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Published in:
Experimental Dermatology

DOI:
10.1111/exd.14159

Publication date:
2020

Document version:
Accepted manuscript

Citation for published version (APA):

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

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Download date: 15. Sep. 2023
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Article type: Focus Theme Issue: Review Article

The Role of the Renin-Angiotensin System in Skin Physiology and Pathophysiology

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Review article for the special issue on Dermatoendocrinology

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This article has been accepted for publication and 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.14159

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Abstract
Since its first description around the year 2000, the local renin angiotensin system (RAS) in skin has been subject of an increasing number of studies with many additions over the last two to three years. A focus of research has been investigations on the role of cutaneous angiotensin receptors and locally synthesised angiotensin II in wound healing, in dermatoses associated with skin fibrosis and in melanoma.
This review will provide an introduction into the RAS with emphasis on information relevant for the cutaneous RAS. It will discuss the role of the RAS in skin physiology, followed by a detailed review of the existing literature addressing the role of local angiotensin II and angiotensin AT$_1$- and AT$_2$-receptors in wound healing and in various skin diseases such as hypertrophic scars/keloids, scleroderma, dystrophic epidermolysis bullosa, Dupuytren’s disease, squamous cell carcinoma, melanoma and psoriasis.
In a final section, the potential relevance of drugs, which interfere with the RAS, for future therapy of dermatological disorders is discussed.
Collectively, research about the RAS in skin can currently be described as an area, which has gained increasing attention by basic researchers, thus resulting in a multitude of preclinical studies pointing to the potential relevance of components of the RAS as drug targets in dermatological diseases. With a few small clinical studies already performed successfully for indications such as hypertrophic scars and keloids, it can be said that the skin RAS is now in the critical phase of translation from preclinical evidence to clinical use.

Keywords: angiotensin, angiotensin AT$_1$-receptor, angiotensin AT$_2$-receptor, ACE-inhibitors, AT$_1$-receptor antagonists, AT$_2$-receptor agonists
Introduction

From the early days until now, textbook knowledge and research about the renin-angiotensin system (RAS) has focused on the renal, vascular and central effects of the main effector hormone of the system, angiotensin II (Ang II). It is still widely unknown that all the RAS components are also expressed in skin, which is why working on the cutaneous RAS is sometimes like being caught between two stools: the scientific RAS community regards the skin as irrelevant as a target organ for the RAS, and the dermatology/skin research community regards the RAS as irrelevant for skin physiology and pathophysiology. Despite this hindrance, the number of publications on the cutaneous RAS has steadily risen since the first description of the expression of all components of the RAS in rodent and human skin and is now in the hundreds.[1,2] These studies have provided strong evidence that the RAS is in fact involved in skin physiology and pathophysiology in various ways such as having an impact on keratinocyte proliferation and differentiation, cutaneous inflammation, fibrosis, scar formation, vasomotion and also a role in skin cancers.

This article reviews the current knowledge on the role of the cutaneous RAS in skin physiology and pathophysiology. As explained in more detail in the following, there are more receptors and hormones of the RAS than just Ang II and its main receptors, the AT$_1$-receptor (AT$_1$R) and the AT$_2$-receptor (AT$_2$R). Such other components are for example angiotensin-(1-7) [Ang-(1-7)], alamandine, Ang IV, the receptors Mas, Mas-related G-protein coupled receptor D (MRgD), the AT$_4$-receptor (AT$_4$R)/insulin-regulated aminopeptidase (IRAP) and also aldosterone. However, for conciseness, we have chosen to focus this article on actions mediated by the classical angiotensin receptors, the AT$_1$R and the AT$_2$R. While to our knowledge there are no data on AT$_4$R/IRAP and alamandine/MrgD in skin, there are some studies on expression and role of cutaneous Ang-(1-7), Mas and aldosterone. For information on these studies, we refer to a recent review article by Aleksiejzuk et al.[3]

The cutaneous renin-angiotensin system under physiological conditions

Although the existence of a complete RAS in skin has already been described as early as 1994 for rodents and 2004 for humans, the physiological role of the RAS in skin is still poorly understood.[1,2]
Generally, the RAS is a hormonal system, which comprises the precursor molecule angiotensinogen and various enzymes, which in multi-step, enzymatic cascades promote the generation of the biologically active hormones (Fig. 1). While Ang II, the first identified biologically active hormone of the RAS, is still regarded as the main hormone of the system, more recent research has revealed that there are in fact more biologically active angiotensin fragments such as angiotensin-(1-9), angiotensin-(1-7), angiotensin III, angiotensin IV and alamandine. Renin is the rate-limiting enzyme for angiotensin peptide synthesis and cleaves the decapeptide angiotensin I from the 485-amino acid protein angiotensinogen. The main enzymes cleaving angiotensin I are angiotensin-converting enzyme (ACE) and ACE2 with ACE leading to formation of Ang II, while ACE2 is responsible for the synthesis of angiotensins with tissue protective properties like e.g. angiotensin-(1-9) and angiotensin-(1-7). Interestingly, ACE2 is also the receptor and “entrance gate” for the SARS-CoV and SARS-CoV-2 viruses. The main receptors for Ang II are the AT1R and the AT2R. While the AT1R mediates all of the well-known cardiovascular actions of Ang II such as vasoconstriction and sodium/water-retention, the AT2R is a counter-player and in many aspects opposes the actions of the AT1R by mediating e.g. vasodilation and natriuresis. Interestingly, the spectrum of actions mediated by hormones and receptors of the RAS is by far not restricted to classical cardiovascular actions, but also includes effects on inflammation, fibrosis, proliferation and differentiation and also effects on the immune response. Generally, with regard to these effects, the AT1R – when overstimulated - mediates rather detrimental actions, which contribute to pathological situations such as inflammation and fibrosis. Again, AT2R-mediated effects are mostly the reverse and include e.g. anti-inflammation and anti-fibrosis, thus supporting that the AT2R can be regarded as a kind of endogenous tissue protection and regeneration mechanism. Interestingly, all above mentioned, biologically active, “non-Ang II” angiotensin fragments acting on their respective receptors (for details see figure 1) – as the AT2R - mediate a set of protective actions, which is why this axis of the RAS is called the protective arm of the renin-angiotensin system. The expression of all above-mentioned components of the RAS has been shown by several independent groups for rodent and human skin. RAS components are present not only in the epidermal and dermal layers, but also in subcutaneous fat tissue, in microvessels and appendages such as hair follicles, sebaceous and sweat glands. A limitation of some of the
cited studies is the use of antibodies for the detection of angiotensin receptors. As many antibodies directed against G-protein coupled receptors, anti-AT1R and anti-AT2R antibodies have come under scrutiny regarding their selectivity for the target.\(^{[16,17]}\) However, expression of RAS components in skin has also been shown on mRNA level including a recent evaluation of microarray data sets derived from Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/), thus confirming the findings on protein level.\(^{[2,18]}\)

Regarding the functional role of the RAS in skin physiology, a recent study by Jiang et al. reported that Ang II promotes differentiation of keratinocytes from bone marrow-derived mesenchymal stem cells (BMdSC) under physiological conditions.\(^{[19]}\) The authors found that this Ang II effect involves p38 MAP kinase, Janus kinase 2/3 and c-Jun N-terminal kinase (JNK), because the differentiation of BMdSC into keratinocytes could be blocked by the respective inhibitors. The authors further stated that the effect was AT1R dependent; however, they did not show any respective data in their publication.

Another physiological role of the cutaneous RAS may be related to the recent finding that skin is a major compartment for the storage of sodium, which in turn has an impact on blood pressure regulation.\(^{[20]}\) However, a modifying role of the skin RAS on cutaneous sodium storage – though likely - has not been experimentally proven. Nevertheless, experimental evidence exists for other mechanisms, by which the skin has an impact on physiological blood pressure control, such as production of vasodilatory nitric oxide by keratinocytes, which is under the control of UV-light (so called photo-relaxation),\(^{[21]}\) a balance between hypoxia-inducible transcription factors, HIF-1\(\alpha\) and HIF-2\(\alpha\),\(^{[22]}\) and potentially also the local RAS, but again, the latter still needs to be proven experimentally.\(^{[23]}\)

Of note, already the first studies reporting the presence of a local RAS in skin showed that skin and in particular keratinocytes are capable of synthesising Ang II (and likely other angiotensins) independent from supply of RAS components by the circulation.\(^{[1,2]}\) Nothing is known so far about how this local Ang II production is regulated. One intriguing question in this context that remains to be solved is whether the cutaneous RAS – like the systemic RAS – is activated in situations of a sudden drop in blood pressure or acute, major volume loss and whether skin-derived Ang II contributes to the stabilising effect of the RAS on the circulation under these conditions.
The cutaneous renin-angiotensin system under pathophysiological conditions

- The cutaneous renin-angiotensin system and wound healing

One of the best-studied pathological conditions of skin with regard to the RAS is cutaneous wound healing. Wound healing happens in sequential, yet overlapping steps, which are the haemostasis, inflammatory, proliferative and remodelling phases with scar formation as the end stage (except for minor lesions, which may heal scarless).\[24\]

Interestingly, angiotensin receptors in cutaneous wounds undergo some very dynamic changes in expression during the healing process (Fig. 2). Immediately after wounding, there is an increase in AT\(_1\)R and AT\(_2\)R expression, which seems to be slightly delayed and smaller for AT\(_2\)Rs when compared to AT\(_1\)Rs.\[14,15,25\] In scratched keratinocytes in culture, this upregulation is detectable on mRNA level as early as 1 hour after injury with AT\(_1\)R expression peaking 3 hours and AT\(_2\)R expression peaking 12 hours after scratch.\[25\]. In thermal wounds in mice, AT\(_1\)Rs and AT\(_2\)Rs are more abundant than in controls as early as 2 hours after injury.\[14\] This early increase specifically in AT\(_1\)Rs could play a role for promoting blood clotting,\[26\] for initiating the inflammatory phase \[27,28\] and for inducing reepithelialisation (the proliferative phase) by stimulating keratinocyte proliferation and migration.\[29–32\] Signalling of the AT\(_1\)R leading to keratinocyte proliferation and migration is not well characterised, but seems to involve EGF-receptor transactivation by HB-EGF shedding and by superoxide formation.\[29,30\] A stimulatory effect of the RAS on reepithelialisation was confirmed by studies showing a significant delay in wound closure in AT\(_1\)R deficient mice and in rats treated with an AT\(_1\)R antagonist.\[30,31,33\] Keratinocyte proliferation was also inhibited through reducing Ang II synthesis by the renin inhibitor Aliskiren.\[34\] However, there is also a study, in which treatment with an AT\(_1\)R-antagonist did not have any effect on reepithelialisation.\[35\]

The majority of studies looking into angiotensin receptor expression during wound healing reported a transient decrease in receptor expression after the initial increase (Fig. 2).\[14,15,25,36\]

This dip in expression, which more or less coincides with the proliferative phase, is followed by another period of receptor upregulation during the remodelling phase, but this time with a dominance of AT\(_2\)Rs over AT\(_1\)Rs (Fig. 2).\[14,15,25\]
Regarding the role of AT₁Rs during the remodelling phase, it is well documented that AT₁R stimulation acts pro-fibrotic by increasing levels of TGFβ, which in turn initiates canonical TGFβ-signalling including phosphorylation of Smad2/3 and enhanced transcription of connective tissue growth factor (CTGF/CCN2) and also non-canonical TGFβ-signalling including MAP-kinase activation. These signalling pathways result in stimulation of ECM synthesis by fibroblasts and in transition from fibroblasts into myofibroblast, which have contractile properties and are essential for wound contraction.

Regarding the role of AT₂Rs in the later phases of wound healing, based on data about AT₂R-mediated anti-inflammatory and anti-fibrotic actions obtained from a multitude of mainly non-dermatological preclinical disease models, it can be assumed that AT₂Rs are involved in dampening the inflammatory response and - most of all – in balancing the formation of granulation and scar tissue. Moreover, since AT₂Rs mediate anti-proliferation and differentiation in non-cutaneous cells, it can be speculated that they may also play a role in the differentiation of the newly formed epithelial layer. Strongest evidence that these AT₂R-mediated mechanisms are indeed relevant in the process of cutaneous wound healing is coming from a study by Faghih et al. in male AT₂R receptor knockout mice (AT₂R⁻/⁻ mice). With regard to keratinocyte proliferation and reepithelialisation, the authors observed that wound closure was faster in AT₂R⁻/⁻ mice, thus supporting an anti-proliferative effect of AT₂Rs in keratinocytes during reepithelialisation. During the remodelling phase, AT₂R⁻/⁻ mice presented with increased AT₁R- and TGFβ1/2-expression, the latter supporting an anti-fibrotic effect of AT₂Rs in skin fibroblasts during remodelling. Although this study did not investigate anti-fibrotic signalling of the AT₂R, it is known from other studies that AT₂R stimulation inhibits TGFβ-expression by inhibition of EGR-1, which is one of the transcription factors responsible for TGFβ-synthesis, and by interference with the kinase-driven, TGFβ-coupled signalling pathways by activation of protein-phosphatases [for details see]. Interestingly, despite the accelerated wound closure and activation of pro-fibrotic mechanisms in AT₂R⁻/⁻ mice, in tensiometry experiments healed wounds in these animals turned out to be more fragile than wounds in wild-type mice. Collectively, the above reviewed data suggest that precise balance and interplay between AT₁Rs and AT₂Rs is essential for fine-tuning the fibrotic process in the remodelling phase of wound healing.
Importantly, the effects of the cutaneous RAS in wound healing are not only determined by changes in receptor expression, but generally, local synthesis of Ang II seems increased after injury as shown by direct measurements of Ang II and by increased expression of ACE.[1,2,15,39] In this context, the source of ACE seems not only local synthesis, but also “import” by bone-marrow derived cells.[39] Moreover, infiltrating mast cells provide chymase, which is another enzyme capable of converting angiotensin I into Ang II.[40,41]

For more details on the role of the RAS in cutaneous wound healing, the reader is referred to a recent, excellent review by Bernasconi and Nyström.[28]

- The cutaneous renin-angiotensin system in hypertrophic scars and keloids

A major medical problem resulting from failed control of proliferative and fibrotic processes in wound healing is the formation of hypertrophic scars or keloids.[42] There is evidence that an overactivated cutaneous RAS is involved in this process, because Ang II via the AT1R is known to act pro-fibrotic[8,43] and because levels of Ang II and expression of AT1Rs and the enzymes catalysing Ang II formation, ACE and chymase, are increased in hypertrophic scars and keloids from rodents and humans.[44–47] As in other fibrotic conditions, pro-fibrotic signalling of the AT1R in hypertrophic scars or keloids involves activation of canonical and non-canonical TGFβ-signalling thus leading to increased ECM production, myofibroblast transition and granulation tissue contraction.[35,37,43]

Consequently, a multitude of studies has tested inhibition of the Ang II - AT1R axis by ACE-inhibitors and AT1R antagonists for the prevention or treatment of hypertrophic scars and keloids in preclinical models.[35,37,48–51] All of these studies – no matter, in which species they were performed, or whether drugs were applied systemically or topically – reported a reduction in scar size. While not all studies went into detail regarding the mechanism of action, those that did found a significant inhibition of canonical and non-canonical TGFβ-signalling.[35,37,48,51] The therapeutic efficacy of these pharmacological approaches is supported by two studies showing a reduction in scar formation in mice with global ACE knockout or with tissue-specific, cutaneous knockout of ACE.[37,39]

To the best of our knowledge, so far, two studies have tested the treatment of hypertrophic scars and keloids in humans by topical application of formulations of the ACE-inhibitor
enalapril or the AT₁R-antagonist losartan in randomised, single- or double-blind clinical trials (Table 1).\textsuperscript{50,52} In the losartan study, patients with hypertrophic scars or keloids from different causes were treated for 3 months with a 5% losartan ointment (n = 20) or placebo (n = 10; 7 of originally 17 patients in this group terminated the study prematurely because of no treatment effect) and followed up for 6 months.\textsuperscript{52} In the enalapril study, 30 patients with hypertrophic scars as result of 2\textsuperscript{nd} or 3\textsuperscript{rd} degree burns were treated for 6 months with a 1% enalapril ointment or placebo (subjects had two same-degree scars on symmetrical sites of body which were randomly allocated to the treatment or placebo groups).\textsuperscript{50} In both studies, at the end of the 6 months observation/treatment period, scars were significantly smaller when treated with losartan/enalapril than the placebo treated scars. Although, these single/dual-centre studies with a small number of patients can only be regarded as preliminary and need to be followed up by multi-centre studies with more statistical power, they still provide important preliminary data about a potential therapeutic effectiveness of RAS interfering drugs for the treatment of hypertrophic scars and keloids.

\textbf{- The cutaneous renin-angiotensin system and other dermatoses with fibrotic component}

There are a few publications about the role and therapeutic potential of the RAS and respective interfering drugs in dermatoses other than hypertrophic scars/keloids, in which fibrosis is a significant component of the pathophysiology.

\textbullet \textbf{Systemic Sclerosis}

It is generally accepted nowadays that autoantibodies directed against the AT₁R can be found in plasma of about 85% of patients with systemic sclerosis (SSc).\textsuperscript{53} These autoantibodies have agonistic properties, stimulate the AT₁R and trigger a multitude of cellular events, which for example promote vascular abnormalities and fibrosis. Key cellular mechanisms coupled to the AT₁R in the context of SSc and triggering fibrosis include the synthesis of TGFβ in microvascular endothelial cells or collagen I synthesis by fibroblast.\textsuperscript{54} Vascular abnormalities are characterised by hypercontractility as a result of increased sensitivity of the autoantibody-bound receptors to their natural ligand, Ang II (which may contribute to the pathogenesis of Raynaud phenomenon in patients with SSc/scleroderma) and hyper-adhesiveness due to increased expression of adhesion factors ICAM and VCAM-1.\textsuperscript{55,56}
Although a multitude of studies have investigated AT_1R autoantibodies in SSc in detail, the pathogenetic relevance is still not entirely clarified. This is for two main reasons: (i) AT_1R autoantibodies are only one type of autoantibody among many others with increased levels in SSc/scleroderma, and (ii) although AT_1R stimulation by AT_1R autoantibodies is suspected to drive SSc pathology, there are basically no clinical data supporting that blockade of AT_1Rs by AT_1R antagonists has therapeutic efficacy in SSc. There is only one small clinical trial in 42 patients, which reported improvement of exercise and lung capacity, skin fibrosis and patient function by a 4 months treatment with irbesartan (Table 1). However, so far this study has only been published as an abstract. As a result of lacking evidence, RAS interfering drugs are currently not recommended for the treatment of SSc/scleroderma in any of the guidelines of the leading societies. The only exception is the use of ACE-inhibitors for the treatment of scleroderma renal crisis (Table 1). Nevertheless, there is evidence from a preclinical study in mice, in which scleroderma-like skin lesions were induced by intradermal hypochlorous acid injections, that RAS inhibition actually may have an effect in SSc/scleroderma, since oral treatment of mice with the AT_1R antagonists Irbesartan significantly attenuated the development of inflammatory and fibrotic scleroderma-like lesions.

**Dystrophic epidermolysis bullosa**

Fibrosis driven by an inflammatory response and by TGFβ activation is also essentially involved in the pathophysiology of dystrophic epidermolysis bullosa (DEB). DEB is a rare, inherited bullous disease caused by a mutation in the COL7A1 gene encoding collagen VII. Scar formation in this disease is primarily driven by overexpression of TGFβ and can be seen as a fibrotic process. Since there is no cure available yet by gene therapy, Nyström et al. attempted to attenuate scar formation, which is the main debilitating problem for DEB patients, by treatment with the AT_1R antagonist losartan in a preclinical DEB mouse model. Seven weeks daily treatment of these mice with losartan significantly diminished TGFβ signalling (inhibited Smad 2/3 phosphorylation), halted fibrosis, reduced levels of inflammatory markers (e.g. TNFα and IL-6) and attenuated digit mutilations. Losartan is currently tested in a Phase I/II clinical study in paediatric dystrophic epidermolysis bullosa (Table 1). This study is completed, but has not reported yet.
• Dupuytren’s disease

Dupuytren’s disease is another fibrotic disease, for which an overactivation of the local RAS has been described. A recent study by Chisholm et al. tested treatment with the AT\(_2\)R–agonist C21 in a xenograft model of Dupuytren’s disease in order to investigate the therapeutic potential of the anti-fibrotic properties of the AT\(_2\)R. For this purpose, Dupuytren’s disease cord specimens from patients undergoing open partial fasciectomy were implanted under the dorsal skin of nude mice. The authors found that a 5-day treatment with C21 significantly inhibited myofibroblast proliferation in the transplanted Dupuytren’s disease cord specimens. In accompanying in vitro studies in human dermal fibroblasts, pharmacological AT\(_2\)R stimulation inhibited gene expression of key factors of TGF\(\beta\) signalling, in particular CTGF/CCN2, fibroblast specific protein-1, TGF\(\beta\)1, Smad3, and Smad4.

- The cutaneous renin-angiotensin system and skin cancer
  • Angiotensin receptor expression in skin cancer

Early studies around the year 2000 using immunohistochemistry found that squamous cell carcinoma stained highly positive, but basal cell carcinoma widely negative for AT\(_1\)Rs. AT\(_2\)R expression was not reported in these studies.

More recent data are available regarding angiotensin receptor expression in melanoma cells, which are consistent inasmuch as melanoma cells usually express both, AT\(_1\)Rs and AT\(_2\)Rs. The B16/F10 mouse melanoma cell line is an exception and possesses only AT\(_2\)Rs. Normal melanocytes express exclusively AT\(_1\)Rs.

• Role of angiotensin receptors in melanoma cell proliferation and migration

Regarding the role of angiotensin receptors in melanoma proliferation and migration, Renziehausen et al. reported that AT\(_1\)Rs act as tumour-suppressors and inhibit cell proliferation, while AT\(_2\)Rs promote proliferation of various melanoma cell lines. Moreover, in melanoma cells - especially in those from melanoma metastasis or tumours with invasive growth – expression of anti-proliferative AT\(_1\)Rs was reduced by transcriptional regulation through methylation of so called CpG-islands on the AT\(_1\)R gene. CpG islands are regions of the genome, which are in close proximity to the promoter and regulate its activity; methylation of CpG-islands leads to promoter inactivation. However, even though this study provided a
whole set of data, which consistently supported the authors’ conclusions, the more common
notion on the role of angiotensin receptors in cancer in the scientific literature is that the AT$_1$R
acts pro-proliferative, while the AT$_2$R is a tumour suppressor that inhibits proliferation,
migration and metastasis.$^{[73,74]}$ In fact, another very recent study by Martinez-Meza et al.
reported that selective stimulation or overexpression of AT$_2$Rs in mouse B16/F10 and human
A375 melanoma cells reduced “normal” and transendothelial migration.$^{[72]}$ The view that
AT$_2$Rs have anti-cancer effects is supported by the fact that an AT$_2$R-binding protein, ATIP (also
termed MTUS) has been identified to be a tumour suppressor protein.$^{[75]}$

- **Role of angiotensin receptors in melanoma metastasis**

In an *in vivo* model consisting of C57Bl/6 mice injected with B16/F10 cells (which only express
AT$_2$Rs, but no AT$_1$Rs), Martinez-Meza et al. found that AT$_2$R stimulation with the selective
agonist CGP42112A attenuated lung metastasis.$^{[72]}$ The authors provided evidence that the
anti-metastatic effect of the AT$_2$R was due to dephosphorylation and thereby inactivation of
the known pro-metastatic scaffolding protein caveolin-1 by tyrosine phosphatase PTP1B.

These effects were absent when the AT$_1$R was silenced. Activation of phosphatases is a
common signalling mechanism of the AT$_2$R, which is a G-protein coupled receptor, but with
unconventional signalling mechanisms.$^{[8]}$ Ishikane et al. used the same *in vivo* model in their
study (C57Bl/6 mice injected with B16/F10 melanoma cells) and found that treatment of mice
with Ang II, which non-selectively stimulates AT$_1$Rs and AT$_2$Rs, exacerbated lung metastasis.$^{[71]}$
They further reported that the increase in lung metastatic colonies in response to Ang II
treatment was due to AT$_1$R-mediated, increased adherence of tumour cells to vascular
endothelial cells, in which AT$_1$R stimulation promoted expression of E-selectin. The effect of
Ang II was diminished by the AT$_2$R-antagonist valsartan and in mice with endothelium-specific
AT$_1$R knockout.

- **Role of angiotensin receptors in melanoma-associated angiogenesis**

The importance of effects mediated by angiotensin receptors in peri-tumourous tissue of
melanoma was demonstrated by Egami et al., who described that melanoma-associated
angiogenesis (in a model of melanoma induced by subcutaneous injection of B16-F1 cells)
could be significantly inhibited with an AT$_1$R-antagonist and was reduced in AT$_1$R knockout.

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mice, thus pointing to a pro-angiogenic effect of the AT$_1$R.[76] The reduction in angiogenesis was associated with reduced tumour growth and improved survival of mice.

Collectively, it can be concluded that the vast majority of data nowadays supports that the AT$_1$R promotes melanoma growth by stimulating proliferation, metastasis (by increasing endothelial adhesion) and angiogenesis and that the AT$_2$R inhibits tumour growth by anti-proliferation and by inhibition of invasion and metastasis through inactivation of the scaffolding protein caveolin-1.

- The cutaneous renin-angiotensin system and psoriasis

Psoriasis is a dermatosis, in which the local RAS is overactivated as a result of increased ACE expression.[77] An increase in serum ACE levels in patients with psoriasis has also repeatedly been reported.[78–80] The increase in tissue ACE in psoriasis seems to be disease specific and not present in other dermatoses with inflammatory component,[77] which makes it more likely that the increase in ACE is rather a driver and not a result of the pathology. However, a mechanism, by which increased ACE or Ang II levels may contribute to the pathogenesis of psoriasis, has not been identified. It can only be speculated that increased Ang II levels may disturb the balance between keratinocyte proliferation and differentiation and also have a pro-inflammatory effect. Of note, successful, RAS-independent therapy of psoriasis leads to a reduction of ACE serum and skin levels back to baseline.[77]

It is intriguing to speculate that the increased serum levels of ACE and also aldosterone in patients with psoriasis contribute to the increased cardiovascular risk of these patients.[81] A very recent study by Abdollahimajd addressed this problem and indeed found a correlation between serum ACE levels and mean carotid intima-media thickness in patients with psoriasis.[80] Mean carotid intima-media thickness is an indicator of generalised atherosclerosis independent of conventional cardiovascular risk factors. According to this study, cardiovascular risk was particularly high in patients with psoriatic arthritis. Another hint towards a role for ACE in the pathogenesis of psoriasis comes from epidemiological studies, which evaluated a potential link between the incidence of psoriasis and ACE gene polymorphisms. The ACE gene contains an insertion (I)/deletion (D)
polymorphism within intron 16, with the D allele being associated with higher ACE levels.[82] A recent meta-analysis of such studies concluded that psoriasis is indeed associated with the DD+ID genotype when compared to the II genotype, thus providing a possible explanation for higher ACE levels in psoriasis patients.[83]

It may seem like a contradiction that ACE-inhibitors can lead to an exacerbation of psoriasis. However, this side-effect of ACE-inhibitors is rather regarded as a result of the increase in bradykinin levels and not as a result of the changes in the RAS, i.e. decreased Ang II levels.[84] However, psoriasis exacerbations in response to treatment with AT1R-antagonists remain unexplained.[85]

The cutaneous renin-angiotensin system: A future therapeutic target?

Despite 20 years of research about the cutaneous renin-angiotensin system and a multitude of preclinical studies, which provided evidence for the involvement of the RAS in the pathomechanism of various skin diseases and for a potential therapeutic effectiveness of RAS interfering drugs, drugs targeting the RAS are currently not a first line treatment for any dermatological disease.

However, a few small clinical studies have been performed which showed therapeutic efficacy of interrupting the potentially detrimental Ang II - AT1R axis of the RAS with ACE-inhibitors or AT1R-antagonists for the treatment of hypertrophic scars and keloids and of systemic sclerosis (Table 1).[50,52,58] A clinical study using the AT1R-antagonist losartan to prevent scarring and mutilations in pediatric recessive dystrophic epidermolysis bullosa has just been completed according to the European trial registry, but has not yet reported.[65]

The relatively small number of clinical trials with RAS interfering drugs in dermatological diseases has several potential reasons: (i) Knowledge about a role of the RAS in the pathogenesis of dermatological disorders is still incomplete and not widespread among clinicians, (ii) the therapeutic effects of RAS-interfering drugs in preclinical models may be weaker and less promising than effects of other drugs, and not the least (iii) development of already approved RAS-interfering drugs with expired patent-protection such as ACE-inhibitors and AT1R-antagonist is of limited economic interest and usually not initiated by pharmaceutical companies.
Drugs targeting the protective arm of the RAS such as AT₂R-agonists, MAS-agonists or human recombinant ACE2, which activate anti-inflammatory, anti-fibrotic and anti-proliferative mechanisms, are still in early phases of drug development and currently not developed for dermatological indications. However, once they will have approval for clinical use in non-dermatological diseases, they may be interesting candidates to be tested for the treatment of dermatoses such as psoriasis, hypertrophic scars/keloids or various forms of skin cancer.

Conclusions
After about 20 years of research on the RAS in skin, it is commonly accepted nowadays that rodent and human skin expresses a local RAS including the main receptors for Ang II. Several diseases have been identified, which are associated with an overactivated cutaneous RAS, such as hypertrophic scars and keloids, Dupuytren’s disease and psoriasis. In these diseases, the local RAS promotes cell proliferation and migration, fibrosis and inflammation. In melanoma, the RAS seems to contribute to invasion and metastasis. Pharmacological inhibition of the classical axis of the RAS by ACE-inhibitors or AT₁R-antagonists showed promising therapeutic efficacy in particular in those dermatological disorders, in which the skin RAS is activated. Pharmacological stimulation of the protective arm of the RAS with agonists for the AT₂R or MAS is a novel concept in early drug development, the relevance of which for diseases of the skin still awaits more thorough investigation, but is certainly of promise for the future.

Acknowledgments
The support of Beate M. Henz for research on the RAS in skin in the very early days is warmly acknowledged.

Conflict of Interest Statement
The authors report no conflict of interest.

Author Contributions Statement

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IMSS, KBA, NNW, JJ, MA wrote parts of the manuscript. IMSS and KBA drafted the figures. IMSS created the table. KBA and MA revised the manuscript. UMS conceptualised the manuscript, wrote major parts and made edits to the figures and the table.
References


43. A. M. Murphy, A. L. Wong, M. Bezuhly, Fibrogenesis Tissue Repair. 2015, 8, 7.


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Figure Legends

Figure 1 – The Renin Angiotensin System: This figure shows an overview of the renin-angiotensin system (RAS) illustrating the formation of angiotensin peptides and their respective receptor. Circulating angiotensinogen is processed by renin, an enzyme synthesised by juxtaglomerular cells in the kidneys, to produce angiotensin I. Angiotensin I is then processed by angiotensin converting enzyme (ACE) or chymase to give rise to angiotensin II (Ang II). Ang II binds to angiotensin AT₁- and AT₂-receptors. Ang I can also be metabolised by angiotensin converting enzyme 2 (ACE2) resulting in formation of angiotensin-(1-9), which is an agonist for AT₂Rs. Ang II is further cleaved at the C or N-terminus. At the N-terminus, Ang II is cleaved by aminopeptidase A (APA) to Ang III, which is another AT₂R agonist. Aminopeptidase N then converts Ang III to Ang IV, which activates the angiotensin AT₄-receptor, which is identical to the insulin insulin regulated aminopeptidase (IRAP). At the N-terminal end, ACE2 metabolises Ang II to angiotensin-(1-7), an endogenous agonist of the receptor Mas and – with lower affinity - the AT₂R. Alamandine, a MrgD agonist, emerges from angiotensin-(1-7) by an unknown decarboxylase. Activation of the classical arm of the RAS arm, which comprises Ang II acting on the AT₂R, is responsible for blood pressure and volume homeostasis, but when overstimulated leads to deleterious effects such as inflammation, fibrosis, proliferation, vasoconstriction and promotion of thrombosis. AT₂R, Mas, MrgD, AT₄-receptors and their related agonists are components of the protective arm of RAS, counteracting the actions of the classical arm and mediating tissue protective effects such as anti-inflammation, anti-fibrosis, anti-proliferation vasodilation and anti-thrombosis.

Figure 2 – Angiotensin receptor expression during wound healing.

A) Haemostasis and inflammatory phase – AT₁- and AT₂-receptors are upregulated during this initial stage of wound healing with the AT₁R dominating and contributing to blood clotting and to the initiation of the inflammatory response and of reepithelialisation.

B) Proliferative phase – Expression of AT₁- and AT₂-receptors is reduced during this phase of wound healing thus reducing the stimulatory effect on inflammation and reepithelialisation.

C) Remodelling phase and scar formation – During the final stage of wound healing, expression of
angiotensin receptors is increased again, but this time with dominance of the AT$_2$R. There is evidence that AT$_2$R is involved in the differentiation of the newly formed epithelial layer, dampening the inflammatory response and balancing the formation of granulation and scar tissue.
<table>
<thead>
<tr>
<th>Indication</th>
<th>Drug</th>
<th>Design</th>
<th>Outcome</th>
<th>Reference</th>
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</table>
| Hypertrophic Scar / Keloid | Enalapril Ointment 1% | - Single-centre, randomised, double-blind  
- 30 patients with hypertrophic scar and itching after 2nd or 3rd degree burns  
- Same-degree scars on symmetrical sites of body treated with either Enalapril or placebo twice daily for 6 months | Significant reduction in scar size and significant improvement of itching score in Enalapril treated compared to placebo treated patients | 50        |
|                     | Losartan Ointment 5%  | - Dual-centre, randomised, single-blind  
- 30 patients (20 on losartan, 10 on placebo) with hypertrophic scar or keloid of various causes (surgery, burn, acne)  
- Losartan or placebo twice daily for 3 months; 6 months follow-up | Significant improvement of Vancouver Scar Scale mainly through reduction of vascularity and pliability; Attenuation of itching and pain | 52        |
| Systemic Sclerosis | Irbesartan 75mg/d p.o. | - Randomised, double-blind  
- 42 patients (21 on irbesartan, 21 on placebo) with SSc  
- Irbesartan or placebo for 4 months on top of standard therapy | Significant improvement in exercise capacity, forced vital capacity, skin fibrosis (modified Rodnan skin score) and patient function (SHAQ-DI score) | 58 (abstract) |
| Scleroderma Renal Crisis | ACE-inhibitors | Multiple studies | Approved as standard treatment | 59,60 |
| Paediatric Dystrophic Epidermolysis Bullosa | Losartan suspension 2.5 mg/ml p.o. | - REFLECT Study  
- Dual-centre, non-randomised, single arm  
- 30 paediatric patients (3 – 16 years) with dystrophic epidermolysis bullosa  
- 10 months treatment | Completed, but results not yet reported | 65 |

Table 1: Clinical Trials with RAS-Interfering Drugs
Figure 1

Angiotensinogen → Renin → Angiotensin I → ACE2 → Angiotensin-(1-9) → Angiotensin-(1-7) → Angiotensin II → ACE2 → Angiotensin III → APA → Angiotensin IV → APN

AT1: Classical RAS
- Inflammation
- Fibrosis
- Proliferation
- Vasoconstriction
- Thrombosis

AT2: Protective RAS
- Anti-inflammation
- Anti-fibrosis
- Anti-proliferation
- Vasodilation
- Anti-thrombosis

Mas, MrgD

Diagram shows the conversion of Angiotensinogen to Angiotensin IV through various pathways involving enzymes and receptors.