Feasibility study of OSCN\(^{–}\) and Lactoferrin (Meveol\(^{®}\)) nebulization for Cystic Fibrosis patients.

S. Perrotto\(^1\), S. Le Guelic\(^2\), L. Vecellio\(^3\), E. Fichant\(^4\), P. Stordeur\(^4\), P. Bordeaux\(^1\) and J.P. Peraudin\(^1\).

\(^1\) Alaxia, Vaulx-en-Velin, France \(^2\) Aerodrug, Tours, France \(^3\) CER group, Marloie, Belgium. Supported by EU and Mucoviscide Innovation.

\(\textbf{Introduction} \ldots\)

The *hypothiocyanite (OSCN\(^{–}\)) and Lactoferrin (Lf) system*, described as part of the major human host defense system against infection, is defective in Cystic Fibrosis (CF) patients (1-4). **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\(^{–}\)/Lf and active on P. aeruginosa mucoid (Pam) and non mucoid, on M. abscessus (Ma), B. cepacia, B. dolosa and on MRSA. **Figure 2**.

\(\textbf{Objectives} \ldots\)

- 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).

\(\textbf{Materials and methods} \ldots\)

\(\textbf{Antimicrobial activity of Meveol}\(^{®}\):

- In vivo test with P. aeruginosa mucoid (Pam): 15 mice CFShb were intratracheally infected with 10\(^{9}\) 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 plate).
- In vitro test on M. abscessus (Ma): In a culture of Ma (10\(^8\) 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 cysteine (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.

\(\textbf{Nebulization of Meveol}\(^{®}\):

- Jet and mesh nebulizers: 
  - The NL9M\(^{®}\) (IDF, France -A)- and the Parli C SPRINT\(^{®}\) TurboBoy (Pari, Pulmomed, France -B) -
  - The E-FLOW\(^{®}\) (Pari, Germany -C) and the Micro-Air\(^{®}\) (Omnova, Japan -D) and the Aeroneb\(^{®}\) Go (Aerogen, Ireland) associated with the Idehaler-Pocket\(^{®}\) 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 Meveol\(^{®}\) and nebulized Meveol\(^{®}\) were simultaneously analyzed by spectrophotometry (Thomas & Aune colometric method) to determine the OSCN\(^{–}\) concentration, [OSCN\(^{–}\)], and, by a competitive ELISA assay to determine the Lf concentration, [Lf].
  - Ratios of the [OSCN\(^{–}\)] and the [Lf] measured before and after nebulization were determined.

\(\textbf{Aerosols characterizations}:

- Particle size distributions of aerosols produced by all devices were measured (Maditrac\(_{®}\), 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 deposited in 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, 50 cm H\(_2\)O) (6). The drug mass of Meveol\(^{®}\) collected (drug mass penetrate into the patient airway was determined using a residual gravimetric method\(^{®}\), Inhalable fraction was calculated as follow: (drug mass collected in the filter) / (drug mass loaded in the nebulizer).
- The respirable fraction of Meveol\(^{®}\) (fraction of Meveol\(^{®}\), in terms of nebulizer charge, which may deposit into patient lung) was calculated as the product between the inhalable fraction and the FPF.

\(\textbf{Results} \ldots\)

\(\textbf{Antimicrobial activity of Meveol}\(^{®}\):

- In vivo trials: 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. 3.08 ± 0.3 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**.

\(\textbf{Conclusions} \ldots\)

- 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.
- 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 %). **Table 1**.

- 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).