Exploring Plasminogen as an inhalable therapy for ARDS: preclinical insights

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INTRODUCTION

Background

Acute Respiratory Distress Syndrome (ARDS) is an acute onset of hypoxemia caused by non-cardiogenic pulmonary oedema secondary to altered alveolo-capillary permeability (1). ARDS leads to severe respiratory failure due to fibrin deposition and the formation of an obstructive hyaline membrane in the lungs, which restrains gas excange. Plasminogen (PLG) is the inactive form of plasmin and it's fundamental to break down the fibrin clots (2).

Aim of the study

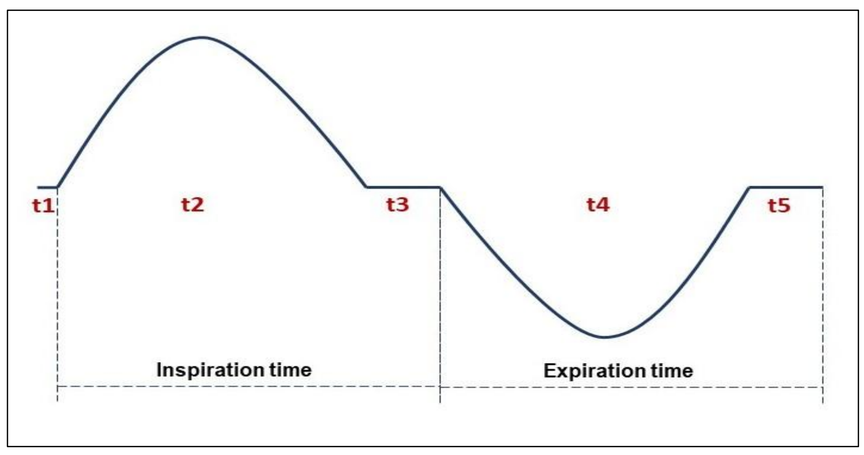
The aim of this work is to assess the profile deposition of plasminogen (PLG), as an off label application of plasminogen eye drops (Orphan Medicinal Product number EU/3/07/461), simulating the injured respiratory system, using a breathing simulator (BRS) and a next generation impactor (NGI).

MATERIALS AND METHODS

The BRS 300i COPLEY (Nottingham, UK) has been used to generate spontaneous breathing to generate a respiratory flow. In order to study the different profiles of spontaneous breathing, three models of breathing have been evaluated.

Applied parameters for simulated ARDS related breathing profiles

	Mild ARDS	*Moderate ARDS*	*Severe ARDS*
Volume	430	**400**	**350**
Rate	14	15	17
I:E	1:1	1:1:1	1:2:1
t 1	0	0	0
t 2	1.69	1.61	1.49
t 3	0.15	0.14	0.12
t 4	1.61	1.47	1.24
t 5	0.85	0.78	0.65
Breathing cycle	4.3 s	4 s	3.5 s

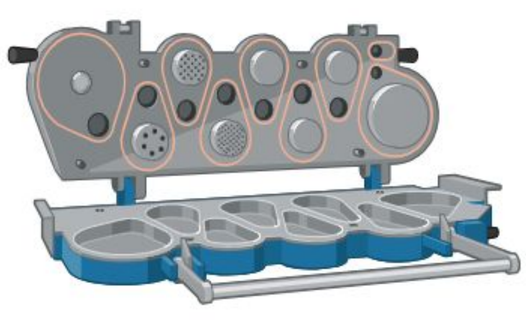


Time segments of the used breathing profiles. t1: pre-inspiratory pause; t2: inspiratory phase; t3: end-inspiratory pause; t4: expiratory phase; t5: end-expiratory pause.

Nebulisation of PLG and Next Generation Impactor (NGI)

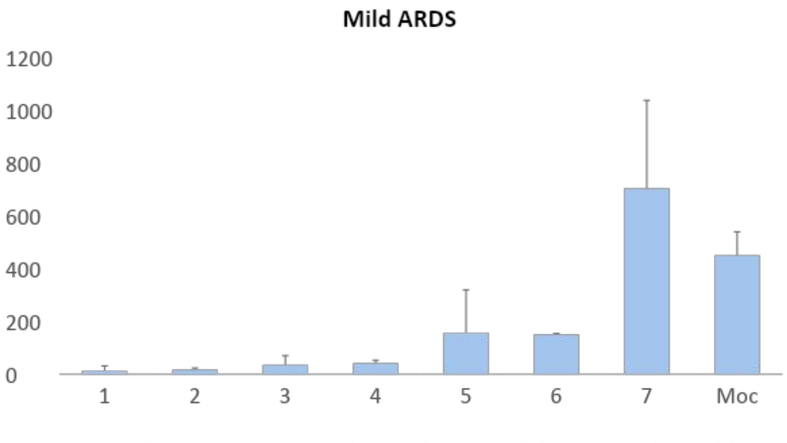
Aliquots of 3 mL of PLG-OMP were nebulized using a Aerogen® SoloTM mesh nebulizer (Galway, Ireland). Nebulization was performed in air for 5 minutes.

The NGI is used to detect the mean aereodynamic diameter and it's composed of seven stages with progressively smaller pores to 0.5 from 5 µm. The flow rate was 15 L/min according to European Pharmacopoeia.

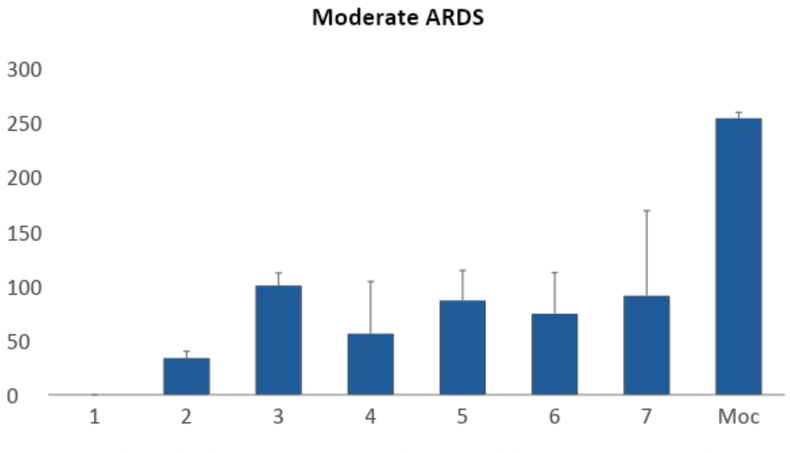


The BRS was connected to the NGI and the latter was connected to the vacuum pump in order to guarantee the aspiration

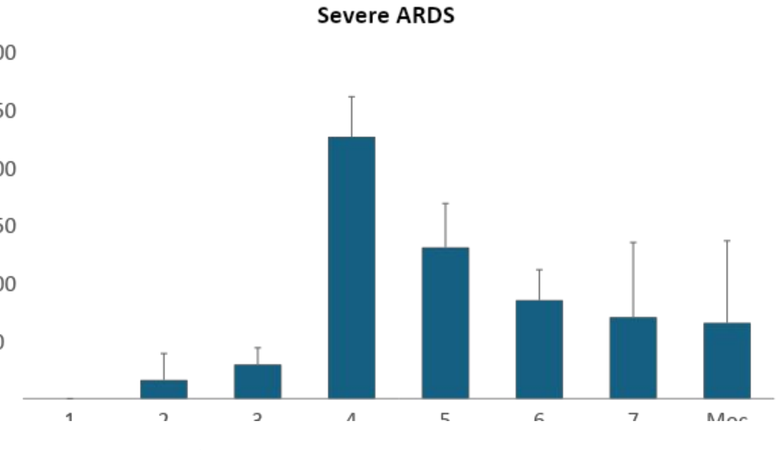
RESULTS



Amount of PLG (µg) recovered in each stage of the NGI under Mild ARDS breathing profile.



Amount of PLG (µg) recovered in each stage of the NGI under Moderate ARDS breathing profile.



Amount of PLG (µg) recovered in each stage of the NGI under Severe ARDS breathing profile.

Average results obtained with Inhalytix® software program, collected with NGI coupled with BRS

	Mild ARDS	Moderate ARDS	Severe ARDS
Dose tot µg	3156	2765	2313
Delivered dose %	61	38	32
FPF	77.9	52.1	73.9
MMAD	1.14	1.38	2.87
GDS	1.2	4.2	2.4
R^2 lenght	0.885	0.990	0.997

FPF: fine particle fraction; MMAD: mass median aerodynamic diameter; GDS: geometric standard deviation

The results show a reduction of the delivery dose, in accordance with the worsening of the disease. The values of MMAD demonstrates that PLG spreads up in the small airways and it is minimally exhaled.

CONCLUSIONS

PLG-OMP eye drops exhibit an optimal deposition profile in the *in vitro* model, confirming that PLG effectively spreads to the lower airways regardless of the different spontaneous breathing models used. An optimal aerosol deposition profile, regardless of respiratory mechanics, allows for the effective use of PLG-OMP via nebulization, maximizing its impact on the lungs while minimizing systemic effects. Further studies on the efficacy of PLG-OMP are ongoing, focusing on its ability to reduce inflammatory and fibrotic processes in an *in vivo* mouse model.

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