An Orally Available Highly Selective 5-Hydroxytryptamine 2B (5-HT_{2B}) Receptor Antagonist Ameliorating Pulmonary and Dermal Fibrosis in Preclinical Models of Systemic Sclerosis

H. Arozenius, L. Pettersson, G. Ekström, C. Wenglén,
AnaMar AB, Lund, Sweden

INTRODUCTION

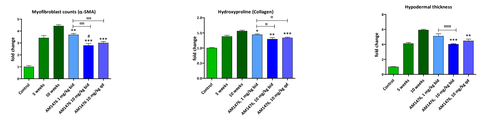
Serotonin or 5-hydroxytryptamine (5-HT) is well known as a stimulator of tissue fibrosis and a significant role of peripheral 5-HT_{2B} receptors has been suggested with the receptor being upregulated in fibrotic tissues. Agonism of the 5-HT_{2B} receptor has been implicated in human tissue fibrosis caused by drugs known to activate the receptor. Pharmacologic inhibition of the 5-HT_{2B} receptor signaling consequently represents a promising treatment strategy for fibrotic disorders including systemic sclerosis. The pro-fibrotic effects of 5-HT and the 5-HT_{2B} receptor are believed to involve activation of the TGF-β/Smad signaling pathway

OBJECTIVE

The objective of the present study was to investigate effects of a selective 5-HT_{2B} receptor antagonist, AM1476 on fibrosis development and on potential biomarkers.

RESULTS

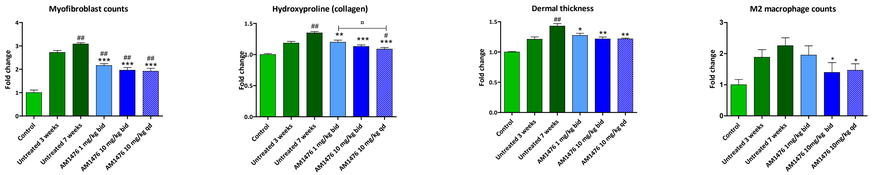
Therapeutic effects in the tight-skin-1 model



Tsk-1 mice developed prominent skin fibrosis with increased myofibroblast differentiation, hydroxyproline content and hypodermal thickening. The skin fibrosis progressed between 5 and 10 weeks. Significant anti-fibrotic effects for all measured parameters were observed after therapeutic treatment with AM1476 at doses of 1 and 10 mg/kg p.o., b.i.d. and 10 mg/kg p.o., q.d..

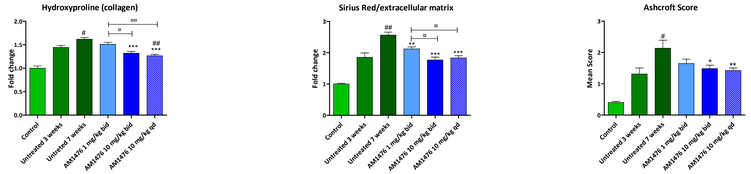
Therapeutic effects in the sclerodermatous chronic graft-versus-host disease model

Skin



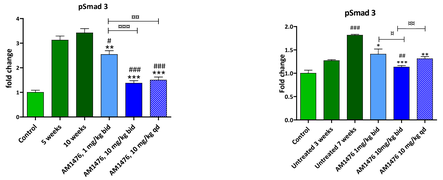
Allogeneic bone marrow transfer (BMT) induced prominent skin fibrosis with accumulation of myofibroblasts, increased hydroxyproline, dermal thickness and M2 macrophage counts with higher levels at 7 weeks compared to the 3 weeks control. Therapeutic, oral treatment with the 5-HT_{2B} receptor antagonist AM1476, once or twice daily, from week 3 to 7 resulted in reduced myofibroblast differentiation, hydroxyproline content, dermal thickening and M2 macrophage counts as compared to the vehicle-treated 7 weeks control. The three different treatment approaches all showed significant antifibrotic effects.

Lung



Allogeneic BMT induced moderate pulmonary fibrosis with increased hydroxyproline content, collagen-covered lung area and Ashcroft score. The changes progressed over time with significantly higher levels at 7 weeks compared to the 3 weeks control. Therapeutic, oral treatment with AM1476, once or twice daily, from 3 to 7 weeks reduced hydroxyproline content, collagen-covered lung area and Ashcroft score as compared to vehicle-treated 7 weeks control.

Reduction of pSmad downstream the TGF-β pathway



The number of pSmad3 positive cells was significantly reduced in AM1476 treated animals (Tsk-1, left and cGvHD right), indicating interference with the TGF-β pathway.

CONCLUSIONS

- The small molecule AM1476 is a highly selective 5-HT_{2B} receptor antagonist
- Oral therapeutic treatment with AM1476 ameliorates dermal fibrosis in the cGvHD and in the Tsk-1 model of fibrosis
- Oral therapeutic treatment with AM1476 ameliorates lung fibrosis in the cGvHD model of fibrosis
- AM1476 reduces pSmad3 suggesting interaction with TGF-β dependent signaling pathways. Further evaluation of pSmad3 as a biomarker is motivated
- AM1476 was well tolerated without obvious signs of toxicity
- The 5-HT_{2B} receptor antagonist AM1476 represents a new treatment opportunity for fibrotic diseases and is currently in early clinical development for systemic sclerosis

Questions? Christina.wenglen@anamar.com

METHOD

In vivo: Sclerodermatous chronic graft-versus-host disease model: Mice were transplanted with bone marrow cells and splenocytes to induce fibrosis. Therapeutic treatment with AM1476 started week 3 and ended week 7. AM1476 was orally administered at 1 and 10 mg/kg b.i.d.or 10 mg/kg q.d.. Skin samples were stained with hematoxylin/eosin and dermal thickness was measured as the distance between the epidermal-dermal junction and the dermal-subcutaneous fat junction. Lung samples were stained with Sirius red and fibrotic area was determined as percent of total lung area and quantification of pulmonary changes were made using the Ashcroft score. Collagen in skin and dermal samples was determined using the hydroxyproline assay. Skin sections were stained with monoclonal anti-α-SMA antibodies to characterize and count myofibroblasts. Skin sections were stained with monoclonal rabbit anti-Smad3 (phospho S423+S425) to determine amount pSmad3. Number of M2 macrophages was determined in skin sections stained with 3 different M2 markers and DAPI.

Tight-skin-1 model: Tsk-1 mice carry a heterozygous mutation in the fibrillin-1 gene and develop fibrosis spontaneously. Oral, therapeutic treatment with AM1476 at 1 and 10 mg/kg b.i.d. or 10 mg/kg q.d. started at week 5 and continued until week 10. Hypodermal thickness was measured on defined areas of the skin of the upper back. Sections were stained with hematoxylin/eosin. Skin sections were stained with monoclonal anti-α-SMA antibodies to characterize and count myofibroblasts. Collagen protein was determined with the hydroxyproline assay using skin biopsies derived from the upper back. Skin sections were stained with monoclonal rabbit anti-Smad3 (phospho S423+S425) to determine amount pSmad3. All *in vivo* data are presented as mean±SEM. Differences between groups were tested for statistical significance by Mann-Whitney U non-parametric test for non-related samples. *P<0.05, **P<0.01, ***P<0.001 vs 7- or 10-week control, #P<0.05, ##P<0.01, ###P<0.001 vs 3-or 5-week control, ¤P<0.05, ¤¤P<0.01, ¤¤¤P<0.001 vs 1 mg/kg bid.

C.W., H.A., L.P., G.E. are all AnaMar AB employees