Dual AMCase/CHIT1 Inhibitor OAT-889 Reverses Pulmonary Inflammation and Airway Remodeling in Two Mice Models of Airway Inflammation

B. Dymek[1], M. Mlącki[1], A.M. Zdziarska[1], M. Mazurkiewicz[1], M. Salamon[1], R. Koralewski[1], A. Gołębiowski[1], J. Golab[1,2], K. Dzwonek[1], P. Sklepkiewicz[1]

[1]OncoArendi Therapeutics SA, Warsaw, Poland; [2]Department of Immunology, Medical University of Warsaw, Poland

Background

Acidic mammalian chitinase (AMCase) and chitotriosidase (CHIT1) are the enzymatically active chitinases, 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

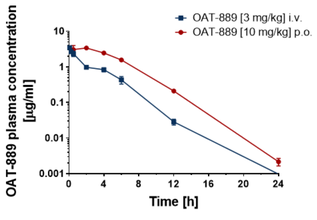
In Vitro Activity

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

	OAT-889 IC50 [nM]
hAMCase	9
hCHIT1	26
mAMCase	8
mCHIT1	29

Pharmacokinetics in Mice

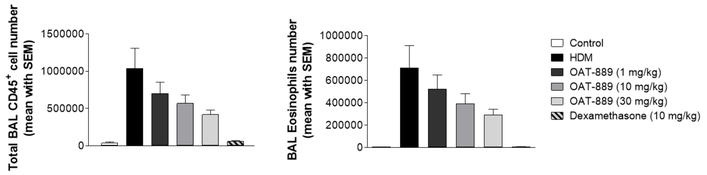
OAT-889 has a favorable pharmacokinetic profile in mice.



OAT-889 Pharmacokinetic Parameters		
Route	IV	PO
Dose (mg/kg)	3	10
$AUC_{0\text{-}inf}$(mg*h/L)	8,60	22,2
AUC,n0-inf(kg*h/L)	2,866	2,22
C0 or Cmax(mg/L)	3,969	3,42
Tmax(h)	n/a	2,0
CL (mL/min/kg)	5,8	n/a
Vss (L/kg)	1,01	n/a
T½ (h)	2,09	1,88
Bioavailability (F%)	n/a	77%

3-week-long HDM-Induced Airway Inflammation Model

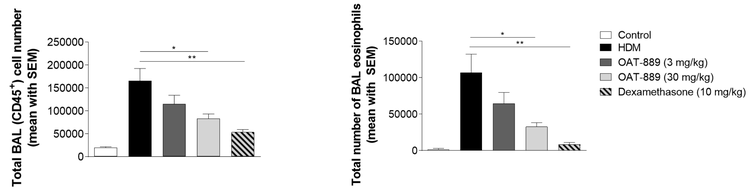
OAT-889 administered qd in therapeutic scheme of treatment reduced the total number of infiltrating leukocytes in BALF in a dose-dependent manner.



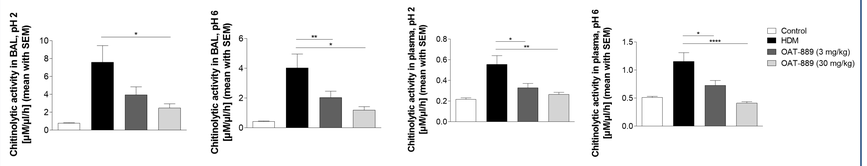
Results

Chronic HDM-Induced Airway Inflammation and Remodeling Model

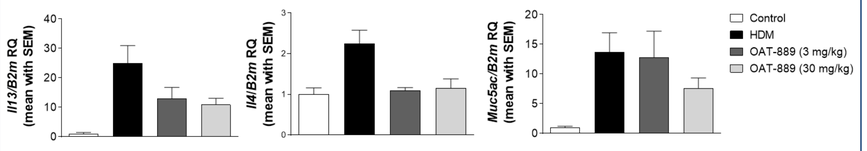
In 7-week-long HDM-induced airway inflammation model OAT-889 administered qd in a therapeutic regiment significantly reduced the total number of leukocytes and eosinophils in BALF.



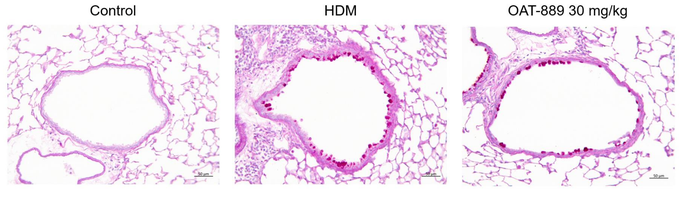
Anti-inflammatory activity of OAT-889 correlated with a significantly reduced chitinolytic activity in BALF and serum.



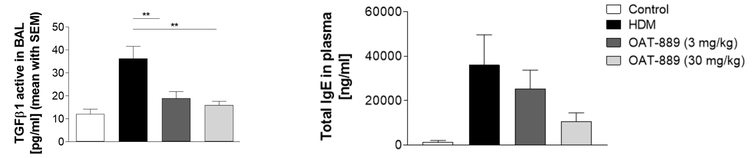
OAT-889 suppressed expression of genes coding inflammatory cytokines IL-13 and IL-4 and mucin MUC5AC in the lungs as assessed with real-time PCR.



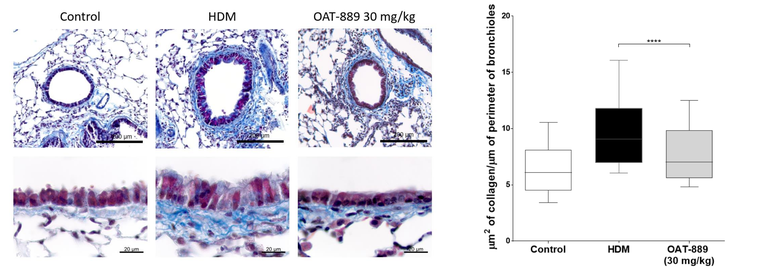
OAT-889 reduced the goblet cell hyperplasia as assessed with periodic acid-Shiff (PAS) staining of mucins in mouse lung tissue.



The significant decrease of active TGFβ1 in BAL was observed in OAT-889-treated animals. OAT-889 reduced the serum IgE concentration in a dose-dependent manner indicating reduction of allergic response.



OAT-889 exhibited significant anti-remodeling activity: decreased airway wall thickness and reduced collagen deposition around bronchioles.



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,N'diacetylchitobioside hydrate or 4-methylumbelliferyl β-D-N,N',N''triacetylchitotrioside substrate, enzyme and varying concentrations of compound in citrate assay buffer pH 5.2 were incubated at 37ºC for 60 minutes. Substrate hydrolysis product - 4-methylumbelliferone was measured fluorometrically.

Pharmacokinetics

The pharmacokinetic properties of OAT-889 were evaluated in female BALB/c mice following single intravenous bolus or oral administration in a 20:80 solutol:5% glucose 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 (n=8) of age-matched 8-week-old 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 3, 10 and 30 mg/kg qd (vehicle: 20:80 solutol:5% glucose in distilled water) starting from day 7 onwards. Dexamethasone was administered by intraperitoneal injection at a dose of 10 mg/kg from day 7 onwards similarly to OAT-889.

Chronic 7-week-long HDM-induced Airway Inflammation Model in Mice

Four groups (n=8) of age-matched 8-week-old 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 a doses of 3 and 30 mg/kg (20:80 solutol:5% glucose in distilled water) starting from week 5 onwards. Dexamethasone was administered by intraperitoneal injection at a dose of 10 mg/kg from week 5 onwards similarly to OAT-889.

Biochemical Assays

BAL cells were analyzed and counted with flow cytometry using appropriate antibodies. ELISA tests (eBioscience) were performed according to manufacturer's protocols. Chitinolytic activity measurements in BAL fluid and plasma were performed in pH 2 (for AMCase activity) and in pH 6 (for both AMCase and CHIT1 activity) using 4-methylumbelliferyl β-D-N,N'diacetylchitobioside hydrate substate similar to assay with recombinant enzymes. For gene expression analysis gene specific TaqMan Assays and TaqMan Gene Expression Master Mix (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 PAS stains, respectively.

Financial Support

„Selective inhibitors of acidic mammalian chitinase (AMCase) as potential therapy for asthma"

„Preclinical research and clinical trials of a first-in-class development candidate in therapy of asthma and inflammatory bowel disease"