Feasibility study of OSCN- and Lactoferrin (Meveol®) nebulization for Cystic Fibrosis patients


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Introduction...

The hypothiocyanite (OSCN) and Lactoferrin (Lf) system, described as the major human host defense system against infection, is defective in Cystic Fibrosis (CF) patients (1-4). Figure 1. Breathing difficulty is the most serious symptom, resulting from frequent lung infections which were mostly treated but not completely cured (5).

Meveol®, the new orphan drug developed for CF patient (N°EU/3/09/654) is an association of OSCN and Lf active on P. aeruginosa mucoid (Pam) and non mucoid (Pa), on β. cepacia, and on MRSA. Figure 2.

Objectives...

- The aim of this study was first to confirm the antimicrobial effect of Meveol® on Pam and on an emerging pathogen M. abscessus (7).
- We have also investigate the feasibility to develop an aerosol Meveol® treatment. The objective was to select, for future clinical trials, the nebulization system which proposes an effective Meveol® treatment (8).

Materials and methods...

Antimicrobial activity of Meveol®:

- In vitro test: 15 mice C57Bl6 were intratracheally infected with 106 CFU of Pam isolated from CF patients, and then treated with Meveol® (50 µL) 24h and 48h after infection, by instillation. The lung colonization (CFU/g) was determined 72h after infection (counting method on agar plates).
- In vivo test on M. abscessus (Ma):

In a culture of Ma (106 CFU/mL) obtained in MH broth at 28°C. 200 µL of Meveol® was directly instilled. A control culture, without Meveol®, was treated in the same conditions. At 0h, 0.5h, 1h, 2h, 4h, 24h and 48h, a sample of both cultures was neutralized with trisulphite (1mL-2mm) and diluted in PBS. Dilutions were then plated in duplicate on agar plates. After 7 incubation days (28°C), present colonies were counted to determine the number of CFU/mL of culture.

Nebulization of Meveol®:

- Jet and mesh nebulizers:
  - The Numa®/Aero® DTF, France (A) and the Pari LCPLUS® (Pari, Pulmomed, France) (B).
  - The E-Flow® (Pari, Germany – C), the Micro-Air® (Numann, Japan – D) and the Aeroneb® Co (Aerogen, Ireland) associated with the Idehaler® chamber (Aerodrug, France) (E).
- Three copies of each nebulizer and their mouthpieces were used and tested in duplicate.

OSCN and Lf stability after nebulization:

- Meveol® (5 mL) was nebulized and aerosols were collected in an Impinger at 12.6 L/min (Ace Glass Inc, USA). Nebulized OSCN and not nebulized Meveol® were simultaneously analyzed by spectrophotometry (Thomas & Aune colorimetric method) to determine the OSCN concentration, 
- Ratios of the [OSCN] and of the [Lf] measured before and after nebulization were determined.

Aerosols characterizations:

- Particle size distributions of aerosols produced by all devices were measured (Malvern, Mother, UK) to determine the volume mean diameter (VMD) and the fine particle fraction (FPF) defined as the % of particles with a diameter smaller than 5 µm predicted a lung deposition.
- Inhalable mass of Meveol® produced by nebulizers was collected in an inhalation filter (PARI, Pulmomed, France) connected to a respiratory pump simulating the patient breath (15 breaths/min, 500 mL, I/E=40/60). The drug mass of Meveol® collected (drug mass may penetrate into the patient airways) was determined using a residual gravimetric method. Inhalable fraction was calculated as follows: (drug mass collected in the filter)/(drug mass loaded in the nebulizer).
- The respirable fraction of Meveol® (Fp fraction of Meveol®) in terms of nebulizer charge, which may deposit into patient lungs was calculated as the part of between the inhalable fraction and the FPF.

Results...

Antimicrobial activity of Meveol®:

- In vitro test: 6/15 mice died in control group and 3/15 mice in treated group. 72h after infection with Pam, mice treated with Meveol® presented a significantly lower level of lung bacterial colonization, when compared to 1.5 ± 0.5 log CFU/g vs. 3.08 ± 0.4 log CFU/g of lungs (p<0.05).
- In vivo kinetic activity of OSCN/Lf: Meveol® has allowed in vivo the total eradication of M. abscessus, within 48h of inoculation. Figure 2.

Nebulization of Meveol®:

- Successfully nebulized, Meveol® was not disturbed by the physical constraints of nebulization. OSCN and Lf were both preserved in the aerosol form of Meveol® nonnebulized (nebulized/not nebulized) determined for [OSCN] and [Lf] were, for all devices, close to 1.
- Aerosols of Meveol® produced by each device were strongly variables in terms of VMD (2.8 µm to 5.9 µm), of FPF (33 % to 63 %), of nebulization time (8.5 min to 41.7 min), of inhalable fraction (18 % to 58 %) and of respirable fraction (6 % to 35 %).

Conclusions...

- Study confirms the antimicrobial effect of Meveol® on P. aeruginosa mucoid, and on M. abscessus, an emerging pathogen.
- The Aeroneb® Go/Idehaler® Pocket® device has been selected to nebulize Meveol® for future clinical trials. The system produces in vivo a high respirable fraction (31 %) during a short nebulization time (9.8 min).