The renin-angiotensin system in cutaneous hypertrophic scar and keloid formation

Hedayatyanfard, Keshvad; Haddadi, Nazgol-Sadat; Ziai, Seyed Ali; Karim, Hossein; Niazi, Feizollah; Steckelings, U Muscha; Habibi, Behnam; Modarressi, Ali; Dehpour, Ahmad Reza

Published in:
Experimental Dermatology

DOI:
10.1111/exd.14154

Publication date:
2020

Document version:
Accepted manuscript

Citation for published version (APA):

Go to publication entry in University of Southern Denmark's Research Portal

Terms of use
This work is brought to you by the University of Southern Denmark. Unless otherwise specified it has been shared according to the terms for self-archiving. If no other license is stated, these terms apply:

• You may download this work for personal use only.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying this open access version

If you believe that this document breaches copyright please contact us providing details and we will investigate your claim. Please direct all enquiries to puresupport@bib.sdu.dk
The renin-angiotensin system in cutaneous hypertrophic scar and keloid formation

Keshvad Hedayatyanfard PhD*1,2, Nazgol-Sadat Haddadi MD2, Seyed Ali Ziai PhD4, Hossein Karim MD2, Feizollah Niazi MD5, U. Muscha Steckelings, MD, PhD6, Behnam Habibi MD4, Ali Modarressi MD7, Ahmad Reza Dehpour PhD3

1Evidence-Based Phytotherapy and Complementary Medicine Research Center, Alborz University of Medical Sciences, Karaj, Iran
2Cardiovascular Research Center, Alborz University of Medical Sciences, Karaj, Iran.
3Experimental Medicine Research Center, Tehran University of Medical Sciences, Tehran, Iran
4Department of Pharmacology, School of Medicine, Shahid Beheshti University of Medical Sciences
5Department of Plastic and Reconstructive Surgery, Shahid Beheshti University of Medical Sciences, Tehran, Iran

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/EXD.14154

This article is protected by copyright. All rights reserved
Abstract

Hypertrophic scar and keloid are two types of fibroproliferative conditions that result from excessive extracellular matrix production. The underlying pathological mechanism is not entirely clear. Activation of the renin-angiotensin system (RAS) is associated with fibrosis in various organs. RAS components including angiotensin II (Ang II), angiotensin AT$_1$ and AT$_2$ receptors, and angiotensin-converting enzyme (ACE) are expressed in the skin and act independently from the plasma RAS. AT$_1$ receptors, which are usually the dominating receptor subtype, promote fibrosis and scar formation, while AT$_2$ receptors inhibit the aforementioned AT$_1$ receptor-coupled effects. Elevated angiotensin II (Ang II) levels acting on the AT$_1$ receptor contribute to skin scar formation through increased expression of inflammatory factors such as interleukin-6 (IL-6), angiogenic factors such as vascular endothelial growth factor (VEGF) and fibrinogenic factors such as transforming growth factor-$\beta$1 (TGF-$\beta$1) and connective tissue growth factor (CTGF), while at the same time
suppressing the anti-fibrotic tissue inhibitors of matrix metalloproteinase (TIMPs). First, small clinical trials have provided evidence that inhibition of the ACE/Ang II/AT\textsubscript{1} receptor axis may be effective in the treatment of hypertrophic scars/keloids. This review provides a detailed overview of the current literature on the RAS in skin, wound healing and scar formation and discusses the translational potential of targeting this hormonal system for treatment and prevention of hypertrophic scars and keloids.

**Keywords**: Angiotensin, Keloid, Hypertrophic scar, Angiotensin-Converting Enzyme blocker, Angiotensin II receptor blockers

### Introduction

Deep cutaneous injuries such as burn injuries, physical trauma, surgical incisions, vaccinations, skin piercings, and even insect bites occasionally produce hypertrophic scars or keloids. It has been estimated that over 100 million people annually suffer from scar-related complications that significantly impair their quality of life\textsuperscript{1,2}. These issues include cosmetic problems, functional disabilities such as contractures, and symptoms including pruritus and pain\textsuperscript{1}. Although the clinical course, physical appearance and pathophysiological features of keloid and scars are different, the differentiation of the two lesions is challenging in the practice, whilst, each of these two conditions requires different therapeutic approaches. Both are two forms of fibroproliferative disorders that appear as a raised lesion above the skin level. Hypertrophic scars stay within the confines of the initial wound, increase in size by pushing out the margins of the scar because of scar contracture and may regress with time. In contrast, keloids extend beyond the confines of the initial wound and never regress\textsuperscript{3,4}.

The pathophysiology of hypertrophic scars and keloids is extremely complex and the current therapies, such as topical formulations (silicone gel, flavonoids: onion extraction), laser treatment, corticosteroid injections, and cryotherapy are far from sufficient\textsuperscript{5,6}. Some recent research has focused on the renin-angiotensin system (RAS) in the process of both wound healing and scar formation as a promising therapeutic target\textsuperscript{7}. Here, we summarize and review the role of the RAS in scar formation as well as the application of these advances in the treatment of hypertrophic scars and keloids.
Scar formation

Hypertrophic and keloidal scars are the sequel of the dysregulated wound healing process, however, their pathogenesis is not fully understood. Wound repair is a dynamic process divided into distinct phases: hemostasis and inflammation, proliferation and angiogenesis, epithelization and contraction, and remodeling consisting of a balanced interaction between different inflammatory and signaling pathways. Initially, hemostasis occurs following injury by activating the extrinsic clotting cascade. A fibrin clot is formed by platelet aggregation and coagulation to minimize the bleeding and to build scaffold for the wound bed. Platelets release different cytokines [e.g. interleukins 6 and 8 (IL-6, IL-8), epidermal growth factor (EGF), insulin-like growth factor (IGF-I), platelet-derived growth factor (PDGF) and transforming growth factor-β (TGF-β)] that recruit an array of inflammatory cells, epithelial cells, mast cells, endothelial cells, and fibroblasts. The inflammatory phase happens within the first 48 to 72 hours after injury. Then the proliferation phase starts and lasts for up to 3 to 6 weeks when fibroblasts produce the extracellular matrix (ECM). The clot is replaced by the granulation tissue in full-thickness wounds, which is mainly made of macrophages, fibroblasts, myofibroblasts, proteoglycans, hyaluronic acid, type III collagen, and elastin. The granulation tissue forms a framework for angiogenesis that guarantees the nutritive perfusion and the delivery of immune cells to the wound as well as re-epithelization. Epithelization and contraction are initiated as early as 6 hours post-injury and necessitate interaction between keratinocytes, dermal and immune cells that are essential for wound closure. The time taken to complete closure of wounds depends on wound specifics including location, depth, size, microbial contamination as well as patient’s characteristics, genetics and epigenetics.

Restoration of barrier function and epithelial layer occurs quickly, while reconstitution of dermal tissue during the remodeling phase usually takes up to several months. In this stage, fibroblasts/myofibroblasts actively reshape the dermal matrix by secreting collagen fibers and matrix metalloproteinase (MMP), the immature type III collagen is modified into mature type I collagen, and immature vessels regress. The balance between apoptosis of existing cells and production of new cells in the granulation tissue contributes to wound maturation during the remodeling phase.

The augmented inflammatory response and overexpression of growth factor signals compromise the extracellular matrix and cause overactivation of fibroblasts with the consequence of an undesirable scar. Keloids and hypertrophic scars have richer vascular tissue and occluded microvessels, increased mesenchymal cell density, more inflammatory...
cells and active fibroblasts, and a thickened epidermal layer compared to normal skin and
scars. The ratio of type I to type III collagen is increased and elastin is decreased within the
dermis. Fibroblasts are the predominant cell type in these two conditions and myofibroblasts
persist, rather than disappearing by apoptosis after 10-15 days after injury before initiation of
the remolding phase \(^3\).

Dysregulation of TGF-β production or the balance between pro-inflammatory cytokines e.g.
IL-6 and IL-8, and anti-inflammatory cytokines e.g. interleukin-10 (IL-10) contribute to
hypertrophic scarring \(^10\). It has been reported that administration of IL-6 increases scarring \(^11\)
while IL-10 administration is associated with scar-less healing \(^12\). Additionally, IL-10
production is decreased in hypertrophic scars compared with normal scars \(^13\). Moreover, IL-
10 knockout mice develop more severe scars than wild-type mice \(^14\). Interestingly, mice
lacking macrophages and functional neutrophils show no obvious scarring while the normal
wound healing is intact \(^15\). Overexpression of TGF-β 1 and 2 and decreased expression of
isoform 3 contribute to hypertrophic and keloid scar formation. TGF-β 1 and 2 signaling
through small mothers against decapentaplegic (SMAD) and Wnt pathways cause activation
of fibroblasts, while TGF-β 3 inhibits the aforementioned signaling cascades \(^10\).
Dysregulation between apoptosis and proliferation of fibroblasts also increases the rate of
collagen synthesis during the healing process resulting in scar formation, and p53, p63, and
p73 genes seem to be involved in this process \(^3,16\). Hypertrophic scars and keloids typically
occur in certain ethnicities. African and Hispanic populations are more prone to keloid and
hypertrophic scar formation compared to other ethnicities. Thus, there might be some genes
involved in the pathology of scarring, which – when identified – may serve as novel targets for
therapy \(^10,17\).

**RAS in the skin and its role in scarring**

The RAS (angiotensin II, renin, ACE, AT\(_1\) receptor, and AT\(_2\) receptor) is the key modulator
of intravascular pressure. Ang II is an eight amino acid peptide formed by renin and
angiotensin-converting enzyme from angiotensinogen. Ang II acts via two specific G protein-
coupled receptors, (Figure 1) the AT\(_1\) and AT\(_2\) receptors, which mediate opposing effects \(^18\).
All components of the RAS are expressed in human skin \(^19\). AT\(_1\) and AT\(_2\) receptors are found
in human keratinocytes, fibroblasts and vascular endothelial cells, while melanocytes express
only AT\(_1\) receptors (Figure 2) \(^19-21\). Both, AT\(_1\) and AT\(_2\) receptors are found in myofibroblasts
and keratinocytes in rodents \(^20,22\).
Stimulation of AT$_1$ receptors activates cell proliferation and migration $^{20,22,23}$, collagen production $^{24}$, and angiogenesis by increasing the expression of angiogenic and fibrogenic factors including TGF-β $^{22,25-28}$. In contrast, the AT$_2$ receptor is an inhibitory receptor for cell proliferation and migration $^{22}$, collagen production $^{24}$ and inflammation by blocking the production of IL-6, tumor growth factor alpha (TNF-α) and TGF-β $^{29,30}$. The expression of AT$_1$ and AT$_2$ receptors is increased in human wounded skin $^{31}$. Morihara et al. reported higher activity of ACE in the human wounded skin compared to the normal skin and the highest activity was detected in the scar tissue $^{32}$. The amount of AT$_1$ receptors is also significantly increased in adult scars compared to the normal skin $^{21}$. In our previous study, we found that Ang II concentration was significantly higher in skin samples isolated from patients with keloid compared to the normal skin. Expression of AT$_1$ receptors was increased in keloid tissue compared with normal skin and hypertrophic scars $^{33}$.

In an in vitro setting, Ang II by acting on AT$_1$ receptors caused migration of keratinocytes and fibroblasts through EGFR transactivation and heparin-binding epidermal growth factor-like growth factor (HB.EGF) shedding $^{20}$. Activation of AT$_1$ receptors also increased re-epithelization and recovery of myofibroblasts in vivo in rats, whereas stimulation of AT$_2$ receptors inhibited these pathways $^{22}$. In addition, activation of AT$_1$ receptors by Ang II led to an increase in collagen production while an AT$_1$ antagonist, valsartan reduced this effect. The anti-fibrotic effects of AT$_2$ receptors were confirmed in another study, in which AT$_2$ receptor activation reduced collagen deposition, while the AT$_2$ receptor antagonist, PD123319, stimulated collagen synthesis in mouse skin $^{24}$.

Treatment of human fibroblasts with losartan, an AT$_1$ receptor antagonist, was found to significantly decrease contractile activity, migration, and gene expression of collagen type 1, TGF-β, and monocyte chemoattractant protein-1 (MCP-1) with a consequent reduction in myofibroblast activity and monocyte trafficking to the scar tissue $^{34}$. Additionally, oral treatment with losartan prevented the development of hypertrophic scars in rats $^{34}$.

In various studies the signaling cascades underlying the fibrogenic effect of AT$_1$ receptors were identified to involve IL-6/ TGF-β and activator protein-1 (AP-1)/TGF-β followed by activation of SMAD 2/3, TGF-β-activated kinase (TAK) 1, MAPK kinase (MKK) 3-p38 and MKK4-JNK $^{25,26,35}$. These mechanisms, which link the RAS with skin fibrosis, are reviewed in detail in the following.
Mechanisms underlying scar formation through RAS

Vascular dysfunction

Abnormalities in blood vessel physiology of various kinds are involved in the pathogenesis of keloids and hypertrophic scars. Vascular dysfunction such as increased vascular density and permeability during wound healing potentiates and prolongs the inflammatory phase resulting in excessive fibroblast activity and ultimately the hypertrophic or keloidal scar formation. These two conditions have a higher number of vessels compared with normal skin and all effective treatments act, at least partly, by suppressing abnormal vascularity. Blood vessels of the skin express AT1 receptors with AT1 receptors generally located in vascular endothelial and smooth muscle cells. Kurosaka et al. found that the expression of vascular endothelial growth factor (VEGF) mRNA is decreased in AT1 receptor knockout (AT1a−/−) mice or in those animals that received AT1 receptor antagonists (ARBs). Additionally, oral administration of the ARB candesartan has been shown to inhibit angiogenesis during wound healing in rats. In our own recent study in humans, one of the first effects of the use of losartan ointment for the treatment of hypertrophic scars and keloids was a decrease in vascularity.

IL-6

An overly strong inflammatory response is a key component in the development of fibrosis. Elevated cytokine expression, mostly IL-6, contributes to fibrosis by enhancing ECM deposition, collagen type I content, and stimulates other fibrogenic mediators including TGF-β and tissue inhibitors of matrix metalloproteinase (TIMP). IL-6 has a role in myofibroblast differentiation. It is found to induce B-cell lymphoma-2 (Bcl2) expression in fibroblasts from idiopathic primary fibrosis patients. Bcl2 may contribute to resistance to apoptosis of myofibroblast isolated from hypertrophic scars. The expression of IL-6, its receptor IL-6Rα and IL-6Rβ (gp130), as well as its various downstream targets such as Janus Kinase (JAK)-1, Signal Transducer and Activator of Transcription (STAT)-3, RAF1, and ELK1 are significantly higher in human primary keloid fibroblasts in vitro compared with surrounding non-lesional skin fibroblasts. The expression of IL-6 is affected by a positive feedback loop wherein the elevated levels of TGF-β boost IL-6 production through Phosphoinositide 3-kinases (PI3Ks) and p38-MAPK and IL-6 promotes TGF-β expression in keloid-resident macrophages. Interestingly, human fetal wound is characterized by minimal inflammation,
lack of IL-6 and scarless healing. Administration of IL-6 to the wounded fetal skin placed subcutaneously in SCID mice was found to cause skin scarring\textsuperscript{11}.

In the heart, Ang II administration increased expression and release of IL-6 by cardiac macrophages in mice by acting on the AT\textsubscript{1} receptor, while IL-6 knockout animals showed reduced Ang II-induced cardiac fibrosis \textsuperscript{46}. In human primary skin fibroblasts, stimulation of the AT\textsubscript{2} receptor inhibited TNF-\alpha-induced IL-6 expression\textsuperscript{47}. Therefore, the Ang/II-IL-6 axis may also work in the skin as a pathway towards fibrosis.

**TGF-\beta and CTGF**

TGF-\beta isoforms 1 to 3 are significant elements within the process of scar formation \textsuperscript{10}. TGF-\beta 1 and 2 activate SMAD 2/3, Transforming growth factor b-activated kinase 1(TAK1), MAPK kinase (MKK) 3-p38 and MKK4-JNK signaling cascades leading to type I collagen and fibronectin synthesis \textsuperscript{48}. Ang II-induced activation of AP-1 is reported to result in TGF-\beta1 expression in human dermal fibroblasts \textsuperscript{26}. ACE inhibitors (ACEI) exert anti-fibrotic properties through suppression of TGF-\beta1/SMAD and TGF-\beta1/TAK1 pathways both \textit{in vitro} and \textit{in vivo} \textsuperscript{48}. ACEIs such as ramipril and captopril are reported to reduce scar size by inhibition of TGF-\beta and PDGF expression \textsuperscript{48}, of TAK1 and SMAD 2/3 phosphorylation \textsuperscript{49}, and of fibroblast proliferation \textsuperscript{48-50}.

CTGF is another important mediator activated by TGF-\beta signaling, which is involved in the process of fibrosis including fibrosis induced by Ang II via the AT\textsubscript{1} receptor \textsuperscript{51}. It was reported that Ang II increased CTGF and ECM via AT\textsubscript{1} receptors in vascular smooth muscle cells of aorta both \textit{in vivo} (normal rats) and \textit{in vitro}, while losartan inhibited this pathway \textsuperscript{52}. Additionally, it was found that two weeks of treatment with the direct renin inhibitor aliskiren or the ARB losartan in transgenic mice expressing active renin from the liver (RenTgMK), inhibited CTGF expression and ECM production in fibrotic lungs \textsuperscript{53}.

**TIMP-1**

Matrix metalloproteases (MMPS) and tissue inhibitors of matrix metalloproteinases (TIMP) 1-4 are important for scar formation since MMPs degrade ECM, while TIMPs inhibit MMPs thus preventing ECM degradation. The expression of TIMP-1 and TIMP-2 was found to be significantly higher in hypertrophic scars and keloids in comparison to regular skin and normotrophic scars, while TIMP-1 was higher in keloids than in hypertrophic scars \textsuperscript{54}. Ang II has been shown to reduce the ratio of MMP-1/TIMP-1 through AT\textsubscript{1} receptors in primary...
cultured fibroblasts isolated from skin of diabetic rats \(^{35}\). In addition, Ang II is found to increase collagen production by increasing the expression of TIMP-1 in mouse fibroblasts. These observations are supported by a study, in which the ARB valsartan inhibited the expression of TIMP-1 in skin fibroblasts of mice \(^{24}\). In the same study, application of the AT\(_2\) receptor antagonist, PD123319, enhanced expression of TIMP-1, thus suggesting that stimulation of the AT\(_2\) receptor may have an anti-fibrotic effect by inhibition of TIMP-1 \(^{24}\).

An anti-fibrotic role for the AT\(_2\)R was further supported in this study by an inhibition of collagen synthesis and TIMP-1 expression by Ang II in fibroblasts derived from AT1Ra-KO mice. The AT2R-coupled inhibitory effect seemed to involve activation of the tyrosine phosphatase SHP-1, since these effects could be blocked by the tyrosine phosphatase inhibitor orthovanadate and in cells transfected with a dominant negative SHP-1 mutant \(^{24}\).

**RAS in the development of fibrosis in other tissues**

ARBs and ACEIs were shown in a multitude of studies to decrease the level of TGF-\(\beta\) (plasma and urine of humans), CTGF (rat aortic tissue), IL-6, interferon-gamma (IFN-\(\gamma\)) (mouse kidney), and VEGF (mouse retina) in tissues other than skin \(^{27,55-60}\). Co-administration of nilotinib and losartan reduced liver fibrosis in rats \(^{61}\). Losartan has also been reported to reduce renal cortical scaring by up to 50\% in mice \(^{57}\). In addition, losartan decreased fibrosis in patients with hypertrophic cardiomyopathy \(^{62}\), chronic hepatitis C \(^{63}\), and idiopathic pulmonary fibrosis of human \(^{64}\). A study found that the combination of aliskiren and losartan inhibited ECM production and fibrogenic factors and pulmonary fibrosis in RenTgmk mice \(^{53}\).

**RAS in wound healing**

There is evidence that the RAS has an important role in normal cutaneous wound healing. For example, angiotensin receptor expression is changing during the course of wound healing, which may modify the “net” effect of Ang II during the different phases of wound healing. A study in rats revealed that three days after wounding, the expression of Ang II receptors, mainly AT\(_2\) receptors, was enhanced in the dermis \(^{65}\). In contrast, the expression of AT\(_1\) receptors decreased within 12 to 24 hours after wounding, but recovered to baseline levels thereafter \(^{66}\). Another study in rats found expression of AT\(_1\) and AT\(_2\) receptors at 0.5, 1, 2, 4, and 24 hours and at the 2\(^{th}\), 3\(^{th}\), 4\(^{th}\), 5\(^{th}\), 7\(^{th}\), and 10\(^{th}\) day’s after wounding with the AT\(_1\) receptor being the dominant receptor at all time points. AT\(_1\) and AT\(_2\) expression followed a
biphasic pattern: receptor expression was decreased during early phases followed by an increase until day 7 after wounding and subsequently gradually reaching normal levels again on day 10. Similarly, Wu et al. found that AT1 and AT2 expression reached a peak on the 7th day after wounding and then decreased, but that AT2 receptor expression increased again during reepithelization.

Accordingly, wound repair was delayed in AT1 receptor knock-out mice and in mice treated with ARBs. In another study, losartan delayed the healing of the wound in the abdominal fascia and reduced its resistance. Taken together, Ang II production and the balance between the local expression of AT1 and AT2 receptors seem essential for the wound healing process.

**Treatments**

**Topical application of ACEI and ARBs**

In one of our own studies, we used topical losartan ointment (5%; water-absorbing bases) to treat hypertrophic scars and keloids. Losartan ointment or placebo ointment were applied twice a day for 3 months to 30 patients, which had been randomly assigned to the treatment (n = 20) or placebo (n = 10) group, respectively. In order to assess the response rate of losartan treatment compared to placebo treatment, the Vancouver Scar Scale (VSS) was used, which includes four indicators: vascularity, pigmentation, pliability, and height. Losartan ointment significantly improved all of these four indicators in hypertrophic scar and keloid tissue. Furthermore, patients reported reduced itching in scar tissue after losartan ointment (Table. 1). No side effects were reported in the Losartan or the treatment group, respectively. In a case report by Ardekani et al., the use of captopril cream (5%) for 6 weeks reduced height, redness, itching and increased the healing rate of keloid from burn injury in an 18-year-old girl. Another clinical trial reported by Mohammadi et al., showed that the use of enalapril (1%) significantly reduced the size of hypertrophic scars. In this double-blind trial, 30 patients with hypertrophic scar and itching after treatment of 2nd or 3rd degree burns and two same-degree scars on symmetrical sites of body, were treated with the enalapril ointment on one, randomly selected side of their body for 6 months, while the other side was treated with placebo. In this study, enalapril treatment significantly improved clinical parameters of hypertrophic scar and also itching.

A beneficial effect of RAS inhibition on scar formation was also reported in a preclinical model in mice by Zehng et al. The authors found that the topical application of ramipril and
This article is protected by copyright. All rights reserved
proliferative effect of the AT\textsubscript{1} receptor is desirable during reepithelization, on the other hand it seems to contribute to excessive fibrosis and contribute to hypertrophic scar and keloid formation. The same is true in reverse for the anti-proliferative effect of the AT\textsubscript{2} receptor. Therefore, it may be essential for a treatment effect to apply the right timing for RAS interference during wound healing and scar formation. This is a problem that has not been addressed experimentally yet and respective studies are needed on preclinical and clinical level.

Another open question is, whether the different ways of interference with the RAS, i.e. ACE inhibition, renin inhibition, AT\textsubscript{1} receptor blockade, AT\textsubscript{2} receptor stimulation or a combination of two or more of these pharmacological approaches results in the best treatment effect. Therefore, head-to-head comparisons of the respective drug classes in preclinical and clinical studies are warranted.

When developing new drugs for topical treatment, it obviously has to be taken into consideration that the epidermis, in particular the stratum corneum and also fibrotic tissue prevent drugs from penetration into deeper layers of the skin \textsuperscript{76}. Therefore, properties such as molecular size, lipophilicity, and solubility of the new drug have to be suitable for topical application \textsuperscript{77,78}. Losartan potassium has a small molecular size (422.91) and seems to be converted into its active metabolite when applied to skin as shown in tests with transdermal system delivery, in which topically applied losartan effectively led to blood pressure lowering \textsuperscript{77}. For better penetration of losartan and other RAS-interfering drugs into deep skin layers or fibrotic tissue, other measures such as the use of nano-carriers or penetration enhancers may be necessary. It also needs to be taken into consideration that when treating cutaneous diseases with topical losartan or ACE inhibitors, patients may experience effects on blood pressure.

**Conclusion and Translational perspectives**

The existing literature strongly supports that RAS components are expressed in various cells of the skin, that the skin RAS can act independently from plasma RAS and that it plays an important role in wound healing. The pro-fibrotic, pro-inflammatory and pro-proliferative effects of Ang II are mostly mediated through AT\textsubscript{1} receptors, while AT\textsubscript{2} receptors act anti-fibrotic, anti-inflammatory and anti-proliferative \textsuperscript{20,28,69,74}. There is now strong evidence that
overactivation of the local RAS in skin contributes to the development of hypertrophic scars and keloids. AT\textsubscript{1} receptor activation induces migration and proliferation of keratinocytes, fibroblasts, and increased collagen production. The pro-fibrotic effects of Ang II are mediated via various pathways including IL-6/TGF-\(\beta\) and AP-1/TGF-\(\beta\) followed by activation of SMAD 2/3, TAK1 and CTGF. In contrast, AT\textsubscript{2} receptor stimulation exerts inhibitory effects on keratinocytes, fibroblasts and collagen production and the respective signaling cascades. Apparently, the net effect of Ang II on wound repair or hypertrophic and keloidal scar formation largely depends on the changing ratio of AT\textsubscript{1} and AT\textsubscript{2} receptor expression at different stages during the healing process.

First steps towards translation of preclinical evidence for a role of the RAS in wound healing and scar formation into the clinical situation has already been made in small clinical studies and case reports indicating that pharmacological inhibition of Ang II acting on the AT\textsubscript{1} receptor by ARBs or ACE inhibitors may be able to reduce or prevent the formation of hypertrophic scars and keloids. Further research and larger, sufficiently statistically powered clinical studies are needed to confirm these still preliminary data. For this purpose, it would be desirable to have an optimized formulation of ARBs or ACE inhibitors for topical application that ensures an effective penetration of these drugs into deeper layers of skin and into the fibrotic tissue. Finally, the therapeutic potential of AT\textsubscript{2} receptor agonists for treating hypertrophic and keloidal scars will have to be tested in the clinical situation as soon as respective drugs, which currently are in later stages of clinical development for fibrotic diseases, will become available.

Acknowledgments

The authors would like to appreciate the Shahid Beheshti University of Medical Sciences. The authors express their grateful thanks to the collaboration of Tayeb Ghadimi, Siamak Farokh Forghani, Mohammadalipour, and Hassan Niknejad.

Author contributions

K.H, S.Z, H.K, F.N, and B.H participated in all aspects of the main file draft, N.SH participated in main file draft writing A.M and A.D participated in the revision of article.
Conflict of interest:

The authors declare that there is no conflict of interests.

<table>
<thead>
<tr>
<th>Condition/Disease</th>
<th>Drug/Dose/Route of application</th>
<th>Number of patients</th>
<th>Duration of treatment</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertrophic scar and keloid</td>
<td>Topical losartan ointment 5%</td>
<td>30</td>
<td>3 months</td>
<td>Reduce vascularity, pigmentation, pliability, and height</td>
<td>7</td>
</tr>
<tr>
<td>Keloid</td>
<td>Topical Captopril</td>
<td>1</td>
<td>6 weeks</td>
<td>Reduced height,</td>
<td>70</td>
</tr>
</tbody>
</table>

ORCID: https://orcid.org/0000-0003-0764-0425

Table 1: The clinical studies with ARBs and ACEi for treatment of hypertrophic scars and keloid
<table>
<thead>
<tr>
<th>Scar Type</th>
<th>Treatment</th>
<th>Duration</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertrophic</td>
<td>Topical Enalapril ointment 1%</td>
<td>30</td>
<td>Reduced size of hypertrophic scar and itching</td>
<td>71</td>
</tr>
<tr>
<td>Keloid</td>
<td>Enalapril (10 mg, once per day p.o.)</td>
<td>2-6</td>
<td>Improvement of keloid</td>
<td>79</td>
</tr>
</tbody>
</table>

**References**


36. Ogawa R, Akaishi S. Endothelial dysfunction may play a key role in keloid and hypertrophic scar pathogenesis—keloids and hypertrophic scars may be vascular disorders. *Medical hypotheses.* 2016;96:51-60.


46. Ma F, Li Y, Jia L, et al. Macrophage-stimulated cardiac fibroblast production of IL-6 is essential for TGF β/Smad activation and cardiac fibrosis induced by angiotensin II. *PloS one.* 2012;7(5):e35144.


This article is protected by copyright. All rights reserved


Renin Angiotensin System in Skin

- Angiotensinogen
- Angiotensin I
- Angiotensin II
- Renin
- ACE
- AT₁ receptor
- AT₂ receptor

Cell proliferation and migration
Angiogenesis
Collagen and ECM production => Fibrosis
Pro-inflammatory cytokines => Inflammation

exd_14154_f1.jpg