A Novel Highly Selective 5-Hydroxytryptamine 2B (5-HT_{2B}) Receptor Antagonist Ameliorating Fibrosis in Preclinical Models of Systemic Sclerosis

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INTRODUCTION

Microvascular injury is one of the first pathological events in systemic sclerosis, and precedes fibrosis. A consequence of vascular damage is the exposure of subendothelial connective tissue that causes activation of platelets and local 5-hydroxytryptamine (5-HT) release. 5-HT function as a regulator of cell proliferation, inflammation, tissue regeneration and repair. A dysfunctional 5-HT activity could consequently lead to diseases associated with tissue fibrosis and inflammation. Recent studies suggest that 5-HT_{2B} receptors have an important role in fibrotic diseases by regulating production of pro-fibrotic mediators and modifying cell differentiation and activation. The increased deposition of extracellular matrix is believed to rely on the activation and differentiation of myofibroblasts. The 5-HT_{2B} receptor has also been suggested to activate the TGF-β/Smad signalling pathway, a pathway of key importance in fibrosis.

In this study a novel highly selective 5-HT_{2B} receptor antagonist, AM1125, was evaluated for its ability to reduce the production of matrix proteins in human dermal fibroblasts and to ameliorate fibrosis in the tight-skin-1 model of systemic sclerosis. The influence of AM1125 on TGF-β signalling pathways was also investigated.

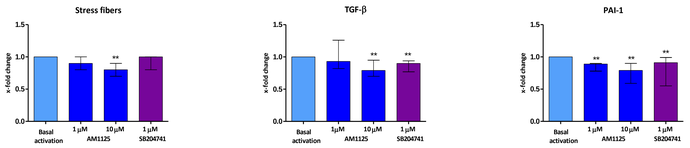
Binding (Ki, nM)			Functionality (IC_{50}, nM)		
$5\text{-HT}_{2A}R$	$5\text{-HT}_{2B}R$	$5\text{-HT}_{2C}R$	$5\text{-HT}_{2A}R$	$5\text{-HT}_{2B}R$	$5\text{-HT}_{2C}R$
310	0.87	53	5080	2.4	1230

AM1125 is a highly selective 5-HT_{2B} receptor ligand and antagonist with no 5-HT_{2B} receptor agonism.

RESULTS

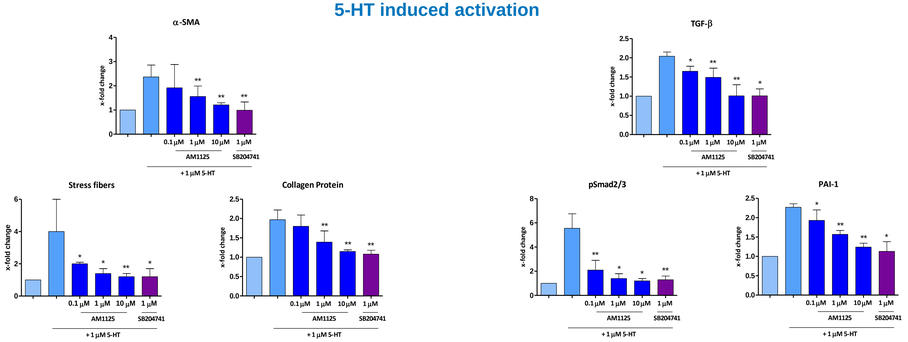
Human dermal fibroblasts

Basal activation



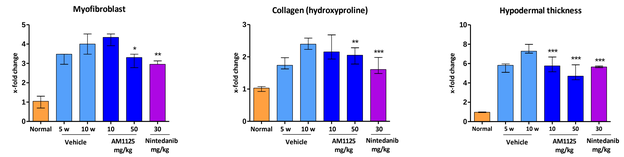
The 5-HT_{2B} receptor antagonist, AM1125, reduced basal activation in dermal fibroblasts isolated from patients with systemic sclerosis. A significantly reduced formation of stress fibers was observed as well a as decreased TGF-β and PAI-1 mRNA production. The effects were comparable to the control SB204741.

5-HT induced activation



5-HT induced the production of several pro-fibrotic markers in human dermal fibroblasts isolated from systemic sclerosis patients. The 5-HT_{2B} receptor antagonist AM1125 inhibited the stimulatory effects of 5-HT on dermal fibroblasts. This was shown as a significant reduction of myofibroblast markers and a decreased collagen release. In addition, a decrease in TGF-β and PAI-1 mRNA production as well as Smad2/3 phosphorylation was observed. The effects were comparable to the control SB204741.

Therapeutic effect 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 the age of five and ten weeks. Significant anti-fibrotic effects were observed after therapeutic treatment with AM1125 at a dose of 50 mg/kg p.o., b.i.d. for all measured parameters. At the lower dose, 10 mg/kg p.o. b.i.d., significant effect was observed for reduction of hypodermal thickness. AM1125 was well tolerated without obvious signs of toxicity. Significant anti-fibrotic effects were observed for the control nintedanib.

CONCLUSIONS

- The 5-HT_{2B} receptor antagonist AM1125 reduces TGF-β expression as well as phosphorylation of Smad2/3 and PAI-1 mRNA production suggesting that 5-HT_{2B} receptor mediated effects are TGF-β dependent
- AM1125 reduces the number of myofibroblasts *in vitro* and *in vivo*
- AM1125 reduces collagen production *in vitro* and *in vivo*
- Therapeutic treatment with AM1125 ameliorates dermal fibrosis in the tight-skin model of fibrosis
- Selective, high affinity 5-HT_{2B} receptor antagonists represent a novel, innovative mechanism for the treatment of systemic sclerosis

METHOD

In vitro, dermal fibroblasts isolated from biopsies from SSc patients were treated with the 5-HT_{2B} receptor antagonist, AM1125 at 0.1, 1 or 10 µM with or without 1 µM 5-HT. After 24 hours incubation the effects were analyzed on RNA or protein level. Total RNA was isolated and RTqPCR was used to quantify the genes for α-SMA, TGF-β and PAI-1. Dermal fibroblasts were fixed and stained with rhodamine-conjugated phalloidin to visualize actin stress fibers. Phosporylated Smad2/3 were detected with antibodies against pSmad2/3 (Acris Antibodies). Total soluble collagen in cell culture supernatants was quantified using SirCol collagen assay (Biocolor). SB204741 was used as a control.The colorimetric MTT assay was used to analyze cell viability. All data are presented as median± range and differences between groups were tested for statistical significance by Mann-Whitney U non-parametric test. All results are expressed as fold changes, normalized to the DMSO control (set as 1-fold), * P<0.05, **P<0.01, ***P<0.001.

In vivo: Tsk-1 mice carry a heterozygous mutation in the fibrillin-1 gene. Treatment of Tsk-1 mice with AM1125 (50 and 10 mg/kg p.o. b.i.d.) and nintedanib (30 mg/kg, p.o. b.i.d.) started at an age of 5 weeks 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 mono-clonal anti-αSMA antibodies (clone1A4, Sigma-Aldrich). Collagen protein was determined with the hydroxyproline assay using skin biopsies derived from the upper back. All *in vivo* data are presented as median±interquartile range. 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.

Disclosure: CW, LP, HA and GE are all AnaMar AB employees