Pharmacological profile and efficacy of GLPG1690, a novel ATX inhibitor for COPD treatment

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Introduction
Autotaxin (ATX), a secreted lysophospholipase, plays a central role in the production of the bioactive lysophosphatidic acid (LPA). These LPA species interact with multiple LPA receptors to control a range of cell activities (migration, contraction, survival...). Recently, a role for the ATX/LPA axis in asthma and fibrosis was reported, suggesting involvement in additional lung diseases.

Objective
This study aimed to characterize the pharmacological profile of GLPG1690, a novel ATX inhibitor, as well as its efficacy in pulmonary lung inflammation induced by tobacco smoke exposure in mice.

Methods
In vitro LPA assay
Cleavage of LPC by recombinant ENPP2 releases LPA which was quantified via an enzymatic method using choline oxidase and peroxidase.

LPA ex vivo plasma assay
GLPG1690 was incubated with plasma at 37°C, 5% CO2, for 2 hours. At the end of the incubation, LPA 18:2 was quantified by LC-MS/MS.

In vivo

Mice C57Bl/6 were exposed to tobacco smoke (TS) in a chamber on a daily basis, with exposure up to 45 minutes per day for 11 days. GLPG1690 (5 or 10 mg/kg) or dexmethasone (Dex, 0.3 mg/kg) were dosed orally (p.o.) on days 8-11, twice a day (b.i.d.), 3h before and 6h after each daily exposure to TS. Roflumilast (Ref, 5 mg/kg) was dosed, p.o. on days 6-11, once daily (q.d.). Mice were sacrificed 24h after the final air or TS exposure and bronchoalveolar lavage (BAL) was performed. Cells recovered from BAL were used for total cell counts using cytosin prepared slides. At day 10, LPA18:2 and GLPG1690 were quantified in plasma by LC-MS/MS for the PK/PD relationship.

Immunohistochemistry
4 μm-thick paraffin sections from TS and control mouse lung were immunostained with anti-autotaxin antibody (Abnova, PA8-520) and detected with anti-rabbit antibody Alexa 488 conjugated. DAPI (Vector) was used for nuclear counterstaining.

Gene expression
RNA extracted from mouse right superior lung lobes were analysed by micro-arrays using Agilent’s custom-designed Gene Expression 8x60 K Sureprint G3 arrays (Agilent).

Calculation and statistical analysis
Data are expressed as mean ± s.e.m. Comparisons among the groups were made using one-way analysis of variance (ANOVA) and Dunnett’s Multiple Comparison Test (StatView software).

Results


Conclusion
GLPG1690, a potent and selective ATX inhibitor, dose-dependently reduced LPA both ex vivo and in vivo. GLPG1690 displayed high efficacy in a preclinical model for COPD, with pronounced effect on the BALF cell count and lung gene expression profile, pointing to a novel therapeutic indication for autotaxin inhibitor.

In vitro pharmacological profile

<table>
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<tr>
<th>Source</th>
<th>IC50 value (nM)</th>
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<tbody>
<tr>
<td>mATX</td>
<td>224</td>
</tr>
<tr>
<td>hATX</td>
<td>131</td>
</tr>
<tr>
<td>K-NATX (competitive)</td>
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Ex vivo plasma assay

LPA18:2

<table>
<thead>
<tr>
<th>Species</th>
<th>IC50 value (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>242</td>
</tr>
<tr>
<td>Mouse</td>
<td>417</td>
</tr>
</tbody>
</table>

Tobacco smoke mouse model

Cell count in BALF

PK/PD in tobacco smoke model

Lung paraffin

Immunohistochemistry in lung at day 11

X400 microscope magnification

Fig. 1. GLPG1690 was shown to be a potent competitive inhibitor of mouse and human ATX in a biochemical assay (IC50 values of 242 and 131 nM, respectively and a Kd of 15 nM). Similar potency was observed ex vivo in the human and mouse LPA18:2 plasma assay. In the human ex vivo plasma assay, GLPG1690 reduced several LPA species with similar potency.

Fig. 2. In a curative tobacco smoke mouse model, GLPG1690 dosed at 5 and 10 mg/kg b.i.d. p.o. reduced significantly the increase of cell numbers in BALF induced by TS exposure (47% and 51%, respectively). Roflumilast at 5 mg/kg p.o. did not display similar inhibition as GLPG1690 (47%). In contrast, dexmethasone at 0.3 mg/kg did not inhibit the total cell numbers in BALF showing the steroid resistance of the model.

Fig. 3. In GLPG1690-treated mice, an inverse relationship was shown between LPA18:2 and GLPG1690 plasma levels reflecting in vivo target engagement.

Fig. 4. Autotaxin expression in lung from sham and TS exposed mice (immunostained in green). After 11 days of TS exposure, a marked increased of ATX expression was observed in the cytoplasm of bronchial epithelial cells (DAPI counterstaining in blue in the window).

Fig. 5. Heat map of top 50 genes list regulated by tobacco smoke exposure, GLPG1690 dosed at 10 mg/kg and Roflumilast in lung. Gene expression analysis displayed a strong modification of the gene profile by TS exposure, which is partially reversed with GLPG1690 at 10 mg/kg and, with a less pronounced impact, by Roflumilast.

References