Introduction...

The hypotiocyanite (OSCN⁻) and Lactoferrin (Lf) system, described as part of the major human host defense system against infection, is defective in Cystic Fibrosis (CF) patients (1-3). Figure 1. Breathing difficulty is the most serious symptom, resulting from frequent lung infections which were mostly treated but not completely cured (3).

Meveol®, the new orphan drug developed for CF patient (N'EU/3/09/654) is an association of OSCN⁻/Lf and active on P. aeruginosa mucoid (Pam) and non mucoid, on M. abscessus (Ma), B. cepacia, B. dolosa 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 (11),

→ 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 (10).

Materials and methods...

- In vivo test with P. aeruginosa mucoid (Pam)
- 15 mice C57Bl6 were intratracheally infected with 10^6 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 vitro test on M. abscessus (Ma)
- In a culture of Ma (10^6 CFU/mL) obtained in MH broth at 28°C, 1 mL of Meveol® per mL of culture was directly added. A control culture, without Meveol® treatment, was also constituted. At 0h, 0.5h, 1h, 2h, 4h, 24h and 48h, a sample of both cultures was neutralized with cetyltrimethylammonium bromide (1mL:2mM) and diluted in PBS. Dilutions were then plated in triplicate on TSA plates. After 5 incubation days (28°C), present colonies were counted to determine the number of CFU/mL of culture.

Nebulization of Meveol®

- Jel and mesh nebulizers:
  - The NUMATARD® (DFT, France-A) and the Port LCSPRINT® nebulizer (Purmormed, France-A)
  - The E-Flo® (Pal, Germany-C), the Micro-A® (Imron, Japan-B) and the Aeroneb® Go (Aerogen, Ireland) associated with the Idehaler-Pocket® chamber (Aerodrug, France) (8).
  - 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 12.5 L/min (Ace Glass Inc, USA). Nebulized Meveol® and not nebulized Meveol® were simultaneously analyzed by spectrophotometry (Thomas & Aure colorimetric method) to determine the concentration of OSCN⁻ and Lf, and, by a competitive ELISA assay to determine the Lf concentration, [Lf].
  - Ratios of the [OSCN⁻] and [Lf] measured before and after nebulization were determined.

- Aerosol characterizations:
  - Particle size distributions of aerosols produced by all devices were measured (Aerosizer, Malvern, 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 predicting a lung deposition.
  - Inhalable mass of Meveol® produced by nebulizers was collected in an inhalation filter (PAR, Purmormed, France) connected to a respiratory pump simulating the patient breath (1.5 breaths/min, 0.50 mL, V=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 filters) / (drug mass loaded in the nebulizer)].
  - The respirable fraction of Meveol® is calculated as the product between the inhalable concentration and the FPF.

This study confirms the antimicrobial effect of Meveol® on P. aeruginosa mucoid, and on M. abscessus, an emerging pathogen.

Other challenge tests have also shown in vitro efficacy on Burkholderia strains (B. cenoceica, B. dolosa)

The Aeroneb® Go/Idehaler-Pocket® device has been selected to nebulize Meveol® for future clinical trials. The system produces in vitro a high respirable fraction (31%) during a short nebulization time (9.8 min).

Results...

- In vivo trial: 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, than control mice: 1.5 ± 0.48 log CFU/g vs. 3.0 ± 0.78 log CFU/g of lungs (p<0.05).

- In vitro kinetic activity of OSCN⁻/Lf: Meveol® has allowed in vitro the total eradication of M. abscessus, within 48h of incubation, Figure 3.

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®. Ratios (nebulized/not nebulized) determined for (OSCN⁻) and for [Lf] were, for all devices, close to 1.

- Aerosol of Meveol® produced by each device was strongly variables in terms of VMD (2.8 µm 5.9 µm), of FPF (33% 63%), of nebulization time (8.5 min to 41.7 min), of inhalable fraction (18% 58%) and of respirable fraction (6% 35%), Table 1.

- The Aeroneb® Go/Idehaler-Pocket® nebulization system, predicting 31% of Meveol® deposited into patient lung within 9.8 min, has been selected for future clinical trials.

Conclusions...