The receptor for advanced glycation endproducts: A potential role in systemic sclerosis

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Chapter

GENERAL INTRODUCTION
Chapter 1

INTRODUCTION

Systemic sclerosis (SSc) is a complex autoimmune disease characterised by vasculopathy, immune activity, and excessive skin and internal organ fibrosis. It is associated with substantial disability, high morbidity, and high mortality rates. However, despite the large amount of research, the exact pathogenesis of SSc is still unknown. Particularly, the early course of the disease remains a major knowledge gap.

Often, patients’ first presentation comprises Raynaud’s phenomenon (RP). This is a vasospastic disorder of the distal arteries, which in most patients is primary—that is, idiopathic. When RP is secondary due to an underlying disease, it may be accompanied by additional symptoms, such as sclerodactyly, digital ulcerations, telangiectasia, calcinosis cutis, pulmonary involvement, and gastro-intestinal involvement. These symptoms should be considered alarming, as they suggest SSc and SSc-overlap syndromes as underlying causes. Pulmonary involvement, including interstitial lung disease (ILD) and pulmonary arterial hypertension (PAH), is a devastating complication that often leads to premature death in the SSc population since no specific disease-modifying treatments are currently available.

SSc can be divided into two subsets: limited cutaneous SSc (lcSSc) and diffuse cutaneous SSc (dcSSc). The first type, lcSSc, consists of non-progressive skin thickening, typically limited distally to the elbows and face. Internal organ involvement generally evolves later in the course of the disease, and RP may be present years to even decades before the SSc diagnosis is made. In contrast, dcSSc consists of widespread and progressive skin thickening, which is typically proximal. This subtype has a strong tendency towards early involvement of internal organs, leading to, for example ILD, cardiac, or renal involvement and to high mortality rates.

Previous research has focused on identifying biomarkers that predict early diagnosis and disease progression. This would allow treatments to be started early in those patients who are at increased risk of developing SSc and/or related complications, thereby eventually preventing internal organ damage. Early diagnosis and treatment are of crucial importance since no curative disease-modifying therapies are currently available and complications are irreversible.

Previously, the combination of RP, autoantibody positivity, and puffy fingers have been identified as red flags for very early SSc. SSc-specific antibodies and/or an SSc-specific nailfold capillary pattern are necessary for definite diagnosis of very early SSc.
Although these criteria comprise a clinically recognisable vignette, the underlying pathophysiological factors that trigger disease initiation and progression have not been clearly identified yet. These factors should be defined in the search for new biomarkers for early recognition of the disease and its subsequent progression towards complications. These biomarkers are also needed to develop new early treatment strategies.

**Pathophysiology of systemic sclerosis**

The activation of endothelial cells is considered a hallmark of SSc. Endothelial cell activation causes dysfunction and damage and may result from different factors, including ischaemia, oxidative stress, antibodies, or environmental factors. However, the definite initiating factor is still unknown. Endothelial cell activation leads to endothelial cell damage, apoptosis, differentiation into myofibroblasts, and defective vascular tone control, thus contributing to vasculopathy (e.g., RP), one of the key elements in the pathogenesis of SSc8,9.

Another crucial cell type in the pathophysiology of SSc is the fibroblast. Fibroblast activation has been suggested to follow endothelial cell activation, and it results in the formation of myofibroblasts. Myofibroblasts have contractile properties and produce extracellular matrix proteins, such as collagen type I and type III, and inflammatory proteins, such as interleukin-6 (IL-6), thereby contributing to a pro-fibrotic and pro-inflammatory state10. However, the exact trigger of fibroblast activation has also not been identified yet.

Davies et al.11 and Yoshizaki et al.12 suggested a possible role of advanced glycation endproducts (AGEs) and high mobility group box 1 (HMGB1) in the pathogenesis of SSc. AGEs are products derived from oxidative stress, and HMGB1 is a nuclear protein released by activated, apoptotic, or necrotic cells. These factors will be discussed later in more detail. Davies et al.11 showed that elevated AGE expression is related to RP duration, while Yoshizaki et al.12 found that upregulated HMGB1 is related to disease severity, including vascular complications and skin involvement, suggesting a possible relation with vasculopathy and fibrosis.

Moreover, Davies et al.11 showed that the expression of AGEs and the receptor for AGEs (RAGE) was increased especially in lcSSc patients with calcinosis cutis. Expression of AGEs correlated with RP duration only in this patient group compared to lcSSc patients without calcinosis cutis and patients with dcSSc. Calcinosis cutis is a skin calcification that arises without disorder in calcium-phosphate metabolism. Patients with calcinosis cutis are predisposed to exaggerated vasculopathy indicated by more
telangiectasia, giant capillaries, and a reduced capillary density and, thus possibly, to more severe disease than those without calcinosis cutis\textsuperscript{13–15}. These findings suggest a contribution of AGE-RAGE in the development of calcinosis cutis, possibly due to the increased level of ischaemia in lcSSc patients with calcinosis cutis, which was also suggested by the correlation between AGE expression and RP duration in lcSSc patients with calcinosis cutis.

Therefore, we aim to define the role of AGES, HMGB1, and RAGE in the early disease course of SSc.

Figure 1 shows a schematic overview of the proposed pathogenic mechanisms that contribute to the development of SSc. We hypothesise that the interaction of AGES and/or HMGB1 with fibroblasts, possibly mediated by RAGE, leads to fibroblast activation (represented by the turquoise arrow). In turn, this activation may stimulate myofibroblast differentiation and deposition of extracellular matrix proteins, such as collagen, which cause a pro-fibrotic environment and progression of SSc.

**The receptor for advanced glycation endproducts (RAGE)**

RAGE is a cell-surface receptor with a variety of ligands, including AGES and HMGB1. RAGE is present on many different cell types, such as fibroblasts, smooth muscle cells, and endothelial cells, and is implicated in several diseases, including vascular, neurodegenerative, and auto-immune diseases\textsuperscript{16,17}.

The soluble form of RAGE (sRAGE) is the extracellular domain of the membrane-bound form (mRAGE). It is produced in several ways: alternative splicing of RAGE mRNA or proteolytic cleavage by matrix metalloproteinase 9 (MMP9) or A-Disintegrin and Metalloproteinase 10 (ADAM10)\textsuperscript{18}. sRAGE acts as a decoy receptor for different RAGE-ligands, preventing their binding to mRAGE and thus preventing subsequent responses by mRAGE. Therefore, it might have therapeutic properties in RAGE-related diseases.
1. SSc possibly starts with EC activation, leading to EC injury, apoptosis, disruption of the endothelial lining, and EC differentiation into myofibroblasts.

2. Subsequently, fibroblasts may be activated, resulting in pro-inflammatory and pro-fibrotic cytokine production.

3. Cytokines induce fibroblast differentiation into myofibroblasts and production of extracellular matrix proteins.

4. Extracellular matrix proteins induce fibrosis, vasculopathy, and loss of organ function.

**Figure 1. A schematic overview of the proposed pathogenic mechanisms that contribute to the development of systemic sclerosis.**

We hypothesise the following: the turquoise arrow represents the interaction of AGEs and/or HMGB1 with fibroblasts, possibly mediated by RAGE, leading to fibroblast activation. In turn, this may stimulate myofibroblast differentiation and deposition of extracellular matrix proteins, such as collagen, which cause a pro-fibrotic state and progression of SSc. This figure was created with BioRender.com.

SSc: systemic sclerosis; EC: endothelial cell; RAGE: receptor for advanced glycation endproducts; TLRs: toll-like receptors; AGEs: advanced glycation endproducts; HMGB1: high mobility group box 1; DAMPs: damage-associated molecular patterns; TGF-β: tissue growth factor-β; IL-6: interleukin-6; PDGF: platelet-derived growth factor; IL-1β: interleukin-1β; Col-1: collagen 1; CTGF: connective tissue growth factor; IL-1: interleukin-1; IL-8: interleukin-8; α-SMA: α-smooth muscle actin.
Advanced glycation endproducts (AGEs)
AGEs are sugar-modified adducts that bind to amino acids or lipids and arise during non-enzymatic glycation or oxidative stress (i.e., the Maillard reaction)\textsuperscript{19,20}. The Maillard reaction is initiated by exposing amino groups to sugar residues, forming a Schiff base. More stable Amadori products result from the intramolecular rearrangement of the Schiff base. After molecular rearrangement of these Amadori products, stable AGEs are formed. Known AGEs include pentosidine, N$^\varepsilon$-(carboxymethyl)lysine (CML), N$^\varepsilon$-(carboxyethyl)lysine (CEL), and N$^\delta$-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine (MG-H1).

AGEs are formed and accumulate during ageing but are also increasingly acknowledged for their role in several chronic diseases through inducement of inflammation and stiffness in tissues. They are formed under the influence of different pathways of stress and can develop inflammation and oxidative stress after binding to RAGE. Moreover, they can generate cross-links leading to increased tissue stiffness.

Systemic AGEs can be non-invasively assessed by skin autofluorescence (SAF) using the AGE Reader. This assessment was validated by Meerwaldt et al.\textsuperscript{21}, who evaluated skin biopsies of age- and sex-matched diabetic and control subjects. Moreover, several studies have shown increased levels of SAF in auto-immune diseases, including rheumatoid arthritis, systemic lupus erythematosus (SLE), and SSc\textsuperscript{22-25}.

High mobility group box 1 (HMGB1)
HMGB1 is a nuclear protein with intracellular and extracellular functions, and it is released after cell injury, infection, or necrosis\textsuperscript{26}. Intracellular, HMGB1 regulates DNA processes, while extracellular, it is a signal for the immune system, where it can bind to different receptors, including toll-like receptors (TLRs) and RAGE, operating as a damage-associated molecular pattern (DAMP). The role of HMGB1 has been described in an increasing number of diseases, including sepsis, cancer, and SLE\textsuperscript{26,27}.

The role of AGEs, HMGB1, and RAGE in the development of systemic sclerosis
Few studies on the role of AGEs, HMGB1, and RAGE on the development of SSc exist. As mentioned above, a study by Davies et al.\textsuperscript{11} suggested a possible contribution of AGEs and RAGE, since the expression of the AGE CML and RAGE was increased in skin tissue of SSc patients, especially in lcSSc patients with calcinosis cutis. Another study by Yoshizaki et al.\textsuperscript{12} found that HMGB1 and sRAGE were elevated in the sera.
and skin of SSc patients. They suggested that increased HMGB1 and sRAGE serum levels were associated with disease severity. However, other studies on SAF have found conflicting results. Our research group demonstrated that SAF was not increased in SSc patients compared to healthy controls (HCs). However, Murray et al. and Dadodiene et al. demonstrated that SAF was increased in SSc patients. Thus, drawing a conclusion concerning SAF is difficult. Nevertheless, AGEs, HMGB1, and ligand-RAGE interactions, as well as the subsequent activation of disease-specific pathways, may play a role in the pathogenesis of SSc. Linking these pathways to SSc might offer an innovative approach to disease-modifying targets and potentially prevent complications, such as ILD and PAH.

AIMS OF THIS THESIS

This thesis comprises seven chapters. We performed in vitro, ex vivo, and in vivo studies on the role of the AGEs/HMGB1-RAGE axis in the pathogenesis of SSc.

In chapter 1, we provided a general introduction of the important topics of this thesis. In this thesis, we aim to identify early markers and the subsequent activation of disease-specific pathways that may precede organ complications in SSc. These might predict development of organ involvement, possibly be targeted to prevent future organ damage, and eventually be used to improve patient outcome.

In chapter 2, we review the AGE Reader device that can non-invasively assess AGEs. The chapter addresses the AGE Reader as a clinical tool to assess accumulation of AGEs in the skin. Furthermore, the chapter addresses different exogenous and endogenous factors that potentially influence the assessment and its interpretation.

Chapter 3 describes a proof of concept study about the AGE Reader assessment in very dark skin, one of the endogenous factors that potentially influences the assessment on an individual basis. This study validates the AGE Reader assessment in very dark skin by comparing the non-invasive AGE Reader assessment with AGE levels measured in skin biopsies of very dark-skinned healthy subjects and patients with diabetes.

Chapter 4 presents an in vitro and ex vivo study, showing the possible role of the AGEs/HMGB1-RAGE axis in the onset of skin fibrosis in SSc through interferon upregulation. We address AGEs and HMGB1 expression in skin and blood and
investigate whether AGEs and HMGB1 stimulation of dermal fibroblasts leads to inflammation, interferon upregulation, and fibrosis, as has been observed in SSc.

**Chapter 5** describes a study on the predictive value of sRAGE and HMGB1 for ILD, PAH, and mortality in SSc. We measured levels of sRAGE and HMGB1 at baseline and assessed the development of pulmonary events and survival rates in 188 patients with SSc from 2013 to 2020 in a retrospective cohort.

**Chapter 6** outlines an observational pilot study in which lcSSc patients with calcinosis cutis underwent an [¹⁸F]Sodium Fluoride (NaF) PET/low-dose CT (LDCT) scan. This scan can detect the active process of calcification formation. Detection of calcinosis cutis formation, which is currently only detected at its irreversible end-stage, might facilitate more effective treatments, potentially giving rise to earlier treatment administration. Therefore, we assessed the feasibility of visualising active calcification formation with the [¹⁸F]NaF PET/LDCT scan in lcSSc patients with calcinosis cutis.

In the final chapter (**Chapter 7**), we summarise the thesis and discuss the data and future perspectives.
REFERENCES


