

REVIEW

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Elevation of the antifibrotic peptide *N*-acetylseryl-aspartyl-lysyl-proline: a blood pressure-independent beneficial effect of angiotensin I-converting enzyme inhibitors

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Abstract

Blockade of the renin-angiotensin system (RAS) is well recognized as an essential therapy in hypertensive, heart, and kidney diseases. There are several classes of drugs that block the RAS; these drugs are known to exhibit antifibrotic action. An analysis of the molecular mechanisms of action for these drugs can reveal potential differences in their antifibrotic roles. In this review, we discuss the antifibrotic action of RAS blockade with an emphasis on the potential importance of angiotensin I-converting enzyme (ACE) inhibition associated with the antifibrotic peptide *N*-acetyl-seryl-aspartyl-lysyl-proline (AcSDKP).

Introduction

In recent decades there has been a tremendous increase in the therapeutic options available for the inhibition of the renin-angiotensin system (RAS). Historically, angiotensin-converting enzyme inhibitors (ACE-I) were the first class of RAS inhibitors identified. The first ACE-I, captopril, was discovered by a scientist at Squibb, a US pharmaceutical company, in 1975 [1]. Captopril was based on the peptide sequence of bradykinin-potentiating factor, which inhibited the conversion of angiotensin I to angiotensin II when perfused into pulmonary circulation [2].

Approximately 15 years ago, a second class of RAS inhibitors was introduced into the market, the angiotensin II receptor blockers (ARBs) [3]. Very recently, a novel class of RAS inhibitor, including aliskiren [4], which directly inhibits renin has been put into clinical use. Most of the literature support the beneficial effects of this novel class of RAS inhibitors as antihypertensive drugs [5,6]. Interestingly, the use of these drugs is not limited to antihypertensive disorders. The clinical use of RAS inhibitors has emerged as beneficial for the prevention of diabetes [7,8],

fibrotic kidney disease [9], heart disease [10], aging [11] and Alzheimer's disease [12].

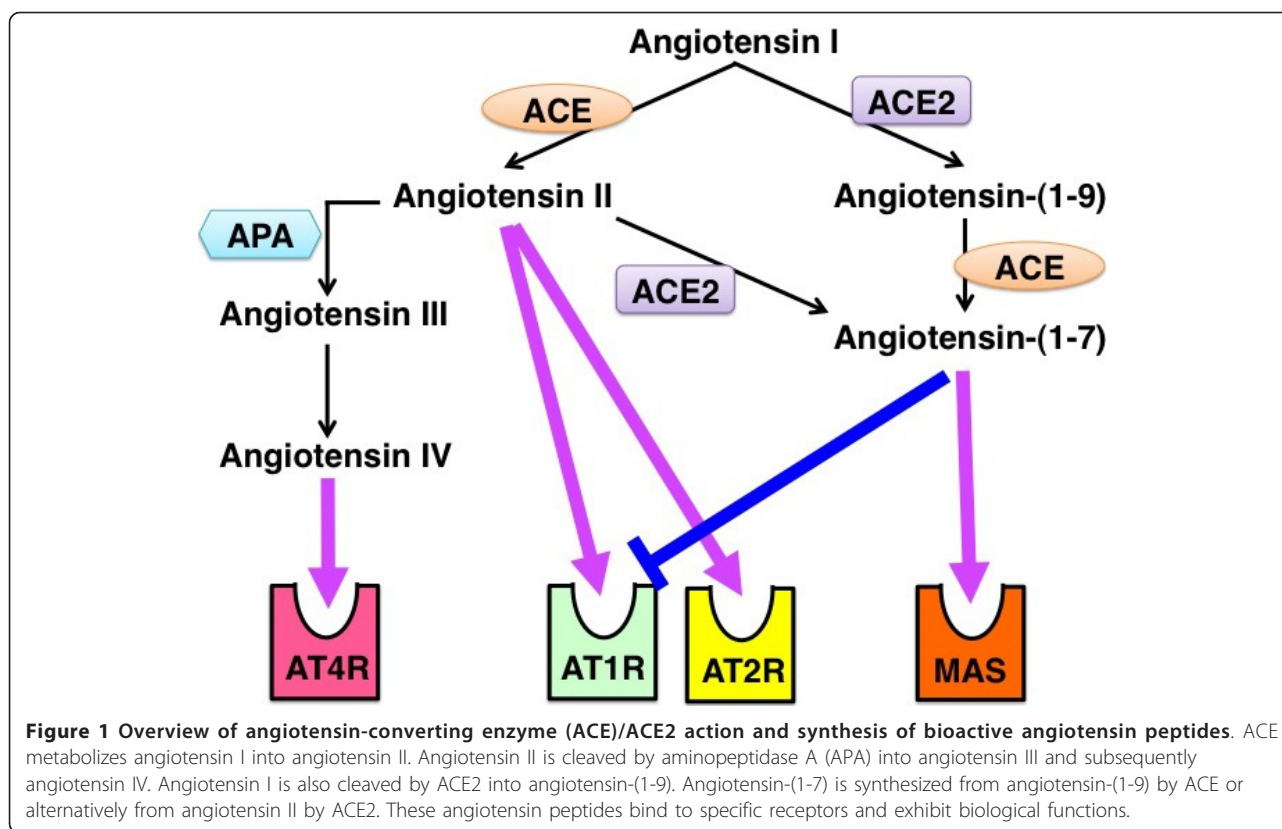
There is no doubt that RAS inhibitors are beneficial drugs; however, the differences between each of these classes of inhibitors are not yet clear. After a brief introduction to the RAS, we analyze the potential differences between ACE-I and ARBs as antifibrotic drugs. Emphasis is placed on the ACE inhibitors and the antifibrotic peptide AcSDKP.

RAS

Renin, an aspartyl protease, was discovered by Robert Tigerstedt at the Karolinska Institute in 1898 [13]. The majority of renin in the body is found in the juxtaglomerular cells of the kidney. Additionally, renin has been found in many other tissues but without clear mechanistic evidence of its function in these locations [14]. Renin cleaves angiotensinogen, which results in the production of the decapeptide angiotensin I. The octapeptide angiotensin II, a potent vasoconstrictor, is formed by ACE-mediated cleavage of angiotensin I.

There are two main receptors for angiotensin II (AT1 and AT2), which are differentially expressed on the cell surface (Figure 1) [15]. Those receptors share the configuration of a seven-transmembrane receptor but exhibit only around 20% protein sequence homology [16].

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These two receptors play distinct physiological roles [16]. AT1 receptors are coupled to G proteins and mediate diverse signaling pathways, such as activation of phospholipases, inhibition of adenylate cyclase, and stimulation of tyrosine phosphorylation [15]. However, the interaction of AT2 receptors and G proteins is controversial [17]. These two receptors are differentially regulated during the development [18]. When analyzed in lamb, AT2 receptors are expressed abundantly in the fetal kidney, especially in the undifferentiated mesenchyme [18]. These AT2 receptors are at decreased expression levels after birth [18]. AT1 receptors are initially expressed in the nephrogenic cortex and developing glomeruli, proximal tubule and vessels; they become more abundant through the development processes [18].

The expression of AT1 receptors is stimulated by several conditions, such as high cholesterol levels and osmolarity changes, but decreased by high concentration of angiotensin II [15]. Such angiotensin II-dependent downregulation is not found for AT2 receptors; instead, AT2 receptors are induced by tissue injury [17]. Indeed, AT2 receptors are re-expressed by renal injury and the nephron remodeling processes [17].

Vasoconstriction, profibrotic action, growth stimulation, aldosterone release and proinflammatory functions are classical angiotensin II-driven physiological functions

that are mediated by AT1 receptors [19]. AT2 receptor-mediated signaling may antagonize AT1-mediated signal transductions [20-22]. However, accumulating evidence indicates that AT2 receptor-mediated signaling also mediates the detrimental action of angiotensin II, including hypertrophy [23,24], and the stimulation of proinflammatory pathway nuclear factor κ B [25,26]. In this regard, blockade of the AT2 receptor by a specific inhibitor was associated with the inhibition of inflammation and renoprotection in subtotaly nephrectomized rats [27].

In addition to classical members, some new bioactive molecules, such as angiotensin IV and angiotensin-(1-7), have been introduced in RAS systems.

Angiotensin II is metabolized by aminopeptidase A (APA) into angiotensin III and finally angiotensin IV (Figure 1) [28]. Angiotensin IV binds to the specific receptor AT4 (Figure 1), which is reported to be an insulin-regulated membrane aminopeptidase [29,30]. It is reported that angiotensin IV can induce plasminogen activator inhibitor (PAI)-1 expression in the proximal tubule and vascular endothelial cells [29,31]. PAI-1 activation has been associated with the reduction of extracellular matrix turnover [32]; angiotensin IV-mediated signaling may be associated with the tissue fibrosis [31]. The angiotensin IV-generating enzyme APA is induced

in conditions of renal injury and high angiotensin II levels [28]; subsequently, more angiotensin II is utilized in the production of angiotensin IV. Angiotensin IV is also associated with the release of nitric oxide and focal adhesion kinase phosphorylations [33,34]. Interestingly, the angiotensin IV/AT4 receptor signaling pathway has been shown to be involved in glucose homeostasis [35,36] and cognitive functions [37], suggesting diverse physiological roles of this pathway.

Another RAS-derived bioactive molecule is angiotensin-(1-7), which has been shown to inhibit the effects of angiotensin II (Figure 1) [38]. For example, angiotensin-(1-7) plays a role as an antihypertensive molecule through the stimulation of the release of vasodilator prostaglandins and nitric oxides [38]. In addition to such antihypertensive effects, angiotensin-(1-7) inhibits the angiotensin II-induced proliferation and growth stimulation signal in vascular smooth muscle cells [39-41]. Most likely, these effects of angiotensin-(1-7) as a negative regulator of angiotensin II are mediated, at least in part, by the downregulation of the angiotensin II receptor AT1 (Figure 1) [42]. Also, it is reported that angiotensin-(1-7) is the endogenous ligand for the MAS receptor (Figure 1) [43]. Studies utilizing MAS receptor deficient mice have indicated that the interaction between angiotensin-(1-7) and the MAS receptor plays vital roles in heart function [43], sympathetic tone regulation [44], aortic relaxation [45], and endothelial function [46].

The synthesis of angiotensin-(1-7) is mediated by a unique RAS pathway involving ACE2 (Figure 1) [47,48]. ACE2 is expressed predominantly in vascular endothelial cells of the heart and kidney [47,49]. Both ACE and ACE2 metabolize angiotensin I. However, the resulting peptides are different (Figure 1). As shown above, ACE converts angiotensin I to the octapeptide angiotensin II, whereas ACE2 cleaves one amino acid from angiotensin I; subsequently, nonapeptide angiotensin 1-9 is synthesized (Figure 1) [47]. Although angiotensin 1-9 itself exhibits no known biologic activity, angiotensin 1-9 is cleaved by ACE, and bioactive angiotensin-(1-7) is synthesized (Figure 1) [50]. ACE2 can also directly cleave angiotensin II to form angiotensin-(1-7) (Figure 1); therefore, this angiotensin II degradation product exhibits properties that are opposite those of angiotensin II [48].

RAS activation and tissue fibrosis

Activation of RAS and production of angiotensin II is associated with tissue fibrosis [51,52]. Angiotensin II stimulates extracellular matrix accumulation and collagen deposition through the induction of mitogen activated protein kinases (MAPKs), such as extracellular signal-regulated kinase (ERK) [53], p38 [54] and c-Jun N-terminal kinases (JNKs) [55], *in vivo* and *in vitro*. Additionally, angiotensin II stimulates the expression of the profibrotic

cytokine transforming growth factor (TGF) β in rat cardiac fibroblasts [56] and connective tissue growth factor in rat tubular epithelial cells [57]. Some reports have also indicated that angiotensin II may directly activate Smad proteins, which are part of the intracellular TGF β signaling pathway [58-60]. Furthermore, angiotensin II stimulates rat cardiac fibroblast proliferation [61]. In addition to the angiotensin II/AT1 receptor-mediated major profibrotic signaling pathways in RAS, the angiotensin IV/AT4 receptor pathway could contribute to tissue fibrosis via the induction of PAI-1 [31], as described above.

Therefore, appropriate inhibition of profibrotic angiotensins such as angiotensin II or angiotensin IV, production pathways, or, alternatively, activation of an antipfibrotic angiotensin pathway such as ACE2 or angiotensin-(1-7) could be a potential route for antifibrotic therapy. In this regard, currently available RAS inhibitors, such as ACE-I and ARB, are somewhat reasonable as antifibrotic drugs. However, there are differences in the antifibrotic molecular mechanisms of these drugs.

ACE inhibitors

ACE inhibitors are members of the first class of RAS inhibitors. The first ACE inhibitor to be used in the clinic, captopril, showed dramatic beneficial effects in type I diabetic patients with nephropathy [62]. Following this study, several clinical trials demonstrated that ACE inhibition could significantly prevent the progression of renal disease [63-65].

The ACE-I class of drugs exhibit their antihypertensive effects by inhibiting the conversion of angiotensin II from angiotensin I. ACE-I inhibition has been shown to decrease fibrosis in experimental models of heart [66] and kidney [67-69] disease. Because angiotensin II is a profibrogenic molecule, it would be logical to conclude that the beneficial effects of ACE-I are mediated through the inhibition of angiotensin II production; however, the beneficial effects of ACE-I cannot be explained by the suppression of angiotensin II production alone because maximal doses of ACE-I may not be sufficient to inhibit all the biosynthesis of angiotensin II [16]. Indeed, systemic administration of ACE-I has little effect on the formation of angiotensin II in the kidney, even though such ACE-I can almost completely inhibit systemic angiotensin II formation from angiotensin I [70]. Therefore, it is likely that the decrease in the production of angiotensin II is not the only mechanism underlying the antifibrotic effects of ACE-I.

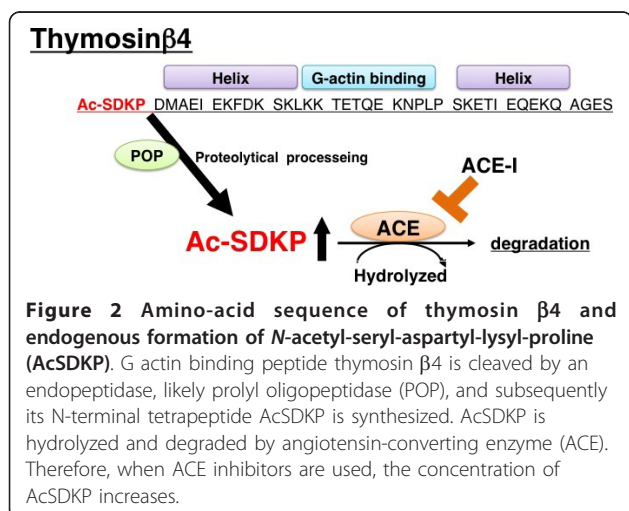
ACE inhibition and elevation of the antifibrotic peptide N-acetyl-seryl-aspartyl-lysyl-proline (AcSDKP)

AcSDKP is a tetrapeptide originally isolated from fetal calf bone marrow [71], and has recently emerged as an antifibrosis molecule.

Details of the endogenous synthesis of AcSDKP are not yet clear; however, available information strongly suggests that thymosin β 4 (T β 4) is the most likely candidate precursor of AcSDKP [72,73] (Figure 2). Lenfan *et al.* showed that incubation of [3 H] T β 4 with bone marrow cells or bone marrow lysate resulted in the formation of [3 H]AcSDKP [72]. Furthermore, T β 4 knock-down utilizing the small interfering (si)RNA for T β 4 led to significant reduction of AcSDKP in HeLa cells [73]. AcSDKP is the N-terminal sequence of T β 4 (Figure 2). AcSDKP was believed to be synthesized by a single cleavage employing Asp-N endopeptidase [72]. However, Asp-N was only found in bacteria; therefore, Cavasin *et al.* tried to find another enzyme responsible for the synthesis of AcSDKP from T β 4 [74]. Subsequently, they found that prolyl oligopeptidase (POP) is responsible for the formation of AcSDKP and that POP inhibitors blocked the formation of AcSDKP from T β 4 [74] (Figure 2).

T β 4 is a ubiquitously distributed 43-amino-acid peptide (4.9 kDa), originally identified as an intracellular peptide, which can sequester G-actin and regulate its polymerization [75,76]. In addition to the role as actin polymerizations, T β 4 exhibits various biologically significant activities [75,76]. Interestingly, Bock-Marquette *et al.* reported that the administration of exogenous intracardiac and intraperitoneal T β 4 significantly restored cardiac functions associated with neovascularization in an experimental myocardial infarction model of mice [77] and epicardial progenitor mobilization [78], suggesting that T β 4 exhibit extracellular organ-protective roles associated with antifibrosis and enhanced angiogenesis.

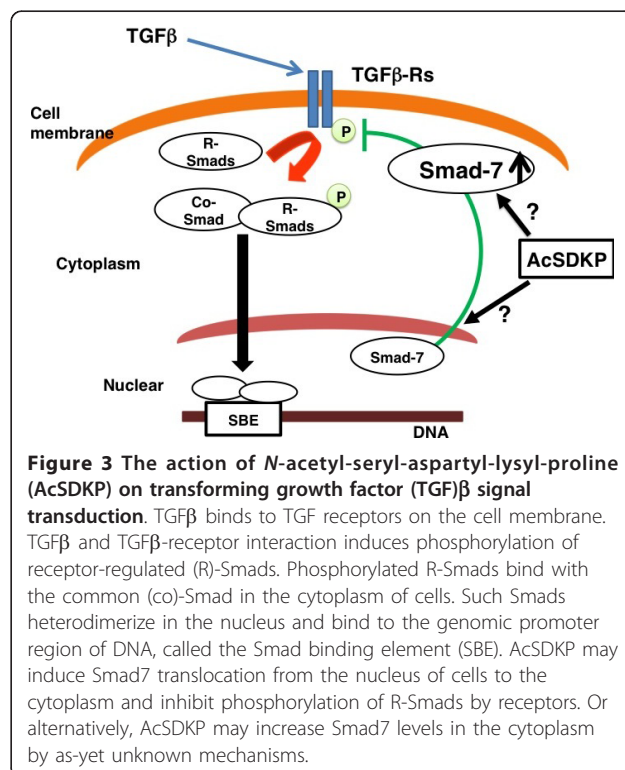
AcSDKP is a natural inhibitor of hematopoietic stem cell proliferation that prevents entry into S phase from G1 in the cell cycle [79]. Interestingly, AcSDKP is



hydrolyzed in the presence of ACE (Figure 2). Therefore, plasma levels of AcSDKP are minimal in normal conditions, whereas ACE-I administration leads to a fivefold increase in its concentration [80]. AcSDKP has been shown to suppress the proliferation of human mesangial cells [81] and renal fibroblasts [82], in addition to inhibiting collagen deposition in mouse cardiac fibroblasts [83]. The administration of AcSDKP ameliorated renal fibrosis and glomerular sclerosis in hypertensive rat models and diabetic and non-diabetic kidney disease models without altering blood pressure [84,85]. These observations suggest that the renoprotective effects of ACE-I are mediated, at least in part, by the accumulation of AcSDKP (Figure 2).

Our group and others have shown that AcSDKP prevents Smad2 phosphorylation (Figure 3) and that this molecular mechanism may mediate its antifibrotic effect [86,87]. This observation identifies AcSDKP as the first circulating, endogenous inhibitor of Smad2 phosphorylation.

The Smads are transcription factors specific to the TGF β family, and they play essential roles in signal transduction from the cell membrane [88,89]. Smads are classified into three types: (1) receptor-regulated Smads, or R-Smads (Smad2 and 3); (2) common Smad, or co-Smad (Smad4); and (3) inhibitory Smads, or I-Smads (Smad6 and 7). Upon TGF β binding, the type II receptor interacts with the type I receptor, which induces



phosphorylation of serine residues in the type I receptor (Figure 3) [90]. Subsequently, the phosphorylated type I receptor recruits R-Smads to be phosphorylated, and phosphorylated R-Smads interact with co-Smad in the cytoplasm of cells (Figure 3). This R-Smad and co-Smad heterodimer is imported into the nucleus (Figure 3) with the help of importin- β [91,92]. The Smad heterodimer binds to Smad-binding elements in the promoter regions of DNA (Figure 3). Under normal conditions, I-Smad is localized to the nucleus (Figure 3) [87]. E3 ubiquitin ligase Smurfs mediate the translocation of nuclear-localized I-Smads to the cytoplasm following TGF β stimulation. Cytoplasmic I-Smad competitively inhibits R-Smad phosphorylation by the type I receptor (Figure 3) [93]. Ubiquitination of receptors by I-Smad-associated Smurfs are also part of the negative feedback loop between TGF β and the I-Smads [94-96].

How does AcSDKP inhibit TGF β -induced phosphorylation of R-Smad? This effect is likely associated with the activation of I-Smads (Figure 3). Incubation of human mesangial cells in the presence of AcSDKP leads to cytoplasmic mobilization of Smad7, one of the I-Smads, in the absence of TGF β stimulation (Figure 3) [87]. Our group and others have reported increased Smad7 levels *in vivo* following AcSDKP administration, supporting this Smad7-mediated anti-TGF β effect by AcSDKP (Figure 3) [97,98]. Additional information related to the mechanism underlying the AcSDKP-mediated translocation and increase in Smad7 concentration is not clear. Interestingly, AcSDKP also inhibits cell cycle progression stimulated by serum-derived or platelet-derived growth factor-B in human mesangial cells by inhibiting the degradation of p53, p27^{kip1} and p21^{cip1} [81]. Similar to Smad7 [96], these molecules are exclusively degraded by the ubiquitin-proteasome pathway [99]; therefore, it is possible that AcSDKP may inhibit the Smad7 degradation pathway.

ACE has N-terminal and C-terminal catalytic domains responsible for interactions with and cleavage of target substrates (Figure 4) [100]. Evidence suggests that these two catalytic domains may be different (Figure 4). Bradykinin is hydrolyzed at approximately the same rate by both of these catalytic sites. Although angiotensin I can be cleaved by either catalytic domain, the C-terminal domain has a threefold higher affinity for angiotensin I (Figure 4) [100,101]. Interestingly, AcSDKP is hydrolyzed exclusively by the N-terminal catalytic domain (Figure 4) [102]. Importantly, each ACE-I exhibits a distinct affinity for each of the catalytic domains; for example, captopril displays a higher affinity for the N-terminal catalytic domain (Figure 4). It is likely that the hydrophobic moieties of ACE-I play an essential role in this domain selectivity [103]. It was recently reported by Li *et al.* that mice deficient for the N-terminal catalytic domain of ACE

exhibited an antifibrotic effect due to an accumulation of AcSDKP, revealing the importance of the N-terminal domain for the antifibrotic actions of ACE-I (Figure 4) [104]. In addition to the full-length somatic form of ACE, there is a transcriptional variant with an N-terminal deletion, known as the germinal form [100]. The testes, which express germinal-type ACE, are associated with higher levels of AcSDKP relative to other tissues [105,106]. AcSDKP and its precursor peptide, T β 4, were able to rescue fibrotic heart disease in a preclinical model [78]. These results demonstrated the importance of the antifibrotic effect of AcSDKP in the inhibition of ACE. Therefore, the N-terminal catalytic domain-specific inhibitor of ACE, RXP407 (Figure 4), has great potential as an antifibrotic therapy [107-110].

Angiotensin type I receptor blocker vs ACE inhibition

There may be potential problems with the long-term clinical use of ACE-I to inhibit the RAS. The prolonged use of ACE-I leads to the compensatory upregulation of angiotensin I [111]. Under these conditions, known as aldosterone escape, chymase may act as the converting enzyme to generate angiotensin II [111]. Therefore, a strategy that prevents angiotensin II from binding to the angiotensin type I receptor is necessary. To address this problem, ARBs, such as losartan, were developed as a novel class of RAS inhibitors [3]. Large clinical trials, such as the RENAAL study, have revealed that losartan exhibits renoprotective effects and inhibits overall mortality in type 2 diabetic nephropathy patients with overt proteinuria [112]. Other studies have also reported similar renoprotective effects associated with an increase in overall mortality. The clinical use of ARB is much easier than that of ACE-I given the side effects typically associated with the latter, such as dry cough, which leads to poor compliance in patients prescribed the drug. Because ARBs inhibit only the AT1 signaling pathway, they were thought of as an ideal strategy to treat hypertensive patients with kidney diseases.

As pharmacological function and effector target are different, ACE-I and ARB exhibit different influences in RAS-dependent and RAS-independent pathways, such as the AcSDKP accumulation by ACE-I described above.

ACE inhibition by ACE-I leads to a suppression of angiotensin II formation, resulting in less angiotensin II binding to the AT1 receptor as well as the AT2 receptor [16]. However, when an ARB is utilized, AT1 receptor signaling is inhibited; angiotensin II accumulates, and subsequently, such increased angiotensin II binds and activates AT2 receptors [22]. As shown above, stimulation of the AT2 receptor may be detrimental for organ protection (and may also antagonize the AT1 receptor-mediated profibrotic signal in some experimental conditions) [22].

Even though ACE-I may not directly suppress ACE2, ACE-I might inhibit the formation of antihypertensive/antifibrotic angiotensin-(1-7) in an indirect fashion, because conversion of angiotensin 1-9 to angiotensin-(1-7) is mediated by ACE [50]. For angiotensin-(1-7), ARB may increase its formation via accumulated angiotensin II directly cleaved by ACE2 [50].

Another difference between ACE-I and ARB is the concentration of plasma bradykinin [113]. Bradykinin breakdown is mediated by ACE; therefore, ACE-I treatment increases bradykinin concentration [113]. Using bradykinin B2 receptor knockout mice, Schanstra *et al.* reported that the bradykinin B2 receptor signaling pathway exhibited antifibrotic roles associated with the induction of plasminogen activators/matrix metalloproteinase-2, enzymes associated with extracellular matrix degradation in the unilateral ureteral obstruction (UUO)

model of renal fibrosis [114]. Moreover, Akita diabetic mice lacking the bradykinin B2 receptor developed overt nephropathy when compared to control mice [115]. However, the role of ACE inhibition and bradykinin B2 signaling pathway activation is still controversial because it was also shown that ACE-I treatment in the UUO model using either bradykinin B2 receptor knockout mice or control mice demonstrated that ACE-I exhibited a significant reduction in renal fibrosis in all groups [116], suggesting that the presence of bradykinin B2 receptor signaling may not be necessary for the tissue protection mediated by ACE-I in this model [116]. Nevertheless, cell biology analysis in human mesangial cells revealed that bradykinin and the bradykinin B2 receptor pathway might contribute to the therapeutic effect of the ACE-I inhibitor perindoprilat during mesangial scarring [117].

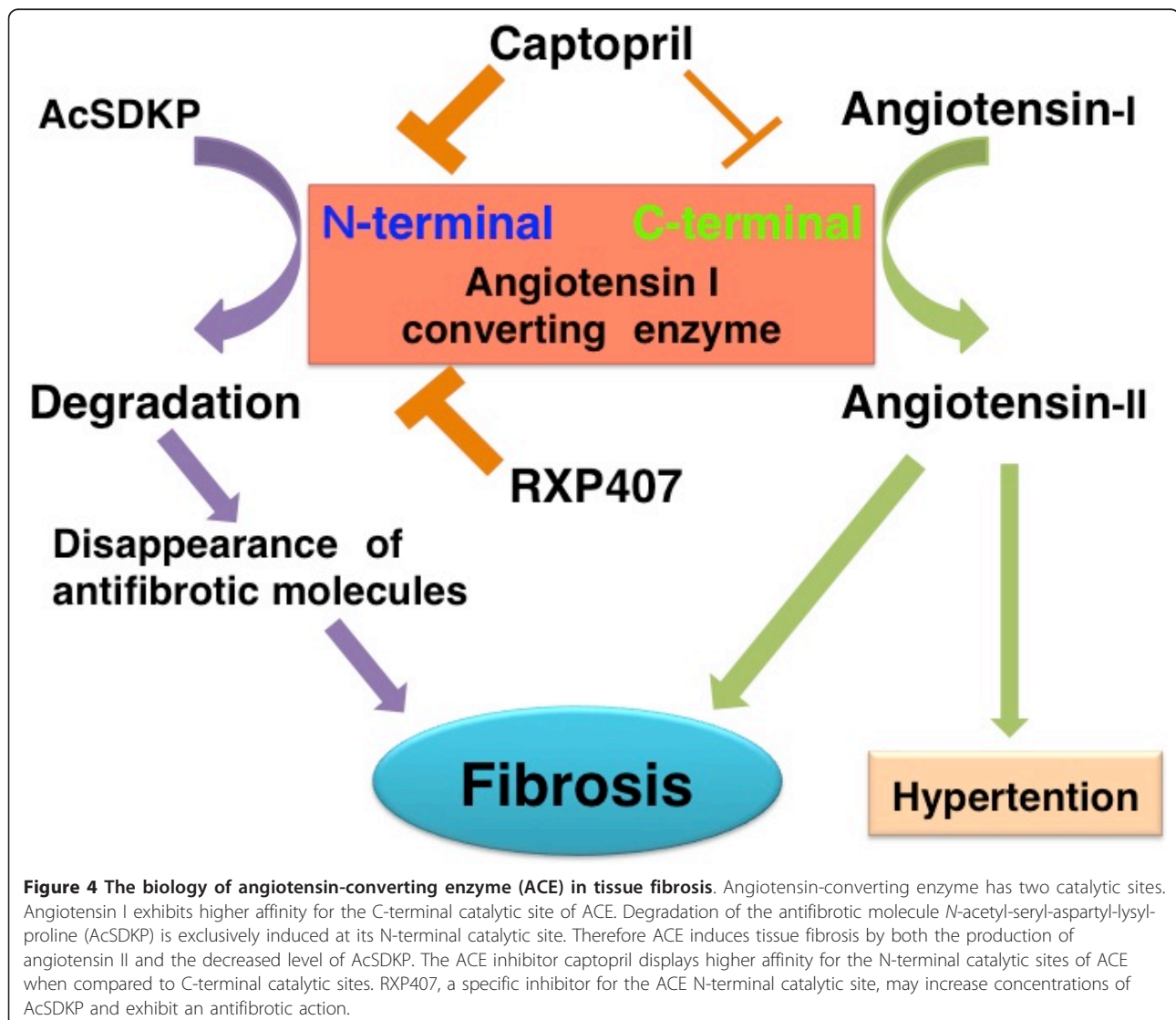


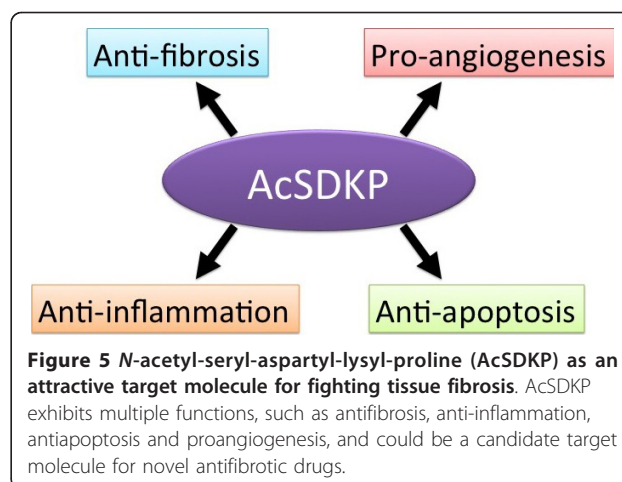
Figure 4 The biology of angiotensin-converting enzyme (ACE) in tissue fibrosis. Angiotensin-converting enzyme has two catalytic sites. Angiotensin I exhibits higher affinity for the C-terminal catalytic site of ACE. Degradation of the antifibrotic molecule *N*-acetyl-seryl-aspartyl-lysyl-proline (AcSDKP) is exclusively induced at its N-terminal catalytic site. Therefore ACE induces tissue fibrosis by both the production of angiotensin II and the decreased level of AcSDKP. The ACE inhibitor captopril displays higher affinity for the N-terminal catalytic sites of ACE when compared to C-terminal catalytic sites. RXP407, a specific inhibitor for the ACE N-terminal catalytic site, may increase concentrations of AcSDKP and exhibit an antifibrotic action.

ACE-I and ARB combination therapy likely show additive antihypertensive and organ protective effects because these two therapies exhibit diverse RAS-dependent and RAS-independent pathway activity [118-123]; however, some trials have shown that combination therapy may not be renoprotective, despite the significant reduction in proteinuria levels [124]. This discrepancy between the trials could be dependent upon the specific drug used and the design of the trials [118-124]. Mauer *et al.* recently reported on important differences between ACE-I and ARBs [125]. They found that ARBs enhanced progression of microalbuminuria in early type I diabetes patients with normotensive and normoalbuminuria. Such enhanced progression of microalbuminuria is associated with a trend of increased mesangial fractional volume in glomeruli in the kidney [125]. Furthermore, treatment with ACE-I showed no significant differences between patients in the treatment or control groups [125]. The conundrum of this study is that the onset of diabetic retinopathy was inhibited by either ARB or ACE-I treatment, suggesting that both drugs efficiently inhibited angiotensin II stimulated signaling pathway in both groups [125]. A follow-up study is clearly necessary to clarify the therapeutic approach for early diabetes patients to prevent the onset of more advanced kidney disease.

One possible explanation for these unexpected results is that the angiotensin II-mediated signaling pathway may not contribute to the onset of microalbuminuria and mesangial matrix accumulation of the kidney in early diabetic normotensive patients with normoalbuminuria. Another possibility is that the administration of ARBs results in local ACE activation [16,126]; in other words, despite blocking the angiotensin II receptor signaling pathway, activation of an angiotensin-independent, profibrotic pathway mediated by ACE may occur, such as the accelerated degradation of AcSDKP (Figure 4). ACE-I, but not ARBs, inhibited murine adriamycin nephropathy, suggesting that diverse pathways may be involved in fibrotic diseases [68].

Anti-inflammatory, antiapoptotic and proangiogenic roles of AcSDKP

Because AcSDKP was originally identified as a hematopoietic stem cell regulator [71,127,128], there have been many studies performed utilizing bone marrow cells. AcSDKP inhibits apoptosis (Figure 5) induced by cytotoxic stresses, including chemotherapy [129,130], radiation [131,132], high temperature [133-135] and photofrin II-mediated phototherapy [136]. Increased apoptosis is associated with tissue fibrosis, and its inhibition has been linked to the restoration of fibrosis in several organs [137-140].



Inflammation is also associated with tissue fibrosis [137-140]. In experimental animal models, AcSDKP inhibited inflammation in the kidney, heart and liver that was associated with the amelioration of tissue fibrosis (Figure 5) [97,98,141-145]. Although the precise molecular mechanisms explaining how AcSDKP inhibits inflammation are not yet clear, it is likely that the suppression of MCP-1 contributes to these anti-inflammatory effects [146]. Inhibition of MCP-1 is likely associated with the inhibition of MAPK activation *in vivo* [147]. However, the effects of AcSDKP on MAPK are cell-type dependent, as suggested by the fact that AcSDKP both inhibits and stimulates ERK phosphorylation in different cellular contexts, such as in rat cardiac fibroblasts [83,148] and human mesangial cells, respectively [81,87]. This finding suggests that AcSDKP is not simply a MAPK inhibitor.

Angiogenesis is important in protection from tissue damage and the promotion of tissue repair. Interestingly, both AcSDKP (Figure 5) [149-151] and its precursor peptide, T β 4 [75,152-154], enhanced angiogenesis and exhibited antifibrotic effects associated with the normalization of organ function [78]. AcSDKP has been shown to improve skin flap survival and accelerate wound healing [151]. The relationship between tumor angiogenesis and T β 4 with AcSDKP has been extensively studied by Wdzieczak-Bakala's research group. They have proposed that high levels of T β 4 and AcSDKP are associated with tumor progression in hematologic malignancies [73,155-157]. Angiogenesis plays a pivotal role in cancer development [158,159], and AcSDKP levels are markedly elevated in both hematologic malignancies and solid neoplasms [73,155-157]. An association between the levels of AcSDKP and tumor angiogenesis was observed in these studies; however, the pathophysiological significance of this result has not been clearly shown.

Finally, AcSDKP infusion inhibited liver injury associated with the inhibition of the TGF β and Smad pathways in carbon tetrachloride-induced liver fibrosis models [141]. Additionally, this treatment was associated with the induction of bone-morphogenetic protein 7 (BMP-7) [141], a promising antifibrotic molecule [160,161]. The antifibrotic, antiapoptotic, anti-inflammatory and proangiogenic properties of BMP-7 have been well established [160,161]. Therefore, it is possible that AcSDKP could function, in part, through the induction of BMP-7 [141]. Furthermore, this same study demonstrated that AcSDKP induced expression of the potent anti-inflammatory transcription factor peroxisome proliferator-activated receptor (PPAR)- γ [141]. PPAR- γ is not only a potent anti-inflammatory transcription factor, but it is also a critical regulator for adipogenesis, lipogenesis and insulin sensitivity [141].

Perspective

We have summarized the potential beneficial effects of AcSDKP in fibrotic diseases. It is obvious that the antifibrotic effect of AcSDKP is associated with its anti-inflammatory, antiapoptotic and proangiogenic properties (Figure 5). Therefore, AcSDKP appears to be an attractive molecule for antifibrotic therapy. One of the problems associated with the use of this molecule as an antifibrotic therapy is its short half-life of approximately 5 min in plasma [162,163]. It would be possible to make an AcSDKP analogue with an extended half-life; however, the hematopoietic effects of AcSDKP are diminished when single amino-acid modifications are made [164]. Therefore, the best approach for converting AcSDKP into a practical antifibrotic agent would be to manufacture a small molecule that mimics AcSDKP function for oral intake. Alternatively, inactivation of the N-terminal catalytic function of ACE could be used as a therapeutic approach; however, some critical information is missing to make this approach feasible. First, the direct target of AcSDKP and its exact function are not clear, even though AcSDKP is known to induce the accumulation of Smad7 and various cell cycle modulators responsible for inhibiting the TGF β /Smad signaling pathway and inducing the antiproliferative effects on fibroblasts and mesangial cells, respectively [81,87, 97,98]. Second, it is not known whether AcSDKP acts as a ligand for its own receptor or if there are any receptors for AcSDKP [165]. Alternatively, AcSDKP may enter cells by phagocytosis and inhibit intracellular signaling pathways. Third, specific inactivation of the N-terminal catalytic domain of ACE by an inhibitor, such as RX407, can be used in the clinic to induce AcSDKP without the side effects associated with conventional ACE-I, such as dry cough [108-110]. Such information is essential if we hope to develop novel antifibrotic

therapies based on enhancing the function of AcSDKP. Additionally, the significance of physiological changes in AcSDKP levels must be analyzed in human patients with fibrotic diseases.

Conclusions

Tissue fibrosis is associated with organ damage and dysfunction, which are the major causes of disability and death in these patients. Specific therapies to treat fibrosis are not yet available in the clinic. Although tissue fibrosis is detrimental to organ function, it may also be a component of homeostasis and repair pathways. Therefore, caution should be used to determine whether AcSDKP is harmful in a subset of patients. We must carefully consider the potential therapeutic utilization of AcSDKP and its role in other diseases [73,155-157]. Clearly, more research is needed into the regulation of AcSDKP levels to show its effectiveness and safety as a therapeutic agent. Nonetheless, AcSDKP remains an attractive target as a potential antifibrotic strategy.

Acknowledgements

The authors' laboratory is supported by grants from the Japan Society for the Promotion of Science to DK and KK, the Uehara Memorial Foundation to DK and KK, individually, and the Kanai Foundation for the Promotion of Medical Science to KK.

Authors' contributions

MK contributed to writing the manuscript. TN made the figures. MK was involved in the discussion. DK made intellectual contributions. KK conceived the project, provided intellectual contributions, and contributed to the manuscript writing and editing. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Received: 21 June 2011 Accepted: 30 November 2011

Published: 30 November 2011

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doi:10.1186/1755-1536-4-25

Cite this article as: Kanasaki et al.: Elevation of the antifibrotic peptide N-acetyl-seryl-aspartyl-lysyl-proline: a blood pressure-independent beneficial effect of angiotensin I-converting enzyme inhibitors. *Fibrogenesis & Tissue Repair* 2011 **4**:25.

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