A novel highly selective 5-HT$_{2B}$ receptor antagonist reduces myofibroblast differentiation and extracellular matrix deposition in models of lung fibrosis

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

INTRODUCTION

Pulmonary fibrosis is a chronic, fatal disease characterized by an excessive accumulation and remodeling of connective tissue which is considered to be the result of an imbalanced wound healing process. The fibrotic tissue disrupts the physiological tissue structure, leading to organ dysfunction and contributes to morbidity and increased mortality of affected patients. The increased deposition of extracellular matrix is thought to rely on the activation and differentiation of myofibroblasts.

Serotonin (5-HT) is known to be associated with fibrosis and recent studies support that 5-HT$_{2B}$ receptors have an important role in fibrotic disease by regulating production of pro-fibrotic mediators and modifying cell differentiation and activation.

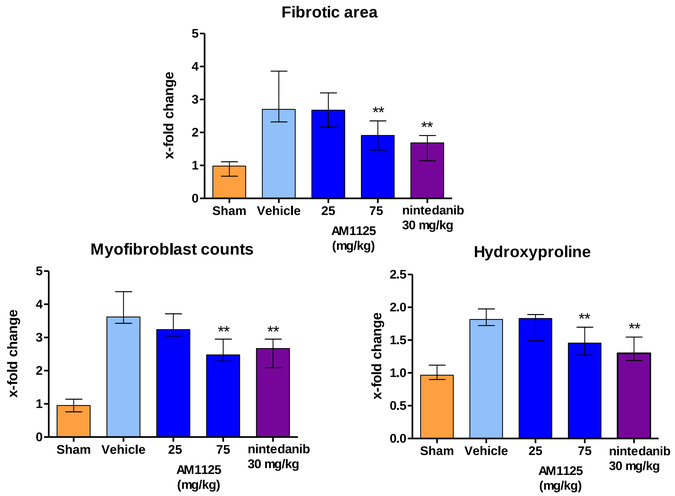
In this study a novel 5-HT$_{2B}$ receptor antagonist, AM1125, was evaluated for its ability to reduce the production of collagen in human lung fibroblasts and attenuate lung fibrosis in the bleomycin-induced lung fibrosis model.

Binding (Ki, nM)			Functionality (IC_{50}, nM)		
5-HT$_{2A}$R	5-HT$_{2B}$R	5-HT$_{2C}$R	5-HT$_{2A}$R	5-HT$_{2B}$R	5-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.

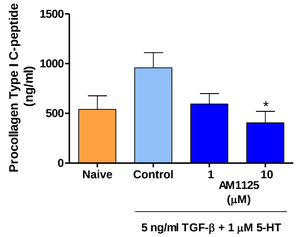
RESULTS

Bleomycin-induced lung fibrosis model



Instillation with bleomycin induced prominent fibrosis with a 2.7 fold increase in fibrotic area, 3.8 fold increase in number of myofibroblasts and 1.8 fold increase in hydroxyproline content compared to non-fibrotic control mice. AM1125 in doses of 75 mg/kg p.o. bid significantly reduced fibrotic area, number of myofibroblasts and hydroxyproline content compared to vehicle-treated, bleomycin-challenged mice. No significant effects were observed with AM1125 in doses of 25 mg/kg p.o. bid. AM1125 was well tolerated without obvious signs of toxicity. Significant anti-fibrotic effects were observed for the control, nintedanib.

Human lung fibroblasts



The 5-HT$_{2B}$ receptor antagonist, AM1125, reduces TGF-β and 5-HT induced collagen production in human lung fibroblasts

CONCLUSIONS

- The 5-HT$_{2B}$ receptor antagonist AM1125 reduces collagen production in human lung fibroblasts
- Preventive treatment with AM1125 significantly reduced fibrosis in the mouse model of bleomycin-induced lung fibrosis
- 5-HT$_{2B}$ receptor antagonism results in significant effects on myofibroblast counts, collagen production and relative fibrotic area *in vivo*
- Selective, high affinity 5-HT$_{2B}$ receptor antagonists represent a novel, innovative mechanism for the treatment of pulmonary fibrosis

METHOD

In vitro, normal human lung fibroblasts were treated with AM1125 at 1 or 10 µM. After a few minutes the cells were stimulated with TGF-β1 in combination with 5-HT. 48 hours post TGF-β1/5-HT stimulation, the effect on collagen synthesis was evaluated by measuring pro-collagen type 1 peptide (PIP) using a standard ELISA kit (Takara Bio #MK1010). Statistical calculations were made by one-way ANOVA with Dunnett's multiple comparisons test, *P<0.05.

In vivo, single intratracheal injection of bleomycin was employed to induce lung fibrosis in eight week old, female C57Bl/6 mice. Oral twice daily treatment (AM1125: 25 and 75 mg/kg; nintedanib: 30 mg/kg) was started at the day of bleomycin instillation and continued throughout the study (28 days). Nintedanib served as a positive control. The whole right lung was extracted, fixed, embedded in paraffin. 3 µm sections were stained with Sirius Red to identify fibrotic areas. The relative fibrotic area, calculated as % of total lung area, was determined using ImageJ software. Myofibroblasts were detected by incubation with mono-clonal anti-α-SMA antibodies. Amount of collagen protein was determined by the hydroxyproline assay. Data are presented as median± interquartile range, difference between groups were tested by Mann-Whitney U non-parametric test for non-related samples, **P<0.01.