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 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* mucoïd (Pam) and non mucoïd, on *M. abscessus* (Ma), *B. cepacia, B. dolosa* and on MRSA. Figure 2.

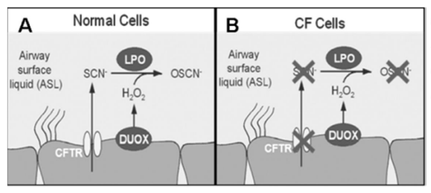
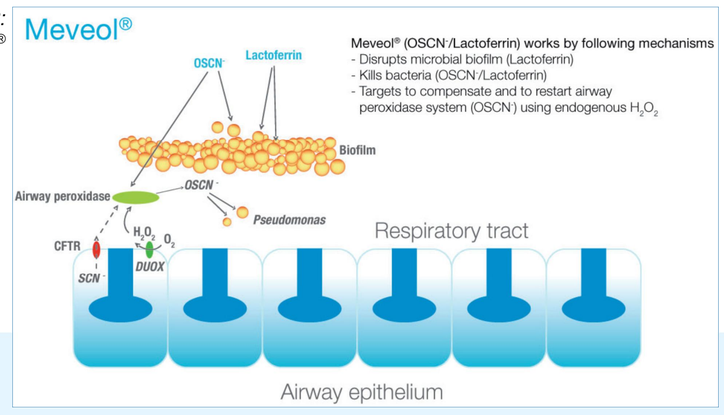


Figure 1:
A: Endogenous synthesis of $OSCN^-$ in normal cell.
B: Absence of synthesis of $OSCN^-$ in CF Cells due to non functional CFTR activity[6].

Figure 2:
Action of Meveol®



➔ 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®:

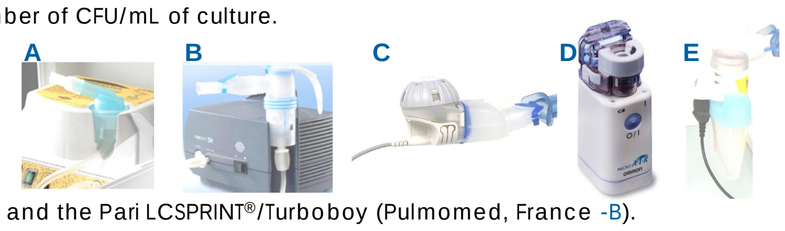
➔ *In vivo* test with *P. aeruginosa* mucoïd (Pam)

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 (10^5 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.

Nebulization of Meveol®:



➔ *Jet and mesh nebulizers*

- The NL9M®/ABOX+ (DTF, France -A) and the Pari LCSPRINT®/Turboboy (Pulmomed, France -B).
- The E-Flow® (Pari, Germany -C), the Micro-Air® (Omron, 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 not nebulized Meveol® were simultaneously analyzed by spectrophotometry (Thomas & Aune colorimetric 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.

➔ *Aerosols characterizations*

- Particle size distributions of aerosols produced by all devices were measured (MastersizerX, 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 (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[9]. 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 lungs) was calculated as the product between the inhalable fraction and the FPF.

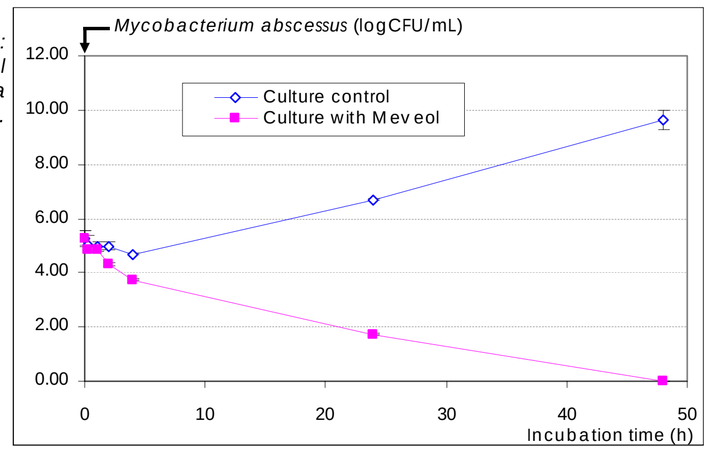
… Results …

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.

Figure 3:
In vitro kinetic of antimicrobial activity of Meveol® on a Mycobacterium abscessus culture.



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.

➔ 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.

Table 1: Characterization of aerosols of Meveol® (5ml) produced by: the Aeroneb® Go/Idehaler-Pocket®, the E-Flow®, the Micro-Air®, the NL9M® and the Pari LCSPRINT®, in terms of the VMD (µm), the inhalable fraction, respirable fraction and nebulization time. Inhalable and respirable fractions are expressed in terms of the % of the loaded Meveol® introduced into the nebulizer.

mean±SD	VMD (µm)	Inhalable fraction (%)	Respirable fraction (%)	Nebulization time (min)
Aeroneb® Go/Idehaler-Pocket®	4.3 ± 0.5	57 ± 1	31 ± 3	9.8 ± 0.4
E-Flow®	4.5 ± 0.9	47 ± 13	26 ± 11	10.6 ± 0.1
Micro-Air®	5.7 ± 0.2	23 ± 5	8 ± 1	36.2 ± 5.0
NL9M®	5.7 ± 0.2	30 ± 5	12 ± 2	9.7 ± 1.1
Pari LCSPRINT®	3.4 ± 0.5	38 ± 6	20 ± 1	24.2 ± 8.1

➔ 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

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

➔ Other challenge tests have also shown *in vitro* efficacy on *Burkholderia* strains (*B. cenocepacia*, *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).

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