**BACKGROUND**

Acidic mammalian chitinase (AMCase) and chitotriosidase (CHIT1) are the enzymatically active chitinas, which have been implicated in the pathology of diverse lung diseases. While the mechanisms through which chitinases promote lung inflammation and airway remodeling have not been fully elucidated, several studies have demonstrated that chitinases mediate both the pro-inflammatory and the pro-fibrotic responses in the lungs. AMCase expression is upregulated in tissue macrophages and epithelial cells in lungs of asthma patients and CHIT1 activity is elevated in the bronchoalveolar lavage fluid (BALF) from patients with the interstitial lung diseases. To assess effects of inhibition of chitinases on inflammation and lung remodeling, we evaluated activity of a dual AMCase/CHIT1 inhibitor in 3- and 7-week-long HDM-induced airway inflammation mouse models.

**RESULTS**

**OAT-889**

OAT-889 is a potent, dual AMCase and CHIT1 small-molecule inhibitor.

**PHARMACOKINETICS IN MICE**

OAT-889 has a favorable pharmacokinetic profile in mice.

**OAT-889 administered qd in therapeutic scheme of treatment reduced the total number of infiltrating leukocytes in BALF in a dose-dependent manner.**

**CONCLUSIONS**

OAT-889 demonstrated a therapeutic efficacy in the mouse models of allergic airway inflammation. Inhibition of chitinases led to a strong anti-inflammatory activity associated with reduced total BAL cell count, decreased cytokine expression, and IgE concentrations as well as anti-remodeling effects determined with morphometric analysis of collagen deposition around bronchioles and airway wall thickness. These data provide a rationale for developing a dual AMCase/CHIT1 inhibitor as a first-in-class oral therapy for asthma with the potential to reduce both airway inflammation and remodeling.

**MATERIALS AND METHODS**

**ENZYMATIC ASSAYS**

For determination of chitinolytic activity of recombinant enzymes (AMCase and CHIT1) 4-methylumbelliferyl β-D-N-acetylglucosaminide (4-MUG) and 4-methylumbelliferyl β-D-N-acetylglucosaminide (4-MU) was incubated with 0.2 mg/mL of enzyme for 10 min at 37°C in distilled water. Enzyme activity was measured fluorometrically.

**PHARMACOCHEMICALS**

The pharmacokinetic properties of OAT-889 were evaluated in female BALB/c mice following single intranasal bolus or oral administration in a 20% glucose solution in distilled water vehicle (3 mice/group/timepoint). Samples were stored frozen at -80°C prior to compound extraction and LC/MS analysis.

**THREE-WEEK-LONG HDM-INDUCED AIRWAY INFLAMMATION MODEL IN MICE**

Five groups of female C57BL/6 mice were exposed to intranasal HDM extract (40 μg in PBS) 5 times per week for 19 days. Control mice were intranasally challenged with PBS at times of HDM challenges (n=8). OAT-889 was administered orally at doses of 10, 30 and 100 mg/kg qd (vehicle: 20% glucose in distilled water) starting from day 7 onwards. A ntiguine was administered by intraperitoneal injection at a dose of 10 μg/kg/day from day 7 onwards similarly to OAT-889.

**CHRONIC 7-WEEK-LONG HDM-INDUCED AIRWAY INFLAMMATION MODEL IN MICE**

Four groups of female C57BL/6 mice were subjected to intranasal exposure of HDM (40 μg in PBS) 5 times per week for 7 weeks. Control mice were intranasally challenged with PBS at times of HDM challenges (n=8). OAT-889 was administered orally once a day at doses of 3 and 30 mg/kg (20% glucose in distilled water) starting from week 5 onwards. A ntiguine was administered by intraperitoneal injection at a dose of 10 μg/kg/day from week 5 onwards similarly to OAT-889.

**BIOCHEMICAL ASSAYS**

BALB/c mice were analyzed and counted with flow cytometry using appropriate antibodies. ELISA tests (IgE) assays were performed according to manufacturer's protocols. Chitinolytic activity measurements in BAL fluid and plasma were performed in pH 3 (for AMCase activity) and in pH 6 (for both AMCase and CHIT1 activity) using 4- methylumbelliferyl β-D-N-acetylglucosaminide substrate similar to assay with recombinant enzymes. For gene expression analysis gene specific TaqMan Assays and TaqMan Gene Expression Master Mixes (Applied Biosystems) were used. For assessment of collagen deposition and goblet cell hyperplasia, lungs were fixed, embedded in paraffin and sectioned followed by histochemical staining Masson's trichrome or PAM stains, respectively.