Pharmaceutical Profiles

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TARGETING AN INHALED ERYTHROPOIETIN-Fc FUSION PROTEIN (Epo-Fc) TO THE HUMAN LARGE CENTRAL AIRWAYS

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Introduction

The inhaled route is an attractive option for the delivery of peptides and proteins that are generally ineffective when given orally. When given by inhalation, these drugs must be delivered to the required target site within the airways. The alveolated region in the deep lung is normally considered to be the main target site for inhaled peptides and proteins. However, efficient and cost-effective delivery of drugs to the deep lung is difficult to achieve, and requires the use of highly specialized inhaler devices and / or formulations.

Syntonix have developed a novel technology for delivery of inhaled proteins, and to allow them to cross the epithelial barrier, which involves linking the protein to the Fc component of the human IgG molecule (Bitonti et al 2002). The receptors for Fc are present in the large airways of man, but are much less prevalent in the deep lung. The present study was conducted to show "Proof of Concept" for this technology using a prototype recombinant fusion protein comprising human erythropoietin and human Fc (Recombinant EpoFc, molecular weight 112000 Da), targeted to the central airways of the human lung.

Methods

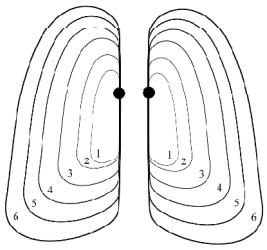
A formulation of EpoFc was radiolabelled with ^{99m}Tc-DTPA, and was administered to three groups of healthy male subjects by AeroNeb Pro nebulizer (Aerogen, Inc.), who inhaled at a targeted volume of 20 % vital capacity (VC) and at a targeted breathing rate of 15 breaths/min. An in-house custom-designed inhalation profile recorder was used to train volunteers and to monitor breathing patterns.

The doses received by the three groups were as follows:

- Group 1 (n=7): Targeted dose 3 µg/kg to central airways
- Group 2 (n=7): Targeted dose 10 µg/kg to central airways
- Group 3 (n=8): Targeted dose 30 µg/kg to central airways

In vitro tests were used to quantify the nebulizer output, and hence to estimate the nebulization time required to deliver the required dose to central airways, which comprised zones 1 to 4 inclusive in a lung model consisting of 6 concentric lung-shaped zones (Pitcairn et al 2002).

Zones 1 to 4 inclusive represent "central airways"



● = Hilum, the point at which the bronchus enters the lungs.

The scintigraphic data were analyzed in order to determine:

- % whole lung deposition
- % oropharyngeal deposition (including esophagus and stomach)
- % retention in delivery device
- Distribution of lung dose across 6 concentric lung-shaped zones

Serum EpoFc concentration was determined at time points up to 120 h post dosing by a microtiter plate ELISA procedure (Quantikine Human Epo Immunassay, R&D Systems).

Results

Dose Fractionation

The fractionation of the dose between whole lungs, oropharynx and device was similar for all three groups. Whole lung deposition averaged approximately 20 % of the nebulizer fill.

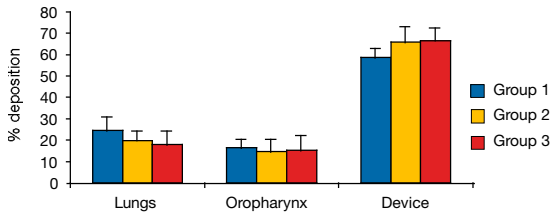


Figure 1. Mass balance: percentage of dose in whole lungs, oropharynx and device

Regional Lung Deposition

Deposition in each lung zone was highest in group 3, and lowest in group 1, and increased in proportion to targeted dose.

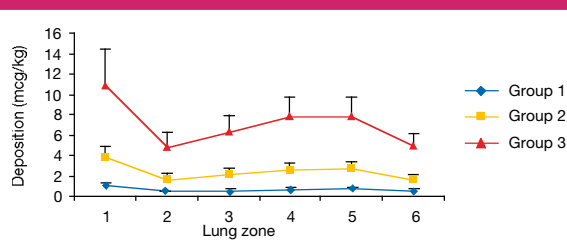


Figure 2. Deposition in 6 Lung Zones

The Lung Penetration Profiles, showing normalized distribution across the six lung zones, were similar for each dose level.

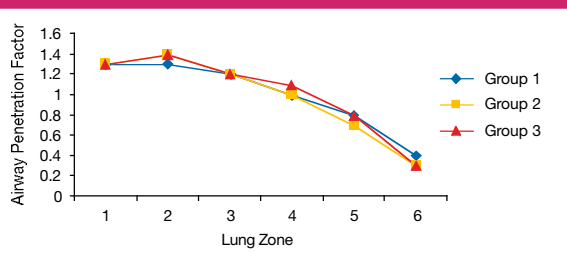


Figure 3. Lung Penetration Profiles

Central lung region dose and inhalation parameters

Mean deposition in central airways (zones 1 to 4 inclusive), and mean inhalation parameters, were close to targeted values:

Central airway dose (µg/kg)		Inhaled volume (%VC)		Breathing rate (Breaths/min)	
Target	Actual	Target	Actual	Target	Actual
3	2.9	20	19.8	15	13.6
10	10.3	20	20.9	15	11.5
30	29.8	20	27.3	15	13.3

Pharmacokinetic data

Pharmacokinetic response (increase in EpoFc serum concentration) was dose dependent.

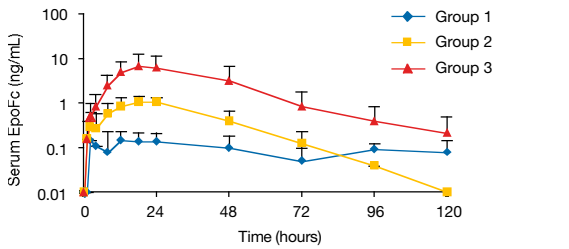


Figure 4. Serum EpoFc Concentrations

Pharmacodynamic response

Pharmacodynamic response (increase in circulating reticulocytes) was dose-dependent, and the EpoFc formulation was well tolerated by all subjects.

Conclusions

Nebulized EpoFc was effectively targeted to the central airways of the lungs at three dose levels, and dose-dependent plasma concentrations of EpoFc were obtained.

These Proof of Concept data suggest that inhaled EpoFc could be clinically effective if targeted to the central human airways, a delivery strategy that is less challenging than targeting inhaled peptides and proteins to the deep lung.

References

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