kidneys, there is undetectable fibrosis and PAI-1; however, PAI-1 is increased in patients who suffer from diabetic nephropathy and arterio-nephrosclerosis (Ma and Fogo 2009). There are studies that showed that Ang II upregulates PAI-1 gene expression by the AT_1 receptor, because all these effects were specifically blocked by an AT_1 receptor antagonist. Moreover, patients with primary hyperaldosteronism had elevated PAI-1 antigen, and aldosterone induced in vitro PAI-1 expression via the glucocorticoid response element in the PAI- promoter (Brown et al. 2000). Interestingly, Ac-SDKP inhibited TGF-β1-induced PAI-1 and α2 type collagen in mesangial cells in vitro, and ameliorated renal insufficiency and mesangial expansion in the db/db mouse model of diabetes (Ma and Fogo 2009).

Tβ4–Ac-SDKP and ACE

It is well established that Ac-SDKP is almost exclusively degraded by the N-domain of ACE (Azizi et al. 1996, 1997; Bernstein et al. 2011). Rats treated with captopril, an ACEI, exhibited increased plasma and urine concentrations of Ac-SDKP, which were inhibited by the co-administration of actinonin, a meprin-α metalloprotease inhibitor (Kumar et al. 2016).

Several trials (e.g., Heart Outcomes Prevention Evaluation, Survival and Ventricular Enlargement, and European Trial of Reduction of Cardiac Events with Perindopril in Stable Coronary Artery Disease) have exhibited improvement in cardiovascular disease outcomes using ACEi (Rosendorff et al. 2015), while the role of Tβ4's N-terminal fragment Ac-SDKP is not yet established in patients. However, many incidences indicate possible involvement of

Ac-SDKP in the protective effects of ACEi. We have shown that treatment with ACEIs is associated with reduced degradation of Ac-SDKP and has resulted in blunted hypertension-induced inflammation and fibrosis in the heart and kidneys without affecting blood pressure or hypertrophy. These protective effects were antagonized with monoclonal antibodies against Ac-SDKP (Hrenak et al. 2015; Peng et al. 2005, 2007). Moreover, in a study of Ser333Trp ACE mutation identified in a patient of African descent who displayed unusual blood ACE kinetics with regards to N-domain-associated peptides, it was noted that the clearance of Ac-SDKP was reduced (Danilov et al. 2014). Another important study by Pokharel et al. has demonstrated that increased cardiac ACE activity by genetically overexpressing cardiac-specific ACE resulted in exaggerated increase in cardiac collagen content; this phenotype has been attributed to selectively increased degradation of endogenous Ac-SDKP, and cancellation of its inhibitory effects on the phosphorylation of TGF-β signaling molecules and its downstream signaling (Peng et al. 2005, 2007; Pokharel et al. 2004). This implies that the antifibrotic effects of ACEIs are mediated in part by increasing cardiac Ac-SDKP. In addition, Tβ4 and its cleavage product Ac-SDKP are reportedly downregulated in advanced chronic heart failure in patients and the canine experimental model of congestive heart failure (Gupta et al. 2014; Sabbah et al. 2015), indicating a substantial clinical significance of the Tβ4–Ac-SDKP pathway in cardiovascular diseases. This suggests that strategies to increase concentrations of the endogenous antifibrotic peptide Ac-SDKP or administration of Ac-SDKP analogs with higher circulating half-life would be safe and physiologically relevant.

Tβ4 is a G-actin binding protein that accelerates wound healing, decreases inflammation, and is found only in mammals (Huff et al. 2004). It was first thought to be located only in thymocytes; however, it is ubiquitously distributed in the body, including lymphocytes, macrophages, cornealcells, and the kidneys (Gómez-Márquez et al. 1989; Nathan 1987). Tβ4 is also known to increase tissue remodeling, vascular endothelial growth factor, and angiogenesis in various tissues like myocardium, cornea, and dermis. Tβ4 has been known to upregulate antioxidative enzymes in human corneal epithelial cells for protection against H_2O_2-induced oxidative damage (Ho et al. 2008). Others have also demonstrated that Tβ4 is upregulated in the fibrotic interstitium found in the distal and proximal tubular cells, and peritubular capillaries of the kidneys in a model of unilateral ureteral obstruction (Ma and Fogo 2009), but the significance of this finding remains to be determined and reconciled against other reports that showed rather downregulation of Tβ4 in heart failure (Gupta et al. 2014; Sabbah et al. 2015).

There is an array of chronic renal diseases that cause chronic nephropathies by damage via progressive renal scarring, fibrosis, increased blood pressure, glomerular hyperfiltration, proteinuria, and loss of renal function (Rodríguez-Lara et al. 2018). In these diseases, the RAS is activated, which releases renin from the juxtaglomerular cells in the proximal convoluted tubule of the kidneys. Renin then converts angiotensinogen (found in the liver) to Ang I, which is in turn converted to Ang II via ACE (Rodríguez-Lara et al. 2018). Ang II then increases sympathetic activity; increases tubular Na^+, Cl^- absorption, and K^+ excretion; causes the adrenal gland to release aldosterone further causing H_2O retention; causes arteriolar vasoconstriction to increase blood pressure; and releases antidiuretic hormone from the posterior pituitary gland to increase H_2O absorption from the collecting duct

(Rodríguez-Lara et al. 2018). All these factors contribute to water and salt retention, and increases circulating volume and perfusion of the juxtaglomerular apparatus in the kidneys (Rodríguez-Lara et al. 2018).

Animal data has shown that inhibition of the RAS can ameliorate the deterioration of the kidneys in these diseases, as the RAS consist of classes of pro-fibrotic factors (van der Meer et al. 2010). The direct effects of Ang II cause increased protein ultrafiltration via ACE (Carlström et al. 2015). Large proteins are eventually lost into the urinary space, and tubulointersitial damage causes renal function to decline. Protein overload in the tubules causes tubular cells to release cytokines, chemokines, growth factors, and vasoactive substances. This leads to abnormal interstitial accumulation of inflammatory cells and interstitial fibrosis (Ma and Fogo 2009). The data suggested that chronic renal therapy with ACEI or Ang II receptor type I blockers can reverse the damaging glomerulosclerosis even in later stages of chronic kidney diseases. In addition, another murine study demonstrated that when the addition of Ang II infusion caused fibrosis, Tβ4 levels were increased (Ma and Fogo 2009). The physiological significance of such finding has not yet been explored.

Interaction of ACE or AT_1 antagonist and Ac-SDKP toward therapy optimization and side effect factors

There were major findings in our laboratories that showed that mice treated with Tβ4 exhibited a reduced mortality rate because of decreased left ventricular rupture post-MI (Peng et al. 2014). We found that excessive inflammatory responses were partially prevented by Tβ4 compared with the control group (Peng et al. 2014). Tβ4 exerts anti-inflammatory actions by inhibiting proinflammatory factors such as ICAM-1 in the left ventricle and inhibiting gelatinolytic activity. Moreover, we showed that Tβ4 treatment for 5 weeks not only reduced interstitial fibrosis and increased capillary density in the myocardium, which lead to improved cardiac function, but also ameliorated left ventricle dilatation and improved cardiac function, as evidenced by an increase in ejection fraction and shortening fraction (Peng et al. 2014). Tβ4 could potentially be a therapeutic regimen for patients with acute MI.

Ac-SDKP is not only present in the spleen and thymus, but also in the lymph node where it has yet not been investigated. T4, Ac-SDKP, and its releasing enzymes meprin-α and POP were measured in the lymph nodes, thymus, spleen of Sprague-Dawley rats (Romero et al. 2017). Cell sorting was used to measure Ac-SDKP in various lymph node cell populations. The highest concentration of Ac-SDKP was found in lymph nodes, followed by testis, thymus, and spleen. Positive immunostaining of TB4 and meprin-α in multi-nucleated giant cells was found in the cortical region, septum, follicular, and germinal centers of the lymph nodes. POP staining was also positive in the cortical region. Ac-SDKP was found to be higher in the lymph nodes than in the T lymphocytes. Macrophages were found to be the main source of Ac-SDKP in the lymph nodes. This data could indicate a key role of lymph nodes and macrophages in the preventative effects of Ac-SDKP on target organ damage of the heart in patients who are also undergoing treatment with ACEIs.

Diabetes affects more than 345 million people worldwide (Forouhi and Wareham 2014). The incidence of diabetes is increasing in the United States and there are long-term complications of diabetes mellitus that includes peripheral neuropathy and diabetic nephropathy. There is no effective treatment or reversal of the progression of diabetic neuropathy. Progression to diabetic nephropathy could be controlled by ACEIs along with tight glucose control; however, there is an urgent need for novel and more efficient methods to combat peripheral neuropathy and diabetic nephropathy. Wang et al. showed that extended Tβ4 treatment of diabetic mice improves neurological function in diabetic neuropathy independent of glucose blood levels (Wang et al. 2015). There was axonal regeneration and remyelination of peripheral nerves in mice treated with Tβ4 mediated by the Ang1/Tie signaling pathway (Wang et al. 2015).

There are studies that have shown that Ac-SDKP could play a protective role against diabetic nephropathy. Ac-SDKP served as an antifibrotic factor in human mesangial cells (Pokharel et al. 2002) and ameliorated renal insufficiency and glomerulosclerosis in diabetic mice via inhibition of the TGF-β/Smad pathway (Pokharel et al. 2002). In recent studies, Nagai et al. (2014) and Nitta et al. (2016) showed that oral administration of Ac-SDKP cured kidney fibrosis. They observed that Ac-SDKP inhibited endothelial-mesenchymal transition ameliorated glomerulosclerosis and tubulointerstitial fibrosis in streptozotocin-induced diabetes in CD-1 mice. The renal protective effect of combined Ac-SDKP and ACEI was better than ACEI or Ac-SDKP alone (Nagai et al. 2014; Nitta et al. 2016). The most common therapy used to combat diabetic nephropathy is based on RAS inhibitors, which have been shown to reduce diabetic nephropathy (Piccoli et al. 2015). ACEI and Ang II type 1 receptor blockers (ARB) are 2 major drug classes prescribed to delay diabetic nephropathy progression (Piccoli et al. 2015). These 2 drug classes could show similar renoprotective effects, but also display significant differences in organ protection from diabetes-associated damage.

It is well known that the maximum dose of ACEI, which abolishes circulatory Ang II, cannot fully inhibit local Ang II production in renal tubules, and yet the ACEI captopril exhibited significant reduction of renal injury and fibrosis in a model of adriamycin-induced nephropathy in mice (Tang et al. 2008). Surprisingly, a high dose of ARB losartan alone or combined with statin therapy failed to overcome adriamycin-induced nephropathy (Tang et al. 2008). A recent meta-analysis revealed that ACEI exhibited stronger organ protection compared with ARB in patients with type 2 diabetes with nephropathy (Strauss and Hall 2018). We and others previously reported that monotherapy with ACEI, ARB, or aldosterone blocker (Anavekar and Solomon 2005; Böhm 2007; Liu et al. 2002; Marcy and Ripley 2006; Russell et al. 1993; Sato et al. 2006; Xu et al. 2002; Yang et al. 2001; Zhu et al. 2012) achieved some cardioprotective effects. Further protective effects were achieved if these monotherapies were to be combined with other drugs such as eplerenone, but not with a p38 inhibitor as we have previously shown (Liu et al. 2005; Wang et al. 2004*b*). Also, monotherapy with Ac-SDKP or ACEI only partly improved cardiac function (Gao et al. 2012; Leuschner et al. 2010; Liu et al. 1997, 2002, 2005; Xu et al. 2004). Moreover, results obtained by Castoldi et al., indicate that Ac-SDKP has an additive effect in reducing cardiac and renal fibrosis with respect to ACE inhibition alone (Castoldi et al. 2009, 2013). In fact, in type 1 diabetic rats, the administration of Ac-SDKP or an ACEI alone reduced cardiac

TGF-β1 and Smad2/3 signaling pathway, and interstitial and perivascular fibrosis; these 2 drugs also reduced glomerular, tubulointerstitial, and perivascular fibrosis. More importantly, the concomitant administration of Ac-SDKP and ACEI resulted in a further significant reduction of cardiac and renal fibrosis when compared with ACE-l alone. Thus, Ac-SDKP, by acting directly on cardiovascular and renal damages such as tissue fibrosis, could represent a new therapeutic tool complementary to classic cardioprotective treatment based on antihypertensive drugs. However, one would stipulate that the additive effects of ACEI and Ac-SDKP could be due to the increased bioavailability of Ac-SDKP by preventing its degradation by ACE; thus, future studies are needed to investigate the therapeutic values of Ac-SDKP alone or combined with ARB, aldosterone receptor blocker, β-adrenergic receptor antagonists, or calcium channel blockers. These reports suggested that the biology of ACE in kidney fibrosis is not limited to “angiotensin-conversion” but may involve some angiotensin-independent effects.

Therapeutic considerations

These reports clearly demonstrate that Tβ4 and its N-terminal tetrapeptide derivative Ac-SDKP could be useful in the treatment of diabetic nephropathy. Tables 1 and 2 summarize most noticeable biological and therapeutic effects, together with proposed corresponding cellular signaling. It would be ideal to develop analogs of Ac-SDKP that could resist degradation, have longer circulating half-life, and that could be administered through single subcutaneous injection such as via special hydrogels for sustained release. ACE inhibition could confer renal protection in diabetic nephropathy by reducing intrarenal Ang II and augmenting AcSDKP expression.

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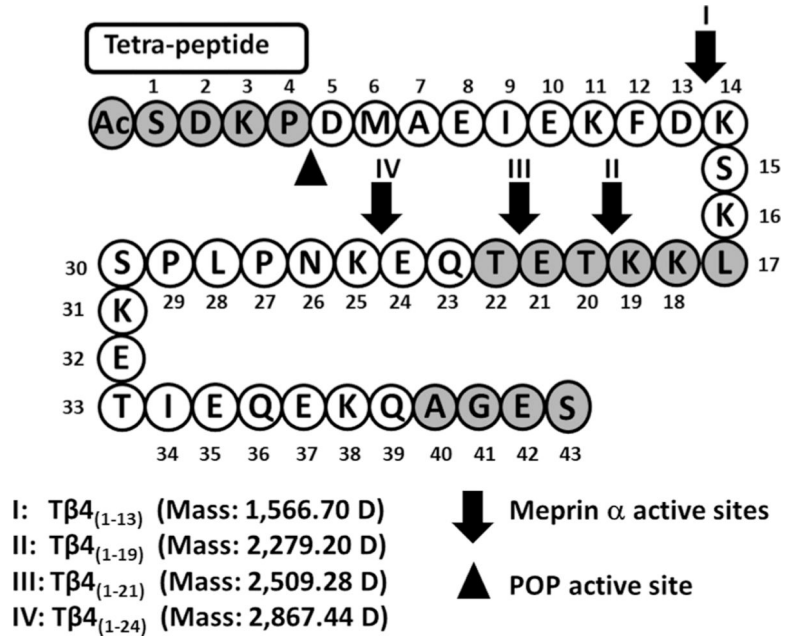

Fig. 1.

Amino acid sequence of thymosin β4 (Tβ4) showing the putative meprin-α cleavage sites. Peptides were released after Tβ4 was incubated with recombinant meprin-α and analyzed by a liquid chromatography – mass spectrometer. Meprin-α cleavage sites are marked by solid arrows. Four NH_2-terminal intermediate peptides <30 amino acids released from Tβ4 by meprin-α are shown. Prolyl oligopeptidase (POP) active site is indicated by a solid triangle.

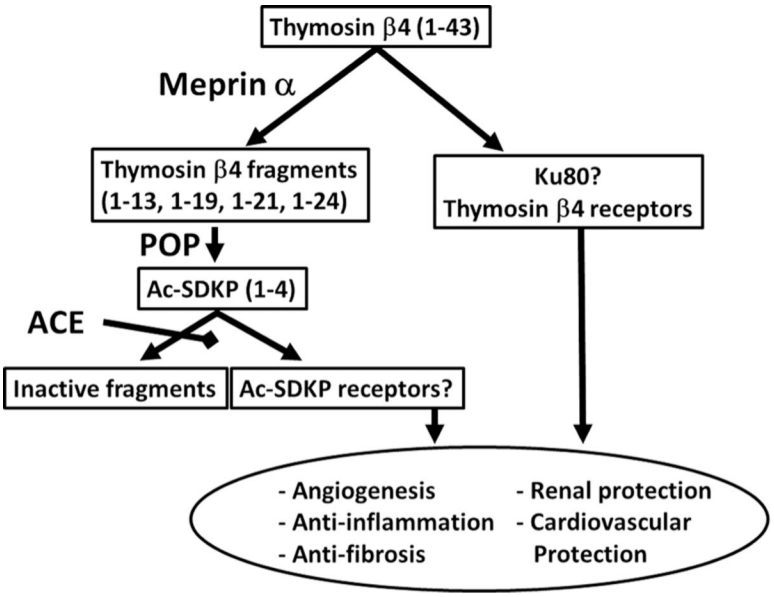

Fig. 2.

Schematic diagram of sequential hydrolysis of thymosin β4 (Tβ4). In the first step, meprin-α hydrolyzes Tβ4 into NH_2-terminal intermediate peptide(s) <30 amino acids. The second step involves prolyl oligopeptidase (POP) hydrolysis of the intermediate peptide(s) that releases *N*-acetyl-seryl-aspartyl-lysyl-proline (Ac-SDKP). Tβ4 and Ac-SDKP, via their putative receptors, provide renal and cardiac protection by reducing inflammation and fibrosis and promoting angiogenesis. ACE, angiotensin-converting enzyme.

Table 1.

Effects of *N*-acetyl-seryl-aspartyl-lysyl-proline (Ac-SDKP) in various diseases and experimental models.

Experimental model/ diseases	Species	Dosage and duration	Observed effects	Mechanisms	References
Traumatic brain injury	Rats, in vivo	0.8 mg/kg per day, 3 days	Improved sensorimotor functional recovery, spatial learning, reduced cortical lesion volume, and hippocampal neuronal cell loss, in the injured brain	Reduced fibrin accumulation, activation of microglia/macrophages, enhanced angiogenesis, neurogenesis, and increased the number of dendritic spines	Zhang et al. 2017
Embolic focal cerebral ischemia	Rats, in vivo	0.8 mg/kg per day, 3 days	Reduced infarct volume and neurological deficits	Inactivation of TGF-β and NF-κB signaling	Zhang et al. 2014
Systemic lupus erythematosus	Mice, in vivo	0.8 mg/kg per day, 3 months	Delayed development of severe hypertension, albuminuria, and early mortality; improved renal function	Prevented glomerulosclerosis, decreased macrophage and T cell renal infiltration, prevented complement C5–9, RANTES, MCP-5 and ICAM-1	Liao et al. 2015; Nakagawa et al. 2017
Lupus nephritis	MRL/lpr mice, in vivo	1.0 mg/kg per day, 12–20 weeks	Reduced proteinuria and improved renal function	Reduced renal T cell and macrophage infiltration; inhibited NF-κB, TNF-α, TGF-β1, α-SMA, fibronectin, and phosphoSmad2/3	Tan et al. 2012
Unilateral ureteric obstruction	Mice, in vivo	1 mg/kg per day, 1 week	Attenuated the gene expression of fibrotic markers	Reduced expression of collagen IV, -SMA, and MCP-1	Chan et al. 2015
Unilateral ureteric obstruction	Mice, in vivo	1.6 mg/kg per day, 5–14 days	Reduced renal collagen 1 expression and fibrosis	Reduced phosphoSmad3; reduced macrophages, PAI-1	Zuo et al. 2013
Silicosis	Rats, in vivo; lung fibroblasts or HAE-A549, in vitro	0.8 mg/kg per day, 4 and 8 weeks. In vitro, 10^{-8} M	Attenuated silicosis-induced increased lung fibrosis in vivo and TGF-β1-induced lung fibroblast differentiation; attenuated epithelial cell apoptosis	Inhibited expressions of TGF-β1 and RAS signaling, inhibited myofibroblast differentiation via decreased SRF and α-SMA-positive myofibroblast localization in siliconic nodules in the lung, inhibited caspase-12 and PERK/eIF2α/ CHOP (ER stress pathway	Xu et al. 2012; Zhang et al. 2018
Dahl salt-sensitive rats	Rats, in vivo	0.8 and 1.6 mg/kg per day, 6 weeks	Prevented renal damage without affecting the blood pressure	Inhibition of macrophage and T cell infiltration and renal fibrosis	Worou et al. 2015
Angiotensin-II-induced hypertensive rats	Rats, in vivo	0.8 mg/kg per day, 3 weeks	Reduces cardiac collagen cross-linking and inflammation in angiotensin-II-induced hypertensive rats	Decreased TGF-β1, LOXL1 and lymphocyte and macrophages infiltration, and NF-κB inhibition	González et al. 2014
DOCA-salt hypertensive mice	Mice, in vivo	0.8 mg/kg per day, 12 weeks	Prevented hypertension-induced inflammatory cell infiltration, collagen deposition, nephrin downregulation, and albuminuria	Decreased DOCA-salt-induced renal collagen deposition, glomerular matrix expansion, and monocyte/macrophage infiltration	Rhaleb et al. 2011
Angiotensin-II-induced hypertension	Rats, in vivo	0.8 mg/kg per day, 3 weeks	Antifibrotic and anti-inflammation effect in thoracic aorta	Enhanced expression of inhibitory Smad7	Lin et al. 2008
5/6 nephrectomy	Rats, in vivo	0.8 mg/kg per day, 3 weeks	Reduced albuminuria, renal inflammation, and fibrosis, and improved glomerular filtration rate	Reduced inflammation and partially restored slit diaphragm nephrin protein expression in the glomerular filtration barrier	Liao et al. 2010

Experimental model/ diseases	Species	Dosage and duration	Observed effects	Mechanisms	References
Galactin-3-induced cardiac injury and dysfunction	Rats, in vivo	0.8 mg/kg per day, 4 weeks	Prevented cardiac inflammation, fibrosis, hypertrophy, and dysfunction	Prevented cardiac inflammation via Gal-3, inhibited TGF-β/Smad3 signaling pathway	Liu et al. 2009
Type 1 diabetes	Rats, in vivo	1 mg/kg per day, 8 weeks	Reduced left ventricular interstitial and perivascular fibrosis	Reduced TGF-β1 and phospho-Smad2/3 protein levels	Castoldi et al. 2009, 2013
Type 1 and type 2 diabetic mice	Mice, in vivo	1 mg/kg per day, up to 4 weeks	Decreased renal fibrosis and protected renal function	Reduced fibronectin, FSP-1, α-SMA proteins expression, and TGF-β/Smad pathway	Nitta et al. 2016; Shibuya et al. 2005
Diabetes mellitus	Mice, in vivo	0.5 mg/kg per day, 8 weeks	Inhibited fibrosis and the EndMT in the heart and kidneys	Inhibition of the EndMT associated with the restoration of FGFR1 and microRNA let-7, P-MAP4K4; inhibition of TGF-β/ Smad3 signaling	Li et al. 2017; Nagai et al. 2014
Myocardial infarction	Mice and rats, in vivo	Ac-SDKP or Ac-SD_DK_DP at 0.8–1.6 mg/kg per day, 4–6 weeks	Decreased cardiac rupture, inflammation, and fibrosis; improved angiogenesis and cardiac function	Inhibited excessive inflammation, fibrosis, and ER stress; increased the expression of angiogenic genes and SERCA2a expression	Ma et al. 2014; Nakagawa et al. 2018; Peng et al. 2013; Song et al. 2014; Wang et al. 2004a; Yang et al. 2004
Autoimmune myocarditis	Rats, in vivo	0.8 mg/kg per day, 4 weeks	Prevented both cardiac dysfunction, hypertrophy and fibrosis	Inhibited innate and adaptive immune cell infiltration, inhibited expression of pro-inflammatory mediators such as cytokines (IL-1α, TNF-α, IL-2, IL-17), chemokines, adhesion molecules ICAM-1, L-selectin, and MMPs	Nakagawa et al. 2012
Anti-glomerular basement membrane nephritis	Rats, in vivo	1 mg/kg per day, 4 weeks	Improved proteinuria and renal dysfunction, and inhibited glomerulosclerosis and interstitial fibrosis	Suppressed gene and protein expression of fibronectin and macrophage infiltration; inhibited TGF-β1, Smad2 phosphorylation; and increased Smad7 expression	Omata et al. 2006
Endothelial cells	Mouse aortic endothelial cell line	0.01–10 nM	Stimulated endothelial cell proliferation and migration and tube formation in a dose-dependent manner	Possible mechanism linked to MMP-1	Liu et al. 2003; Wang et al. 2004a
Cardiac fibroblasts	Human cells, in vitro	0.1–10 nM	Inhibited TGF-β1-induced differentiation of fibroblasts into myofibroblasts	Inhibited all the effects of TGF-β1, and inhibited ET-1-induced TGF-β1 production	Peng et al. 2010
Cardiac fibroblasts	Rats, in vitro	0.1–100 nM	Inhibited collagenase expression and activation	Inhibition of MMPs by Ac-SDKP was associated with increased TIMP-1 and TIMP-2 expressions	Rhaleb et al. 2013
Cardiac fibroblasts	Rats, in vitro	0.1–100 nM	Inhibited ET-1-stimulated collagen production and cell proliferation	Preserving SHP-2 activity and thereby preventing p44/42 MAPK activation	Peng et al. 2012; Rhaleb et al. 2001a
Mesangial cells	Human cells, in vitro	10 nM	Inhibited cell proliferation	Increased p53 and $p27^{kip1}$	Kanasaki et al. 2006
Cancer cell lines	Human and mouse cell lines, in vitro	5–100 μg/mL	Regulated cell proliferation	PI3KCA/Akt pathway mediates Ac-SDKP regulation of cell proliferation	Hu et al. 2013

Note: CHOP, CCAAT-enhancer-binding protein homologous protein; C5–9, complement 5–9; DOCA, deoxycorticosterone-acetate; eIF2, eukaryotic initiation factor 2a; EndMT, endothelial-mesenchymal transition; ER, endoplasmic reticulum; ET-1, endothelin-1; FGFR1, fibroblast growth factor receptor 1; FSP-1, fibroblast-specific protein 1; Gal-3, galectin-3; HAE-A549, human alveolar epithelial cell line-A549; ICAM-1, intercellular adhesion molecule-1α; IL-1α, interleukin-1α; IL-2, interleukin-2; IL-17, interleukin-17; LOXL1, lysyl oxidase like1; MAPK, mitogen-activated protein kinase; MCP-1,

monocyte chemoattractant protein-1; MCP-5, monocyte chemoattractant protein-5; MMPs, metalloproteinases; NF-κB, nuclear factor-κB; PAI-1, plasminogen activator inhibitor-1; PERK, protein kinase R ER kinase; PI3KCA/Akt, phosphatidylinositol-3 kinase-calcium/serinethreonine protein kinase; P-MAP4K4, mitogen activated protein kinase kinase kinase 4; $p27^{kip1}$, member of the universal cyclin-dependent kinase inhibitor; p53, tumor protein (EC:2.7.1.37); RANTES, Regulated on Activation Normal T Cell Expressed and Secreted; RAS, renin–angiotensin system; SERCA2a, sarcoplasmic endoplasmic reticulum ATPase; α-SMA, α-smooth muscle actin; SHP, Src homology 2-containing protein tyrosine phosphatase-2; Smad, Suppressor of Mothers Against Decapentaplegic Miscellaneous; SRF, serum response factor; TGF-β1, transforming growth factor-β1; TIMP-1 and TIMP-2, metallopeptidase inhibitor-1, or −2; TNF-α, tumor necrosis factor-α.

Table 2.

Effects of thymosin β4 (Tβ4) in various diseases and experimental models.

Experimental model/ diseases	Species	Dosage and duration	Observed effects	Mechanisms	References
Developing early fetal heart	Human	NA	T4 in the human heart is primarily localized to endothelial cells of the cardiac microvasculature and coronary vessels, as well as to the endothelial-like cells of the endocardium and to the epicardium	NA	Saunders et al. 2018
Cardiac-specific knockdown of Tβ4	Mice, in vivo	Knockout mice	Reduced coronary vasculogenesis and angiogenesis during embryogenesis and during cardiac injury	Ac-SDKP release	Smart et al. 2007
Global knockdown of Tβ4	Mice, in vivo	Knockout mice	Loss of endogenous Tβ4 had no effect on developing heart or cardiac structures and function in adult heart, but Tβ4 has antiinflammatory and antifibrotic role on heart and kidneys in Ang-II-induced hypertension	Decreased renal and cardiac infiltration of CD68 macrophages, ICAM-1 in hypertensive animals	Banerjee et al. 2012, 2013; Kumar et al. 2018
Global knockdown of Tβ4	Mice, in vivo	Knockout mice	Loss of endogenous Tβ4 accelerates glomerular disease	Tμ4 knockdown in cultured podocytes also increased migration in a woundhealing assay, accompanied by F-actin rearrangement and increased RhoA activity	Vasilopoulou et al. 2016
Liver injury induced by ethanol and LPS	Mice, in vivo	1 mg/kg per day, 1 week	Reduced liver oxidative stress, inflammation, and fibrosis	Inhibited NF-κB; reduced TNF-α, IL-1β, and IL-6; suppressed the epigenetic repressor MeCP2; and increased PPARγ	Shah et al. 2018a, 2018b
Unilateral ureteral obstruction	Rats, in vivo	1 and 5 mg/kg per day, 2 weeks	Decreased proteinuria and reduced renal injury	Decreased TGF-β and α-SMA protein expression, increased E-cadherin	Yuan et al. 2017
Acute stroke	Aged rats, in vivo	12 mg/kg every 3 days, 2 weeks	Reduced infarct volume but Tβ4 treatment did not improve functions, myelination, or gliosis	Increased astrocytic gliosis	Morris et al. 2017
Myocardial infarction	Mice, in vivo	1.6 mg/kg per day, 6 weeks	Prevented cardiac rupture, improved survival rate Reduced cardiac fibrosis and improved cardiac function	Decreased cardiac apoptosis and inflammation, and increased cardiac capillary density	Peng et al. 2014
Myocardial infarction	Mice, in vivo	400 ng/μL intra-cardiac or 150 g/300 μL intraperitoneal	Improved cardiac function and decreased scar tissue volume	Increased ILK and Akt phosphorylation together with decreased cardiomyocyte apoptosis	Bock-Marquette et al. 2004
Myocardial infarction	Rats, in vivo	5.37 mg/kg immediately after surgery and every third day, up to 4 weeks	Reduced infarct size, improved hemodynamic performances; no improvement on volume and ejection fractions	No suggested mechanism	Bao et al. 2013
Myocardial infarction with transfer of Tβ4-EPCs treated	Rats, in vivo	0.05, 0.1, and 0.2 M to treat EPCs	Promoted the survival and angiogenesis of transplanted endothelial progenitor cells in the infarcted myocardium	Increased p-Akt expression in the endothelial cells	Quan et al. 2017
Ang-II-induced hypertension	Mice, in vivo	1.6 mg/kg per day, 6 weeks	Tβ4 knockout mice had increased albuminuria and decreased nephrin expression in the kidney In Tβ4	Exaggerated increased renal and cardiac infiltration of CD68	Kumar et al. 2018

Experimental model/ diseases	Species	Dosage and duration	Observed effects	Mechanisms	References
			knockout mice, Ang II reduced cardiac ejection fraction and increased cardiac hypertrophy and left ventricular dilatation, compared with wild type mice	macrophages, ICAM-1, and total collagen content hypertensive Tβ4 in knockout versus wild type mice	
Pulmonary hypertension	Mice, in vivo	200 μg/200 μL PBS; Tβ4 every day for 3 days prior to MCT, followed by MCT daily for 1 week, then MCT twice weekly for 4 weeks	Protected mice from MCT-induced pulmonary hypertension and right ventricular hypertrophy and fibrosis	Selectively targets Notch3-Col 3 A-CTGF gene axis in preventing MCT-induced PH and RVH	Wei et al. 2014
Patients with acute ST segment elevation myocardial infarction	Human, in vitro then in vivo	1 μg/mL 24 hours before EPC injections	Optimized EPC transplantation appeared to be feasible and safe	NA	Zhu et al. 2016
Murine colitis (model for Crohn's disease	Mice, in vivo	Intracolonic AAV-Tβ4 (4×10^{10} viral genome)	Protected from 2,4,6-trinitrobenzene sulfonic acid-induced colitis and suppressed proliferation of colonic epithelial cells	Reduced inflammation; relieved oxidative stress; modulated TNF-A, IL-1, and IL-10	Zheng et al. 2017
Cardiac fibroblasts	Rats, in vitro	1 μg/mL	Reduced H_2O_2-induced ROS levels and pro-fibrotic genes (CTGF, Col-1, and Col-3)	Increased Cu/Zn SOD/catalase; reduced Bax/Bcl2	Kumar and Gupta 2011
Cultured HCEC, HCET, HCO597, COS-7, a7r5, PAC-1 and HEKa cells	Human, in vitro; rats, in vitro	Overexpression	Inhibited injury-induced pro-inflammatory cytokine and chemokine production	Inhibited TNF-A-induced NF-B activation, IL-8 gene transcription, focal adhesion proteins PINCH-1 and ILK	Qiu et al. 2011

Note: a7r5, rat artery smooth cell line; Ac-SDKP, *N*-acetyl-seryl-aspartyl-lysyl-proline; Ang II, angiotensin II; Bax/Bcl2, Bcl-2-associated X protein/B-cell lymphoma 2; CD68, cluster of differentiation 68; Col 3 A, collagen type III A; CTGF, connective tissue growth factor; Cu/Zn SOD, cupper/zinc superoxide dismutase; EPCs, endothelial progenitors cells; HCEC, adult human coronary epithelial cells; HCET, immortalized HCEC; HCO597, human corneal conjunctival epithelial cell line; HEKa, adult human epidermal keratinocytes; ICAM-1, intercellular adhesion molecule-1; ILK, integrin-linked kinase; IL-1α, interleukin-1α; IL-1β, interleukin-1β; IL-6, interleukin-6; IL-8, interleukin-8; MCT, monocrotaline; MeCP2, methyl-CpG-binding protein 2; NA, not applicable; NF-B, nuclear factor-B; Notch3, neurogenic locus notch homolog protein 3; PAC-1, procaspase activating compound-1; PH, pulmonary hypertension; PINCH, focal adhesion protein; PPARγ, peroxisome proliferator-activated receptor-γ; RhoA, Ras homolog gene family, member A; RVH, right ventricular hypertrophy; α-SMA, α-smooth muscle actin; TGF-β1, transforming growth factor-β1; TNF-α, tumor necrosis factor-α.