STIMULATION OF SOLUBLE GUANYLYL CYCLASE (sGC) FOSTERS ANGIOGENESIS AND BLUNTS ENDOTHELIAL-TO-MESENCHYAL TRANSITION (ENDOMT) OF SYSTEMIC SCLEROSIS (SSc) DERMAL MICROVASCULAR ENDOTHELIAL CELLS

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Background: In SSC, early abnormalities in microvessel morphology and angiogenic impairment in parallel advance with the development of tissue fibrosis orchestrated by myofibroblasts. Increasing evidence suggests that the EndoMT process, in which endothelial cells transdifferentiate into profibrotic myofibroblasts, may take centre stage in SSc pathogenesis [1, 2]. sGC is an enzyme regulating cell growth/proliferation and vascular tone/remodelling by catalysing the production of cyclic guanosine monophosphate. Previous studies reported that sGC stimulation inhibits TGF-β-induced fibroblast-to-myofibroblast differentiation and collagen synthesis by blocking non-canonical ERK-dependent TGFβ signal, and that sGC stimulators (sGCS) may exert anti-fibrotic effects in experimental models of fibrotic disorders.

Objectives: To investigate the possible modulatory effects of sGC stimulation on impaired angiogenesis and EndoMT of SSc dermal microvascular endothelial cells (SSc-MVECs).

Methods: To evaluate the effects of treatment with sGCS on endothelial cell viability/proliferation, 5 lines of SSc-MVECs and 5 lines of healthy dermal MVECs (H-MVECs) were challenged with sGCS (here MK-2947) and assayed with both annexin V/FITC flow cytometry and WST-1. To analyse the modulation of angiogenesis by sGCS, SSc-MVECs were challenged with MK-2947 and subsequently tested for wound healing and capillary-like tube formation capabilities. To study the effects of MK-2947 on EndoMT, the same cells were assessed for the expression of endothelial and mesenchymal/myofibroblast markers by quantitative real-time PCR, western blotting and immunofluorescence, as well as for their contractile ability by collagen gel contraction assay. Phosphorylation of ERK1/2 was assessed by western blotting.

Results: Treatment with MK-2947 did not affect viability/proliferation of H-MVECs, while it significantly increased the proliferation of SSc-MVECs (p<0.001 vs. basal). Compared to basal condition, the MK-2947 challenge ameliorated both wound healing capability (p<0.001) and angiogenic performance (number of nodes: p<0.01; segments: p<0.001; meshes: p<0.01; and junctions: p<0.001) of SSc-MVECs. Upon stimulation of sGC, SSc-MVECs exhibited increased gene expression of proangiogenic matrix metalloproteinase (MMP)-9 (p<0.05) and decreased expression of both antiangiogenic MMP-12 (p<0.05) and pentraxin-3 (p<0.001) respect to basal SSc-MVECs. A significant increase in both gene and protein expression of the endothelial markers CD31 and VE-cadherin, and a parallel decrease in the expression of the mesenchymal/myofibroblast markers αSMA, S100A4, and type I collagen were found in MK-2947-treated SSc-MVECs. MK-2947 also downregulated the EndoMT-driving transcription factor SNAIL1 in SSc-MVECs. Stimulation with MK-2947 was able to significantly counteract the intrinsic ability of myofibroblast-like SSc-MVECs to contract collagen gels (p<0.001) and effectively reduce phosphorylated-ERK1/2 protein levels (p<0.01) respect to basal cells.

Conclusion: Stimulation of sGC effectively ameliorates the angiogenic performance and blunts the pathogenic myofibroblast-like profibrotic phenotype of SSc-MVECs.

REFERENCES:

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TARGETING INFLAMMATION RESOLUTION IN SYSTEMIC SCLEROSIS: PRECLINICAL DATA OF A NEW DISEASE MODIFYING BIOLOGIC DRUG CANDIDATE RESOLVIX

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Background: Systemic sclerosis (SSc) is a complex immune-mediated connective tissue disorder characterized by microvascular damage, inflammatory cell infiltration, and excessive deposition of extracellular matrix proteins (ECMs) resulting in fibrosis in the skin and various internal organs.

Limited treatment options are currently available in the clinic and are mostly focusing on symptom control with limited impact on disease progression. New treatment approaches are needed to address the complex dysregulated pathways and disease drivers to develop effective therapies.

While the pathophysiology of SSc remains incompletely understood it is well-recognized that progressive chronic inflammation is a part of the disease and its progression. Accumulation of apoptotic cells in tissues and defective clearance of these cells contributing to unresolved tissue repair is emerging as a root cause leading to sustained uncontrolled chronic inflammation and fibrosis. Naturally ending and resolving chronic inflammation to initiate healing and repair processes could present a novel therapeutic approach for SSc.

Resolvix, a next generation biological drug candidate under development, harnesses a complex mix of naturally occurring pro-resolutive factors emitted by human cells, has demonstrated promising disease modifying effects in models of experimental arthritis and inflammatory bowel disease.

Objectives: The objective of our preclinical study presented here, was to evaluate the potential therapeutic effects of Resolvix in experimental models of SSc.

Methods: Two established inducible preclinical models of SSc (bleomycin (BLM) and HOCl) were used to monitor the impact of Resolvix mouse equivalent versus a control treatment on skin (thickness and collagen deposition), lung (leucocyte infiltration and alveolar macrophage efferocytosis) and lymphoid organs/blood (Treg) as well as selected plasma proteins. A single treatment or respective controls were administrated intra-peritoneally at 5 weeks post disease induction and mice were monitored daily and sacrificed for analysis after 3 weeks post treatment.

Results: In our hands both preclinical models clearly show phenotypes associated with sclerosing processes of SSc. In both models we could show a significant reduction of skin thickening and collagen deposition in skin samples after a single treatment with Resolvix when compared to controls. Leucocyte infiltrates particularly evident in the HOCl model were significantly reduced by the Resolvix treatment in skin and lung tissues. Interestingly, alveolar macrophages collected from broncho-alveolar lavage (BAL) fluids of the diseased mice in both model systems demonstrated a significant reduction of efferocytosis capacity (eliminating apoptotic cells) a sign of uncontrolled ongoing chronic inflammation and recently reported in SSc patients. Resolvix treatment successfully restored the efferocytosis activity to comparable levels detected in healthy mice.

In addition, Foxp3+ regulatory T cells were increased in the blood of BLM-mice treated with Resolvix.

Plasma concentrations of IL-6 and TNF-α were not modified between controls, treated and non-treated mice.

Conclusion: Collectively our preliminary data demonstrate that harnessed pro-resolutive factors making up Resolvix are able to revert skin fibrosing processes, control inflammatory cell infiltration and restore defective macrophage efferocytosis as a novel a therapeutic approach in SSc.

Resolvix should be considered as a next generation disease modifying biological drug candidate for further development for the treatment of SSc.

REFERENCES:

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IN-VITRO STUDY ON THE EFFECT OF SELECTIVE JAK-INHIBITORS ON PBMCs STAT3 PHOSPHORYLATION FROM SYSTEMIC SCLEROSIS PATIENTS

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Background: Systemic sclerosis (SSc) is a rare autoimmune connective tissue disease characterized by autoimmunity-driven damage and vasculopathy leading