effusion 6 years after administration of BCG treatment, although in that patient, no mycobacteria could be isolated from pleural fluid.[7]

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In Vitro and In Vivo Evaluation of the Combination of Oscillating Positive Expiratory Pressure and Nebulization: A Randomized Cross-Over Study

Evaluación in vitro e in vivo de la combinación de presión espiratoria positiva oscilante y nebulización: estudio aleatorizado cruzado

To the Editor:

Positive expiratory pressure (PEP) as airway clearance technique prevents airway closure during expiration, reduces gas trapping in the lung,[1] increases collateral ventilation[2] and improves spatial ventilation distribution.[3] The addition of oscillations promotes mucus mobilization based on the reduced mucus viscosity[4] and distributed flows in more airways.[5] Oscillating PEP devices have been proposed in cystic fibrosis.[6] Simultaneous use of PEP and nebulization is sometimes performed in cystic fibrosis patients even if its clinical benefits are still not well established.

As the drug amount reaching the lungs is essential for the clinical response of drug with a dose-dependent effect, this study aims at evaluating the effect of a new oscillating PEP device combined to a nebulizer on the drug delivery to the lungs.

We connected a jet nebulizer (Sidestream®, Philips-Respironics) (NEB) at the distal end of an oscillating PEP device (Aerobika®, Trudell-Medical) (NEB-OPEP) with a T-piece blocking the extra vent. Each nebulizer was filled with 250 mg of amikacin diluted with 3 mL of normal saline.

In the in vitro part, a dual-chamber test lung (5600i Dual Adult Test Lung; Michigan Instrument Inc.) driven by a ventilator (Servo-i®, MAQUET) (Fig. 1) simulated an adult breathing pattern (Vt = 500 mL-RF = 12 breaths/min-*I*/*E* ratio = 1:2-breath-hold time = 0.25 s). An absolute bacterial/viral filter (Air Safety Ltd, Lancashire) was placed between the lung model and NEB-OPEP/NEB to measure the inhaled dose (IND). Another filter placed on the expiratory port collected the exhaled dose (ExD). The mass of amikacin collected on filters during nebulization was quantified using the residual gravimetric method.[7] The nebulizer output was calculated by dividing the IND by the nebulization duration. The residual volume was quantified.

In the in vivo part, after ethical approval (B40320107908) and registration of the trial (NCT02535130), six non-smoker healthy males were recruited. They signed a written informed consent form. They were excluded if they received any antibiotic or aerosolized drug during the month preceding the experiments, for history of cardiovascular and/or pulmonary disease, for allergy to aminoglycosides and for abnormal pulmonary function.

After (1) selection visit, spirometry and medical examination and (2) training to inhale correctly, (3) inhalation and (4) urine sampling were performed. Each subject repeated the steps 3 and 4 in similar conditions using a randomized crossover setting (www.randomizer.org) with a one-week washout period between the two configurations (NEB or NEB-OPEP). The subjects breathed spontaneously through mouthpiece wearing a noseclip.

Just before the experiments, the urinary bladder of the subjects was emptied. Then, urine samples were collected at each spontaneous micturition during the 24 h following the nebulization. The volume and timing of micturition were recorded.

After sampling by fluorescence polarization immuno-assay, the total daily amount of amikacin excreted in the urine (Cu max) reflecting lung deposition and the elimination rate constant was calculated using the Michaelis–Menten kinetic model from cumulating amikacin amount measured at each micturition (Cu) and represents the lung dose (LD) ($Cu\ max = Cu \times (1 - e^{-Ke \cdot x \cdot t})$).

The residual amount of drug was calculated by multiplying residual volume and its final concentration.

In vitro, no difference was found between NEB and NEB-OPEP for IND (34.4% (30.0–38.0) vs 29.6% (26.8–32.0)) and ExD (20.8 (19.6–23.2) vs 25.2% (22.4–28.4)). Nebulizer output was higher for NEB than for NEB-OPEP (1.49 ± 0.14 vs 1.10 ± 0.12 mL min^{-1}; $p = 0.032$).

Six subjects completed the study (21.8 ± 1.0 y – 179.2 ± 8.8 cm – 76.5 ± 11.7 kg – FEV1: $97 \pm 7\%$). The lung dose was reduced by 40% and the time required to finish the nebulization was 1.2 min longer with NEB-OPEP than with NEB (Table 1).

Lung delivery around 5% of the ND with NEB confirmed previous results.[8] In addition, our results showed that interposing this new OPEP device between the nebulizer and the patient's mouth reduced the efficiency of the nebulization similarly to results found with other PEP devices under different conditions.[9,10] This is clinically important when dose-dependent drugs are administered. The reduced lung delivery could be explained by the impaction of particles in the PEP device that filters the larger particles.[9,11] However, this hypothesis was neither verified in our study (similar IND and ExD) nor in a previous study.[12]

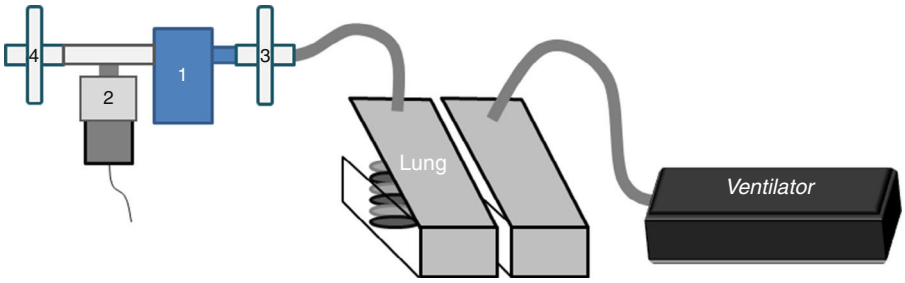

Fig. 1. Illustration of the experimental model. 1 – Aerobika, 2 – nebulizer, 3 – filter collecting the inhaled dose, 4 – filter collecting the exhaled dose.

The duration of the nebulization was inversely related to the adherence in CF[13] and it is therefore considered as a key factor for an optimal treatment.[14,15] In the present study, the small increase in time (+1.2 min) related to the NEB-OPEP is compensated by the time saved on the total duration of the treatment administered separately (3.1 min). This advantage could be important for the patients needing several daily sessions. However, as suggested by our results, the necessity to nearly double the nebulization duration to reach a similar lung delivery with NEB-OPEP must be taken into account. As Berlinsky showed variable delivery depending on the PEP devices,[11] each configuration must be evaluated.

The modified pattern of breathing related to PEP is unfavorable to the nebulization function and explains probably the worst lung delivery related to the combination. The reduction in breathing frequency (7.8 cycles by minutes) when healthy subjects used PEP mask could be considered as advantageous[16] but it was associated to a prolonged expiration that contributes to loose particles during the expiratory phase.[17] The concomitant reduction in the inspiratory–expiratory ratio contributes certainly to the reduced lung delivery by losing particles during the expiratory phase and it can explain the difference with in vitro results. Some particles are probably exhaled and others are delivered in the atmosphere or the device during this phase.

Some limitations of the study should be discussed. First, the nebulizer was placed at the distal outlet of the PEP device. This configuration is less efficient than that in which the proximal outlet is used for connection,[10] although the latter is recommended by the manufacturer. Second, testing healthy subjects omits the clinical and pathophysiological outcomes related to positive expiratory pressure and its oscillations but it permits to evaluate the nebulization function[8,18,19] and reduces the variability resulting from mucus plugging or lung disease. Furthermore, even if lung delivery in healthy subjects cannot be extrapolated to patients with respiratory diseases, a similar lung deposition was recently found between healthy subjects and patients with CF.[20] Third, each subject was its own control to reduce anatomical, anthropometrical and respiratory influences on lung delivery.

This study suggests that connecting Aerobika at the proximal outlet of a nebulizer is less efficient than using the nebulization alone for drug delivery. However, the gain in time is an argument in favor of the combination.

Table 1
Lung delivery comparison between nebulization used alone and coupled with an oscillating positive expiratory pressure device in 6 healthy subjects.

	NEB	NEB-OPEP	*p* value
Lung dose (% ND)	4.7 ± 1.4	2.8 ± 0.2	0.029*
Time of nebulization (min)	4.3 ± 0.7	5.5 ± 0.6	0.012*
Residual volume (mL)	0.85 ± 0.08	1.04 ± 0.12	0.044*

Results are expressed as mean ± SD. NEB: nebulizer; NEB-OPEP: nebulizer couple with an oscillating positive expiratory pressure device; ND: nominal dose.
* $p < 0.05$.

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In Vitro and In Vivo Comparison of Two Nebulizers Used for Inhaled Pentamidine Delivery

Comparación in vitro e in vivo de dos nebulizadores utilizados para administrar pentamidina inhalada

Dear Editor,

Pneumocystis jirovecii pneumonia (PJP) represents a significant cause of morbidity and mortality in immunosuppressed patients.[1,2] Pentamidine, used in secondary prevention of PJP, is administered via inhalation and requires a specific nebulizer.[3,4] In the recent ERS/ISAM Task Force Consensus Statement, the RespirgardII® (Vital Signs) was considered as the reference nebulizer to deliver pentamidine.[5] Nebulizers with comparable properties are required because RespirgardII® is no longer available since its recent withdrawal from the market in some countries.[3] Most of the nebulizers compared previously to the RespirgardII were ultrasonic nebulizers.[6] In this study, we compared a jet nebulizer (ISO-NEB®, Teleflex) to RespirgardII® for pentamidine delivery. Both nebulizers possess one-way valves on the inspiratory and on the expiratory way and an expiratory filter as recommended.[3,4] Both were driven by a similar air flow (8 L/min) and deliver particles with similar size (MMAD: 1–2 μm).[3,7,8] In vitro, nebulizers were connected to a dual chamber lung model (5600i Dual Adult Test Lung®, Michigan Instrument Inc.) driven by a ventilator (SERVO-I®, Maquet) in volume-controlled mode simulating an adult breathing pattern (Vt = 500 mL; RF = 15 breaths/min; I/E ratio = 1:2; no end-inspiratory pause) (Fig. 1). Artificial lung compliance and resistance were set to 70 mL/cmH_2O and 5cmH_2O, respectively. Nebulizations of a pentamidine solution (300 mg/6 mL sterile water) were performed in triplicate for each model in accordance with manufacturer's and guidelines recommendations until one minute after the appearance of the sputtering point.[1,2,7,9] Inhaled dose, expressed in percentage of the nominal dose (ND), corresponding to the nebulized doses deposited on the filter interposed between nebulizer and lung model (weighed before the nebulization and after drying for 24 h) multiplied by the relative mass of pentamidine. The residual volume was also quantified.

In the in vivo part, after ethical approval (2013/27JUI/375) and registration of the trial (NCT02277808), five non-smoker healthy male volunteers were recruited and signed a written inform consent. Each subject performed a spirometry according to the ATS/ERS guidelines.[10] This was a randomized cross-over study based on CONSORT statement for clinical trials. Nebulizations of amikacin sulfate (Amukin®, Bristol-Myers Squibb) dissolved in 4 mL of normal saline (125 mg/mL) were made during 10 min with both devices. During nebulization, tidal volume (Vt; L), respiratory frequency (RF; min^{-1}) and minute ventilation (VE; $L\,min^{-1}$) were monitored by inductance plethysmography (Respitraces®, Ambulatory Monitoring Inc.). Participants were requested (1) to empty their bladder before nebulization, (2) to inhale spontaneously through the mouthpiece with a nose clip in a sitting position, (3) to collect their urine for 24 h following nebulization and (4) to observe a wash-out period of one week between both nebulizations. Then comparison was performed by sampling the daily urinary excretion of nebulized amikacin following the technique previously described by Dequin et al.[11] and analyzed by High Performance Liquid Chromatography. The total daily amount of amikacin excreted in the urine (Cu max) was calculated from cumulating amikacin amount measured at each micturition (Cu) and represents the lung dose. The elimination constant (K_e) was calculated from the fitted curve of the cumulated amount of amikacin excreted in the urine plotted versus the time. The equation is $Cu = Cu\ max(1 - e^{-K_e t})$.

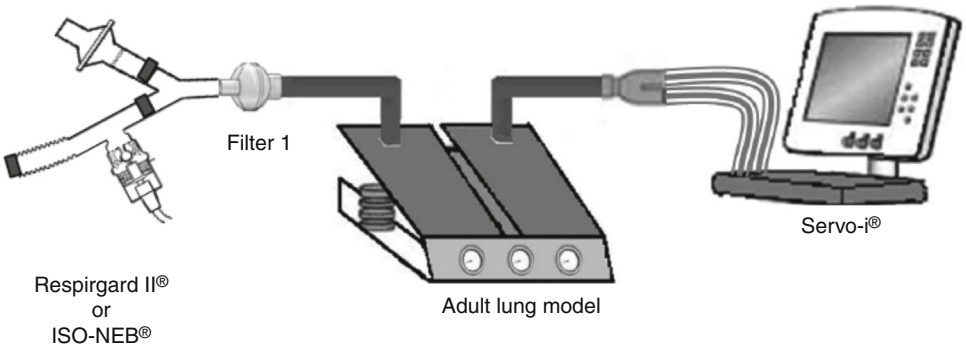

Fig. 1. Schematic diagram of experimental devices used in the in vitro study.