CDDO-Me Attenuates Inflammation in Healthy and Systemic Sclerosis Macrophages

Saemi Han[1,2], Rajan Bhandari[2], and Dr. Patricia A. Pioli[2] (Advisor)

1 Department of Biology, Dartmouth College, Hanover, New Hampshire, United States of America,
2 Department of Microbiology and Immunology, Dartmouth
Medical School, Lebanon, New Hampshire, United States of America

BACKGROUND

- Systemic sclerosis (SSc) is a chronic autoimmune disease characterized by fibrosis, inflammation, autoantibody formation, and vascular abnormalities.
- There are approximately 100,000 SSc patients in the US. The incidence and prevalence rates are much higher in females than in males.
- There are currently no validated diagnostic markers, disease altering FDA-approved treatments or cures for this disease.
- Mechanism of disease pathogenesis is poorly understood.

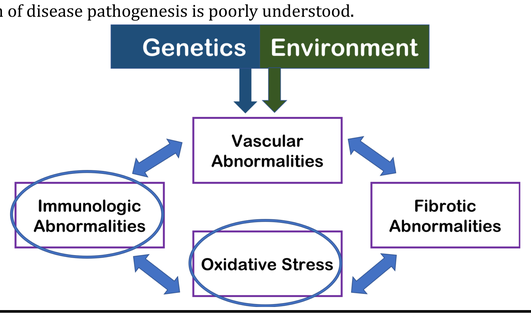


Figure 1: Model of SSc pathogenesis. Genetic and environmental factors converge to cause the onset of SSc. There are four hallmarks of the disease that include vascular abnormalities, fibrosis, oxidative stress, and aberrant immune function.

- Macrophages are innate immune cells that play important roles in phagocytosis, antigen presentation, and cytokine and chemokine production.
- Macrophage activation is plastic and is influenced by the local tissue micro-environment, including cytokines, chemokines, growth factors, and hormones.
- Genes associated with monocyte and macrophage recruitment and differentiation are overexpressed in SSc patients.
- Expression of TGF-β, CCL2, and IL-6, which are known to induce fibrosis, inflammation and oxidative stress, is elevated in SSc macrophages.

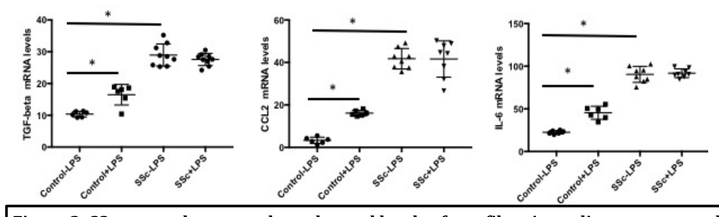
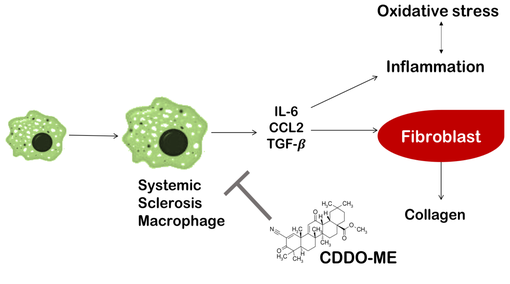


Figure 2: SSc macrophages produce elevated levels of pro-fibrotic mediators compared with control cells. Macrophages from healthy controls or SSc patients were differentiated in autologous plasma and stimulated or not with 10 ng/ml lipopolysaccharide (LPS) for 24 hours. mRNA was analyzed by qRT-PCR. n=7-8 SSc patients and 7 healthy controls; p<0.05

- CDDO-Me is an orally-bioavailable semi-synthetic triterpenoid with anti-inflammatory and anti-carcinogenic properties.
- CDDO-Me is undergoing Phase III clinical trial testing for the treatment of pulmonary arterial hypertension, which is the leading cause of death in SSc patients.

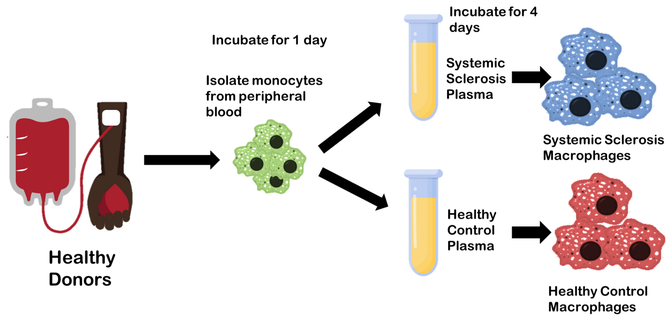
HYPOTHESIS

CDDO-Me inhibits inflammatory and fibrotic mediator production in both healthy and SSc macrophages, resulting in decreased collagen production and inflammation.



MATERIALS & METHODS

- Healthy human peripheral blood derived monocytes were isolated by cold aggregation. Monocyte purity was > 90%.
- Cells were differentiated in healthy or control plasma and treated with 15ng/ml of MCSF and 300nM CDDO-Me or vehicle control as indicated.
- Total RNA was extracted using miRNAeasy Mini Kit. cDNA was synthesized with random hexamers and analyzed by qRT-PCR.
- Flow cytometry was performed using an 8-color MACSQuant 10 with three laser sources (405 nm, 488 nm, 635 nm) and FlowLogic 501.2A software.



RESULTS

CDDO-Me attenuates CCL2 levels in both healthy and systemic sclerosis macrophages

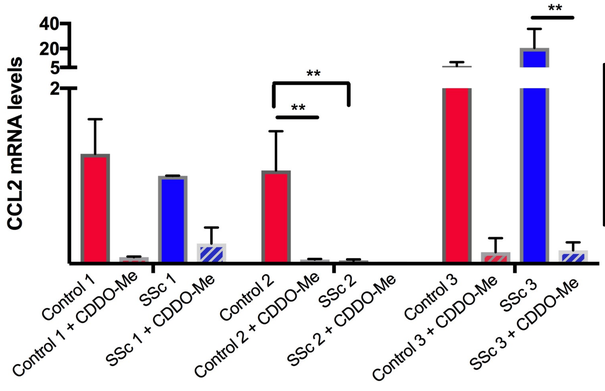


Figure 3: Control and SSc macrophages were treated with DMSO or 300nM CDDO-Me for 24 hours. Total RNA was extracted, reverse transcribed into cDNA and target genes were amplified using qRT-PCR. Values were normalized using β-actin. n=3 for each experiment.

CDDO-Me activates the NRF2 signaling pathway

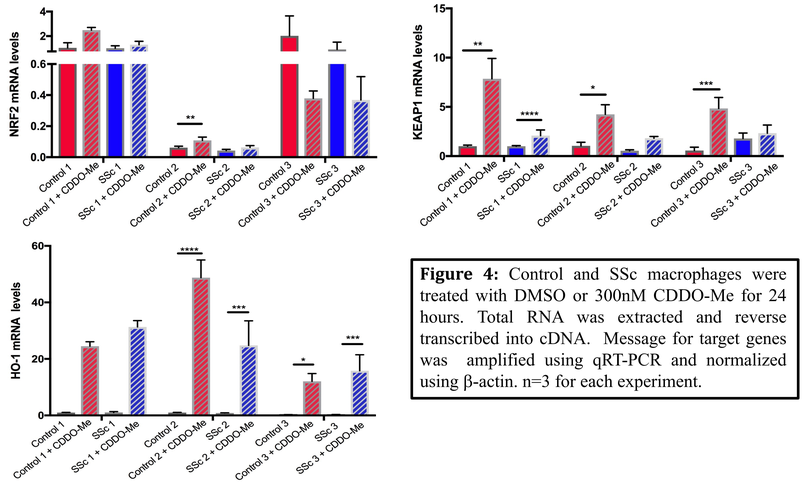


Figure 4: Control and SSc macrophages were treated with DMSO or 300nM CDDO-Me for 24 hours. Total RNA was extracted and reverse transcribed into cDNA. Message for target genes was amplified using qRT-PCR and normalized using β-actin. n=3 for each experiment.

RESULTS CONTINUED

CDDO-Me decreases % CD206+ healthy and SSc macrophages

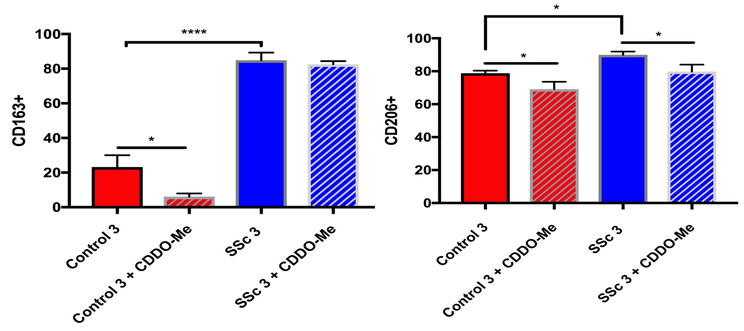


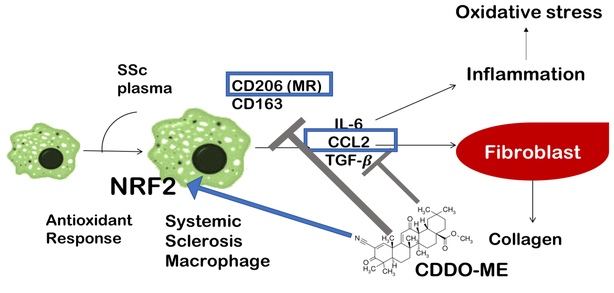
Figure 5: Multicolor flow cytometry was used to analyze effect of CDDO-Me on surface expression of CD163 and CD206. Healthy and SSc macrophages were treated with DMSO or 300nM CDDO-Me for 24 hours before staining with antibodies. n=3 for this experiment.

CONCLUSIONS

- CDDO-Me inhibits expression of CCL2 in both healthy and SSc macrophages.
- CDDO-Me induces Nrf2 activation in SSc macrophages, increasing expression of KEAP1 and HO-1
- CDDO-Me treatment decreases the number of healthy and SSc macrophages that express CD206. CDDO-Me also decreases the percent of CD163-expressing healthy control macrophages, but does not mediate changes in SSc cells.

MODEL

CDDO-Me attenuates expression of pro-fibrotic CCL2 and induces Nrf2 activation in SSc macrophages, suggesting it may be useful as a therapeutic for SSc patients.



ACKNOWLEDGEMENTS

I would like to acknowledge Dr. Patricia Pioli for her mentorship and guidance throughout this whole project , Rajan Bhandari for his help in performing experiments and analyzing data, and Dr. Karen Liby for providing the drugs that were used for this project.

Collaborators included Monique Hinchcliff (Northwestern), Kathleen Aren, Esperanza Arroyo, and Mary Carns.

Funding for this research came from the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), the Scleroderma Foundation, and the Department of Defense.