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

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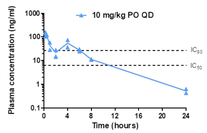
INTRODUCTION

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 in fibrosis 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 5-HT$_{2B}$ receptor signalling represents a promising treatment strategy for fibrotic disorders including systemic sclerosis. 5-HT is released from platelets activated upon vascular damage. The local 5-HT concentration is increased and leads to activation of 5-HT$_{2B}$ receptors on *e.g.* fibroblasts. The pro-fibrotic effects of 5-HT and the 5-HT$_{2B}$ receptor are believed to be mediated through activation of the TGF-β/Smad signaling pathway.

The objective of the present study was to evaluate the highly selective orally available 5-HT$_{2B}$ receptor antagonist, AM1476, for its ability to reduce pulmonary and dermal fibrosis in the sclerodermatous chronic graft-versus-host disease model and dermal fibrosis in the tight-skin-1 model of systemic sclerosis.

RESULTS

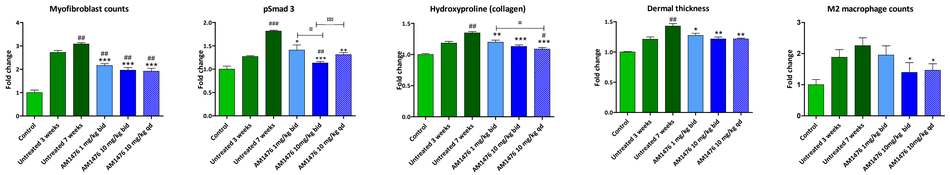
Exposure supports proper target engagement



AM1476 has an IC$_{50}$ of 11.5 nM in mouse functionality assays. Plasma concentrations of AM1476 supported 5-HT$_{2B}$ receptor engagement.

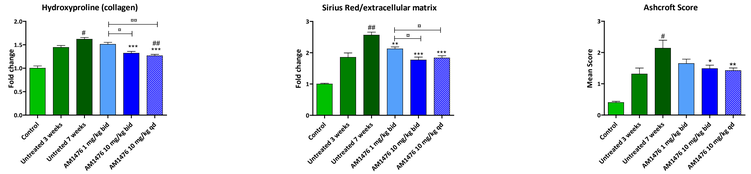
Therapeutic effects in the sclerodermatous chronic graft-versus-host disease model

Skin



Allogeneic bone marrow transfer (BMT) induced prominent skin fibrosis with increased accumulation of myofibroblasts, phosphorylated Smad3, hydroxyproline, dermal thickness and number of M2 macrophages with higher levels at 7 weeks compared to the 3 weeks control. Oral treatment with the 5-HT$_{2B}$ receptor antagonist AM1476 from week 3 to 7 resulted in reduced myofibroblast differentiation, pSmad3, hydroxyproline content, dermal thickening and M2 macrophage counts as compared to the vehicle-treated 7 weeks control. Both once and twice daily treatment resulted in significant antifibrotic effects.

Lung

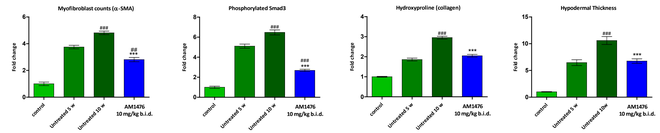


Allogeneic BMT induced moderate pulmonary fibrosis with increased hydroxyproline content, collagen-covered lung area and Ashcroft score. The changes progressed over time with significantly higher levels at 7 weeks compared to the 3 weeks control. Oral treatment with AM1476 from 3 to 7 weeks reduced hydroxyproline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

CONCLUSIONS

- Inhibition of 5-HT$_{2B}$ receptor activity resulted in pronounced anti-fibrotic effects in both pulmonary and dermal fibrotic tissues
- AM1476 reduces pSmad3 downstream the core TGF-β pathway, which motivates further evaluation of pSmad as a biomarker
- AM1476 reduces the number of critical pro-fibrotic M2 macrophages
- The highly selective 5-HT$_{2B}$ receptor antagonist AM1476 represents a promising drug candidate for treatment of fibrotic conditions and is currently in development for systemic sclerosis

Therapeutic effect in the tight-skin-1 model



Tsk-1 mice developed prominent skin fibrosis with increased myofibroblast differentiation, phosphorylated Smad3, hydroxyproline content and hypodermal thickening. The skin fibrosis progressed significantly between 5 and 10 weeks. Significant anti-fibrotic effects for all measured parameters were observed after therapeutic treatment with AM1476 at a dose of 10 mg/kg p.o., b.i.d..

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. Plasma was collected for exposure analysis at different time points the last day of experiment.
Tight-skin-1 model: Tsk-1 mice carry a heterozygous mutation in the fibrillin-1 gene and develop fibrosis spontaneously. Treatment of Tsk-1 mice with AM1476 (10 mg/kg p.o. b.i.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. Myofibroblasts were characterized and counted as fibroblasts positive for α-SMA. Sections were stained with monoclonal anti-α-SMA antibodies. Collagen protein was determined with the hydroxyproline assay using skin biopsies derived from the upper back. 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.

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