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

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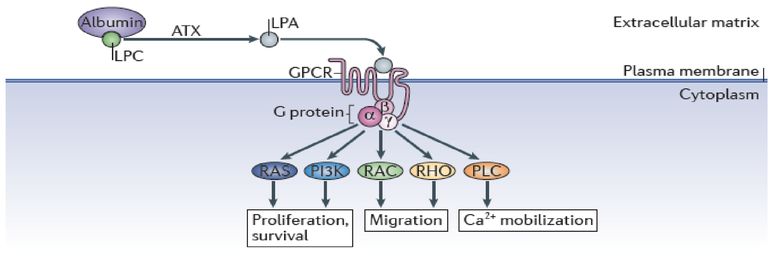
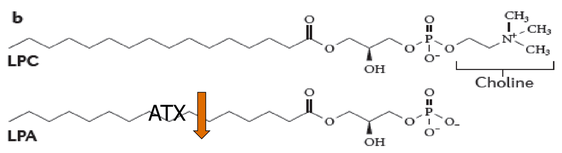
Introduction

Autotaxin (ATX), a secreted lysophospholipase, plays a central role in the production of the bioactive lipid lysophosphatidic acid (LPA). LPA signals through 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 were 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 mouse.

Methods

In vitro LPA assay
Cleavage of LPC by recombinant ENPP2 releases choline 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 dexamethasone (Dex, 0.3 mg/kg) were dosed orally (p.o.) on days 6-11, twice a day (b.i.d.), 1h before and 6h after each daily exposure to TS. Roflumilast (Rof, 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 cytospin 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, PAB-8520) 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 lobs were analysed by micro-arrays using 8*60 K Sureprint G3 arrays (Agilent).

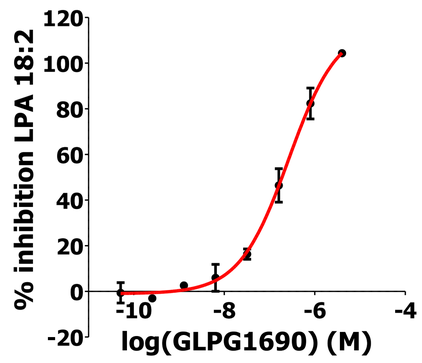
Calculation and statistical analysis
Data are expressed as mean ± sem. Comparisons among the groups were made using one-way analysis of variance (ANOVA) and Dunnett's Multiple Comparison Test (StatView software). *p<0.05, **p<0.01, ***p<0.0001 vs 'TS Vehicle' group.

References

1. B. Heckmann, R. Blanqué, N. Desroy, S. Dupont, C. Cottereaux, A. Monjardet, D. Dirven, T. Christophe, B. Hrvacic, J.Ralic, F. Marsais, E. van der Aar and R. Brys Pharmacological profile and efficacy of GLPG1690, a novel autotaxin (ATX) inhibitor for IPF treatment .*FASEB Science Research Conference , 2015*
2. E. van der Aar, L. Fagard, J. Desrivot, S. Dupont, B.Heckmann, R. Blanqué, L. Gheyle, J. Ralic and F. Vanhoutte. Favorable human safety, pharmacokinetics and pharmacodynamics of the autotaxin inhibitor GLPG1690, a potential new treatment in COPD. *ERS International Congress, 2015*

In vitro pharmacological profile

	Source	IC_{50} value (nM)
Biochemical assay	mATX	224
	hATX	131
	K_i hATX (competitive)	14.7
***Ex vivo* plasma assay LPA18:2**	Human	242
	Mouse	417



LPA *ex vivo* human plasma assay

LPA species	GLPG1690 IC_{50} value (nM)
C14:0	96
C16:0	117
C18:1	115
C18:2	112
C18:3	102
C20:4	93
C22:6	94

Fig. 1. GLPG1690 was shown to be a potent competitive inhibitor of mouse and human ATX in a biochemical assay (IC_{50} values of 224 and 131 nM, respectively and a K_i 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.

Tobacco smoke mouse model

Cell count in BALF

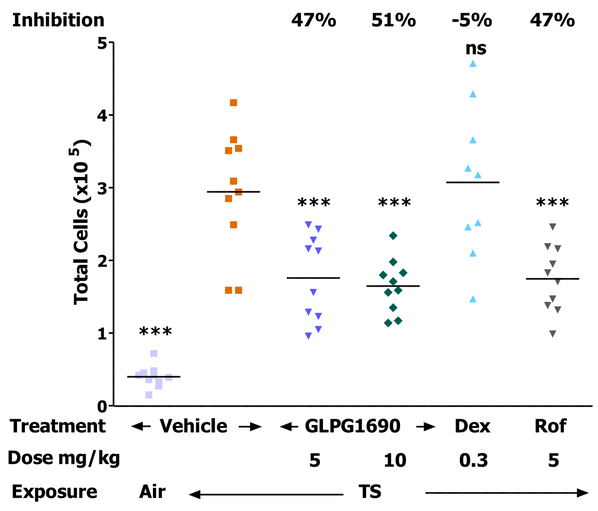


Fig. 2. In a curative tobacco smoke mouse model, GLPG1690 dosed at 5 and 10 mg/kg b.i.d. p.o. reduced significantly the increase of cell numbers in BALF induced by TS exposure (47% and 51%, respectively). Roflumilast at 5 mg/kg q.d. p.o. displayed similar inhibition as GLPG1690 (47%). In contrast, dexamethasone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

PK/PD in tobacco smoke model

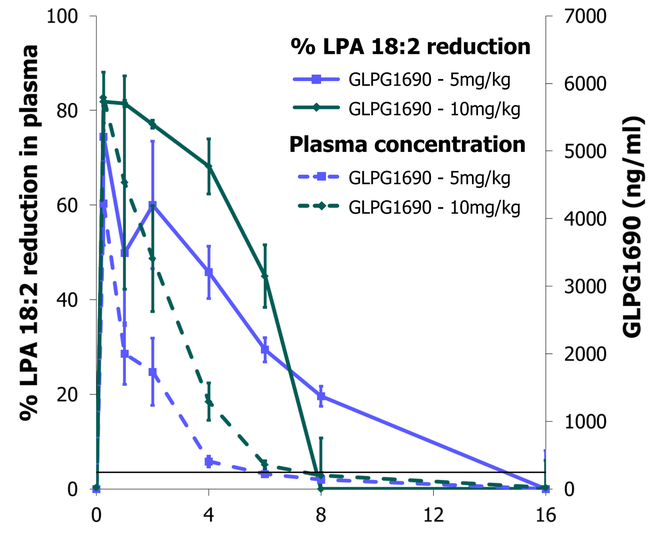


Fig. 3. In GLPG1690-treated mice, an inverse relationship was shown between LPA18:2 and GLPG1690 plasma levels reflecting *in vivo* target engagement.

Immunohistochemistry in lung at day 11

X400 microscope magnification

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).

Lung µarray

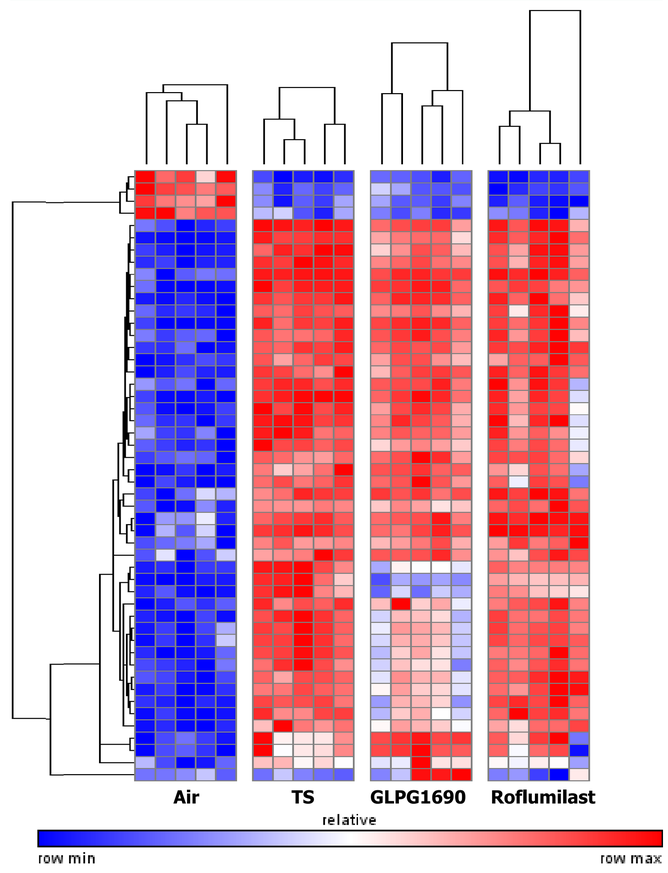


Fig. 5. Heat map of top 50 genes list regulated by tobacco smoke exposure, GLPG1690 dosed at 10 mg/kg and Roflumilast in lung. Gene expression analysis displayed a strong modification of the gene profile by TS exposure, which is partially reversed with GLPG1690 at 10 mg/kg and, with a less pronounced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

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.
GLPG1690 has shown a favorable profile for development, efficacy in a preclinical model for IPF(1) with supportive clinical Phase 1 data(2) and is moving to a exploratory Phase 2a study in IPF.

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