The Therapeutic Efficacy of OAT-889 (Dual AMCase/CHIT1 Inhibitor) in Comparison to Montelukast in HDM-induced Model of Chronic Airway Inflammation in Mice

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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 and to compare it directly with montelukast – first-line oral therapy in asthma, we evaluated activity of a dual AMCase/CHIT1 inhibitor OAT-889 in a 7-week-long HDM-induced airway inflammation mouse models.

Results

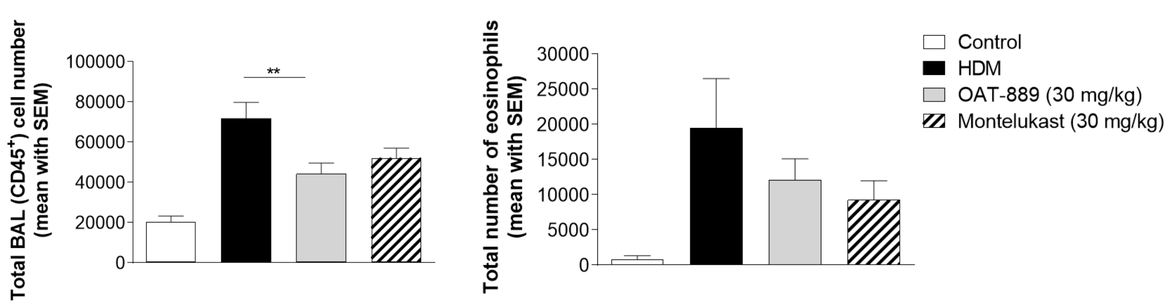
In Vitro Activity

OAT-889 is a highly potent dual AMCase and CHIT1 small molecule inhibitor with a nanomolar activity.

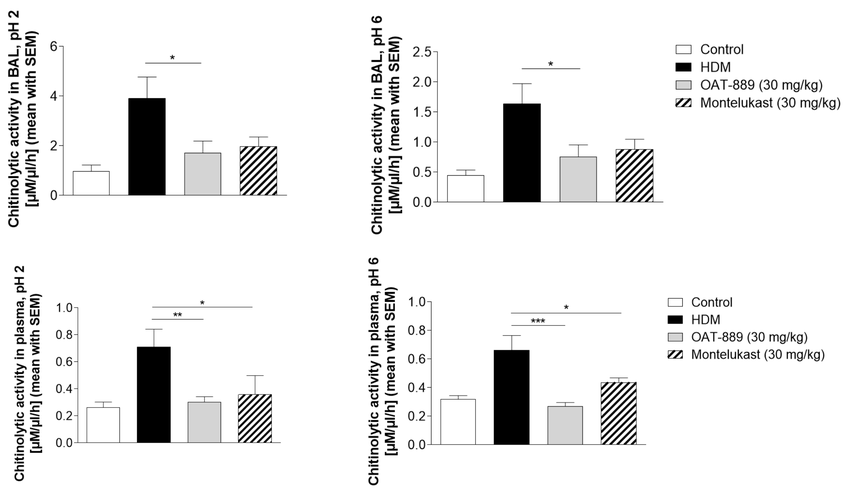
	OAT-889 IC_{50} [nM]
hAMCase	9
hCHIT1	26
mAMCase	8
mCHIT1	29

Chronic HDM-Induced Airway Inflammation and Remodeling Model

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



The anti-inflammatory effects of OAT-889 and montelukast correlated with a significant reduction of chitinolytic activity in BALF and plasma.



References

1. Zhu Z, Zheng T, Homer RJ, Kim YK, Chen NY, Cohn L, Hamid Q, Elias JA. Acidic mammalian chitinase in asthmatic Th2 inflammation and IL-13 pathway activation. *Science*, 2004
2. Boot RG, Hollak CE, Verhoek M, Alberts C, Jonkers RE, Aerts JM. Plasma chitotriosidase and CCL18 as surrogate markers for granulomatous macrophages in sarcoidosis. *Clin Chim Acta,* 2010

Acknowledgments

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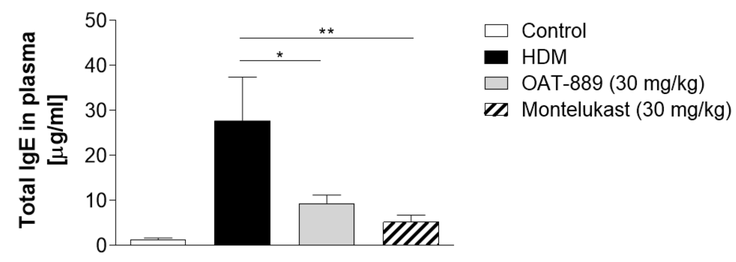
„Selective inhibitors of acidic mammalian chitinase (AMCase) as potential therapy for asthma"

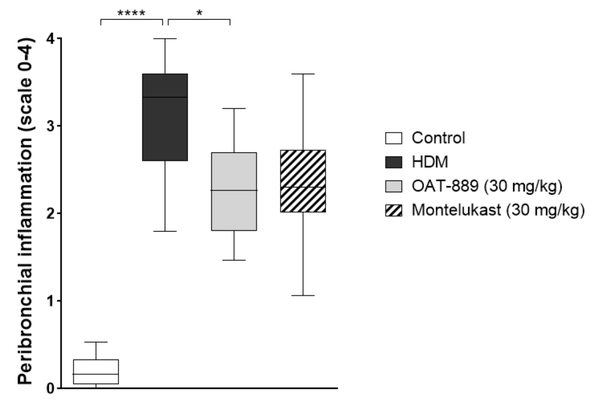
„Preclinical research and clinical trials of a first-in-class development candidate in therapy of asthma and inflammatory bowel disease"

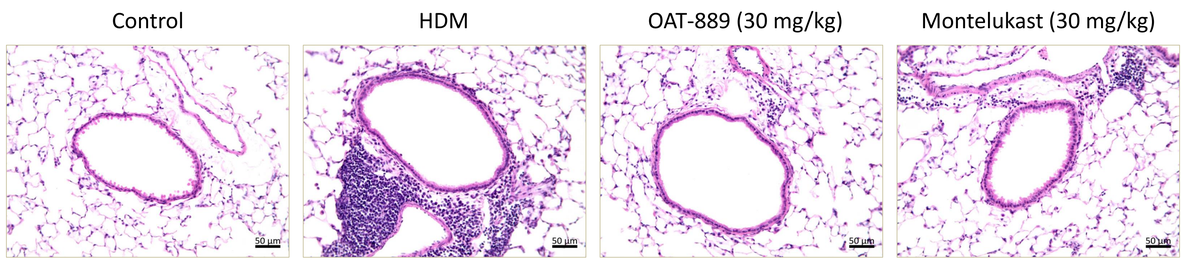
Results

Additionally, OAT-889 and montelukast reduced serum IgE concentration to a comparable level indicating suppression of the allergic response.

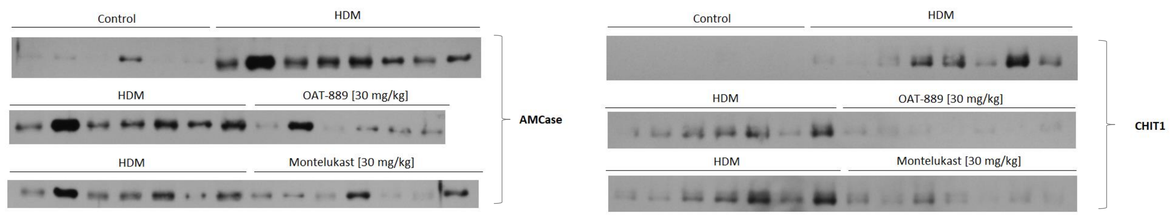


The anti-inflammatory effects of OAT-889 were further confirmed by histological assessment of peribronchial inflammation assessed using a previously published inflammation score.





The reduction of chitinolytic activity in BALF correlated with a significant decrease in total levels of both chitinases in BALF suggesting a feedback loop mechanism which may regulate expression of chitinases in the lungs during inflammation.



Conclusions

OAT-889 demonstrated a profound anti-inflammatory activity in the chronic asthma model in mice as demonstrated by a reduction of the total cell number in BALF and IgE concentration in plasma. These effects were further confirmed by a histopathogical assessment of peribronchial inflammation in the lung tissue. These results provide a rationale for developing a dual AMCase/CHIT1 inhibitor for the treatment of asthma.

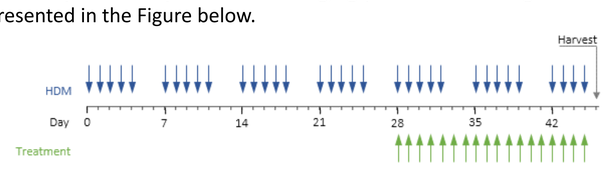
Materials and Methods

Enzymatic Assays

For determination of inhibitory activity of the compound, the 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 the inhibitor in citrate assay buffer pH 5.2 were incubated at 37°C for 60 minutes. Substrate hydrolysis product - 4-methylumbelliferone was measured fluorometrically.

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 exposed to intranasal 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 and montelukast (Biorbyt) were administered orally once a day at dose of 30 mg/kg (20:80 solutol:5% glucose in distilled water) starting from week 5 onwards. The scheme is presented in the Figure below.



Biochemical Assays And Histology

BAL cells were analyzed and counted by flow cytometry using appropriate antibodies. IgE ELISA test (eBioscience) was 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 substrate similar to assay with recombinant enzymes. For immunoblot detection of AMCase and CHIT1 proteins in BALF antibodies from Abcam and R&D Systems, respectively, were used according to manufacturers' protocols. For assessment of a peribronchial inflammation, lungs were fixed, embedded in paraffin, sectioned and stained with hematoxylin and eosin. Scoring was performed blindly by professional pathologist based on a published scale 0-4 (doi: 10.1371/journal.pone.0085839). For each animal, 3 sections were prepared and 5 bronchioles of a similar size per section were analyzed.