Impact of acoustic airflow on intrasinus drug deposition: New insights into the vibrating mode and the optimal acoustic frequency to enhance the delivery of nebulized antibiotic

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Suggested running head: Enhancement of the delivery of nebulized antibiotics

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ABSTRACT

Aim: We investigated the impact of vibrating acoustic airflow, the high frequency ($f \geq 100$ Hz) and the low frequency ($f \leq 45$ Hz) sound waves, on the enhancement of intrasinus drug deposition.

Methods: ^{81m}Kr-gas ventilation study was performed in a plastinated human cast with and without the addition of vibrating acoustic airflow. Similarly, intrasinus drug deposition in a nasal replica using gentamicin as a marker was studied with and without the superposition of different modes of acoustic airflow.

Results: Ventilation experiments demonstrate that no sinus ventilation was observed without acoustic airflow although sinus ventilation occurred whatever the modes of acoustic airflow applied. Intrasinus drug deposition experiments showed that the high frequency acoustic airflow led to 4-fold increase in gentamicin deposition into the left maxillary sinus and to 2-fold deposition increase into the right maxillary sinus. Besides, the low frequency acoustic airflow demonstrated a significant increase of 4-fold and 2-fold in the right and left maxillary sinuses respectively.

Conclusion: We demonstrated the benefit of different modes of vibrating acoustic airflow for maxillary sinus ventilation and intrasinus drug deposition. The degree of gentamicin deposition varies as a function of frequency of the vibrating acoustic airflow and the geometry of the ostia.

Keywords: vibrating acoustic airflow, high frequency, low frequency, intrasinus deposition, ^{81m}Kr-gas ventilation.

1. Introduction

Rhinosinusitis is an inflammatory disorder involving the lining of the nasal passages and the paranasal sinuses. Sinuses are poorly ventilated cavities within the bones of the face communicating with the nasal fossa via the maxillary ostium (Tarhan et al., 2005; Jones, 2001; Fokkens et al., 2012). Chronic rhinosinusitis (CRS) is one of the most common chronic diseases, affecting about 10-15% of the total population with significant impact on quality of life and health care expenditure, and economic impact in terms of absenteeism and productivity (Durand et al., 2011a; Laube et al., 2011). CRS is being defined as the presence of two or more symptoms, such as nasal blockage, obstruction, congestion or nasal discharge (anterior or posterior) (Dykewicz and Hamilos, 2010). Compared with people without rhinosinusitis, those with this disease reported more days spent bedridden and more visits to healthcare providers. These findings highlight the significant impact of this health problem on patient quality of life, as well as costs of care to patients and society (Benninger et al., 2010). One of the primary interests of treatment of this disease is to improve the quality of life of the patients and to reduce the considerable financial burden. CRS patients are currently treated according to guidelines, where finally surgery is performed after various preparative treatments, i.e. using nasal pump sprays. There is no standard management of CRS. Significant advances in management have been achieved, including endoscopic surgery, newer antibiotics, better diagnostic criteria, image-guided surgical navigation, and the avoidance of external procedures. These advances have improved the treatment of this disease process and, for some patients, may lead to a decrease in the frequency, severity, and duration of symptoms and infections. Medical therapy of CRS is a key strategy, with surgery playing a vital adjunctive role. There are, however, several negative factors that are still present after surgery, including potential offending bacteria, fungi, viruses, and the patients' immunologic responses. These factors and others cause many patients to have frequent, recurrent acute

infections compounding their chronic sinusitis. To avoid this, studies have been redirected towards determining better treatments with fewer implications. An efficient topical therapy may allow more effective treatment of upper respiratory diseases, preventing sinus surgery or at least delaying the need for sinus surgery. The most frequently recommended medications for treatment of CRS consist in the systemic administration of antibiotics by oral route. Gentamicin, an aminoglycoside active on Gram-negative bacteria and staphylococci, is considered as one of these frequently recommended antibiotics due to its recognized efficacy on rhinosinusitis associated bacteria (Desrosiers et al., 2011; Kalogjera et al., 2003). The effectiveness of topical delivery of pulmonary antibiotics and corticosteroids through the nebulization process has been previously revealed in many studies (Bhattacharyya et al., 2011; Leclerc et al., 2014a). Topical delivery, particularly nebulization, has the advantage over systemic therapy as it minimizes the risk of systemic side effects while increasing the local dose. Therefore, better targeting of nebulized antibiotics into the common sites of infection, maxillary sinuses, could improve clinical outcomes in patients suffering from CRS and minimize the risk of antibiotic resistance in non-targeted areas. Despite the fact that maxillary sinuses are poorly-ventilated hollow organs, several studies have shown the efficacy of nebulized drug to treat sinus disorder (Leclerc et al., 2014a, 2014b; Durand et al., 2012). Recently, we have studied the gentamicin deposition in the maxillary sinuses using 100 Hz acoustic airflow nebulization. In addition, several works focused on the mechanism involved in sinus ventilation, which is a fundamental requirement of aerosolized drug delivery to the sinus cavities. The sinus ventilation and then the intrasinus drug deposition can be provided by medical devices enabled to generate vibrating airflows. The main objective of these medical devices is to add an acoustic airflow to the airborne droplets in order to generate a sufficient pressure gradient to allow the sinus ventilation (Durand et al., 2011a; Möller et al., 2011, 2010). The aim of this study is the investigation of the impact of different

modes of acoustic airflow on the maxillary sinus ventilation (using a radioactive gas) and the intrasinus drug deposition (using a gentamicin aerosol).

2. Materials and Methods

2. 1. Anatomic nasal replica

2.1.1. Human plastinated cast

A technique that allows the preservation of most physical properties of anatomical specimens is known as plastination. The human plastinated cast used provides an easy access to the maxillary sinuses due to lateral-paramedian sections (Durand et al., 2011b). Its anatomical and aerodynamic behavior closely resembles *in vivo* patterns. Such models were previously characterized anatomically, geometrically and aerodynamically and were successfully used in several aerosol deposition studies (Durand et al., 2011a; Leclerc et al., 2014b; Moeller et al., 2009). In this study, the plastinated nasal cast was used to perform radioactive gas ventilation experiments using ^{81m}Kr-gas.

2.1.2. Nasal replica

A nasal replica was created using a stereolithography technique leading to manufacturing of a transparent, water-resistant, non-porous resin replica of the human plastinated cast (Leclerc et al., 2014b ; co-development with the DTF medical company). Anatomical and aerodynamic similarities between the nasal replica of the plastinated nasal cast, particularly the geometry of the ostia and maxillary sinuses, was confirmed after performing endoscopy and CT scans. The differences in the dimensions of the individual maxillary ostia (6 mm long with a 2 mm diameter for the RMS and 2 mm long with a 5 mm diameter for the LMS) possessed by the plastinated human cast were reproduced in the nasal replica thanks to a stereolithography technique (Leclerc et al., 2014b). In this study, the nasal replica was used to perform gentamicin aerosol deposition experiments. Compared to the human plastinated cast and human *in vivo* experiments, nasal replica was approved to be used in aerosol deposition study facilitating the washing technique and reducing the drying time (Le Guellec et al., 2014).

2.2. Nebulizing systems

Two nebulizing conditions were studied. The first one delivers a high frequency vibrating acoustic airflow; we define a high frequency acoustic airflow as an acoustic airflow of 100 Hz and above. The commercial medical device used was the ATOMISOR NL11 jet nebulizer associated with an AOHBOX compressor (DTF Medical, Saint-Etienne, France). A nasal plug (C28, DTF Medical, Saint-Etienne, France) attached to the nebulizer ensured its interface connection with nasal replica's nostrils. Nebulization can be performed with or without the addition of the high frequency acoustic pressure waves by using these two possible modes offered with this commercial medical device.

The second nebulizing condition consists in superimposing a low frequency vibrating acoustic pressure wave to the aerosol production; we define low frequency acoustic airflow as an acoustic airflow of frequency of 45 Hz and above. The commercial device used was the PARI SINUS jet nebulizer associated with a PARI SINUS compressor (Pari GmbH, Starnberg, Germany). Vibrating and non-vibrating airflow nebulizations were performed by connecting and disconnecting the vent tubing from the vibration output of the compressor. For aerosol delivery to the nasal fossa, this nebulizer was coupled to the same nasal plug used in the first nebulizing conditions. Thus, the low frequency device was not used according to producer specifications (*i.e.* one nostril in, the other out). In fact, as the aim of this work was the impact of acoustic airflow on intrasinus drug delivery; we needed a same experimental protocol of aerosol administration to the nasal fossa for both low and high frequency devices to avoid biases due to the impact of the nasal plug. Thus, we must underline that the results of intrasinus drug deposition for the low frequency device cannot be representative of data obtained in vivo according to the producer specifications.

2.3. Scintigraphic study of the sinus ventilation

^{81m}Kr-gas continuously administered *via* nasal plug through the human plastinated cast was performed in order to study the impact of the different modes of acoustic airflow on the radioactive gas penetration into the maxillary sinuses (^{81} Rubidium / ^{81m} Krypton generator, COVIDIEN IMAGING FRANCE). Ventilation experiments were performed in front of a single-head planar gamma camera (Millennium MPR Gamma Camera; GE healthcare) equipped with a low-energy, high-resolution collimator.

Each nebulizer was coupled to both nostrils of the model *via* a nasal plug (Fig. 1). The output channel of the ^{81m}Kr-gas generator was directly connected to the compressed air pressure supply tubing of the nebulizer. Two movable plates were used to close the maxillary sinuses hermetically during the ^{81m}Kr-gas ventilation experiments (Durand et al., 2001). Several experiments were performed with and without the two types of acoustic airflow (low and high frequency acoustic airflow) with each nebulizer. Anterior static images of ^{81m}Kr-gas ventilation were acquired (matrix 128*128 with a target of 100 Kcnts per acquisition). Trachea of the model was tightly clogged to avoid the radioactive ^{81m}Kr-gas contamination and thus to reduce the noise in scintigraphic images. Acquired images of the gamma camera were analyzed using a Xeleris 2 workstation (GE Healthcare). Images were processed with the same windowing and filtration level.

2.4. Aerosol depositions into the maxillary sinuses

Gentamicin (GENTAMICINE PANPHARMA 160 mg – 2 ml; 80 mg/ml) was used as a marker. It was chosen due to its efficacy recognized on rhinosinusitis associated bacteria. The nebulizers were filled with 4 ml of a gentamicin solution operating at a flow rate of 8 L.min^{-1}. The maxillary sinuses of the nasal replica were hermetically sealed during the experiments. The nasal replica was connected to a 15 cm long tube simulating the trachea equipped with an

absolute filter. Nebulization lasted 10 minutes. After each nebulization, the plates closing the sinuses were then removed and the maxillary sinuses were flushed four times by syringe containing 1 ml of physiological water (Durand et al., 2011a). Each sinus was flushed 4 times using the same physiological water. The region close to the maxillary ostium was never flushed thus avoiding the overestimation of the amount of gentamicin deposited in maxillary sinuses (Durand et al., 2011a). For subsequent measurement of the gentamicin concentration, the collected liquid was kept at -20°C. After each nebulization, the nasal replica was disconnected from the assembly and copiously washed with tap water and dried with pulsed air. Physiological water nebulizations were randomly performed throughout the experiments by filling the nebulizers with 4 ml of physiological water instead of gentamicin to verify the efficiency of model washing; *i.e.* the gentamicin content in the flushing liquid is lower than the detection level of the analyzer. Gentamicin concentrations in samples were quantified by fluorescence polymerization immunoassay (FPIA) with a TDxFLx® analyzer (Abbott Diagnostics Division, USA), which has a lower detection limit of 0.2 $mg.L^{-1}$.

2.5. Particle size-distribution and aerosol output characterization

Aerosol generated by the different studied nebulizers and compressors with and without the effect of superimposed sound pressure waves was characterized using an electrical low-pressure impactor (ELPI, Dekati Ltd., Finland). These experiments allow determining the particle sizing in terms of Mass Median Aerodynamic Diameter (MMAD). The precise protocol was described in previous works (Durand et al., 2011b). Briefly, we used sodium fluoride (NaF; 2.5%; 4 ml) as a chemical tracer according to European standard procedure (NF EN 13544-1). Airborne particles impact on the 12 stages of the cascade impactor; which allows the simultaneous measurement of the aerodynamic size. The ELPI cascade impactor allows the collection of nebulized particles from 7 nm to 10 µm into 12 size fractions and

operated with airflow of 10 $L.min^{-1}$. The nebulizer was connected to the cascade impactor via a metal United States Pharmacopeia (USP) like artificial throat. Hence, for the particle size measurement, NaF was aerosolized by the nebulizer for 5 minutes after which each stage was washed with 5 ml of distilled water. The concentration of NaF samples was then assayed by electrochemical means (PerfectION™ combined with a SevenGo pro™ F- electrode, Mettler Toledo, France). Finally, the MMAD of nebulized particles was calculated using electrochemical measurements of sodium fluoride.

Likewise, a gravimetric study of aerosol was conducted to determine the effect of acoustic airflow of the different nebulizing systems on the total aerosol mass output during a nebulization experiment. The study was performed using sodium fluoride (NaF; 1%; 1.5 ml) as recommended by European standard procedure (NF EN 13544-1) and following the previously described experimental procedure (Durand et al., 2011). The nebulizer was filled with NaF solution and connected to its corresponding compressor. An absolute filter (Inhalation Filter Pad, Pari GmbH, Germany), interposed between the nebulizing system and the respiratory pump (Compas2, Pari GmbH, Germany), was used for the collection of aerosol. The respiratory pump was regulated according to the mentioned above European standard (Sinus patter, Tidal volume of 500 ml, 15 breath/min, Inspiration: Expiration= 1:1). The amount of deposited aerosol was determined by weighing dry filters following a residual gravimetric method (Vecellio et al., 2004).

2.6. Signal processing characterization of acoustic airflow

The acoustic airflow superimposed on the aerosolized drug was characterized in order to understand its effect on the intrasinus drug deposition. C-weighted Sound level meter (EXTECH Instruments, USA), used for the measurement of the maximum sound pressure level, assembled to the data acquisition and recording system (ROGA Instruments, Germany) have been used to measure the acoustic airflow coming out of the nebulizers. After

measurement of the acoustic signal and recording it, spectral analysis was performed using short-time Fourier transform (STFT), that is a Fourier related transform used to determine the sinusoidal frequency of local sections of shorter segments of a signal as it changes over time, for the determination of the frequency components (fundamental amplitude and potential harmonics).

2.7. Statistical analysis

Data of gentamicin concentration deposited into the maxillary sinuses obtained by FPIA as well as metrology study data were analyzed using Prism 5.0 software (GraphPad, SanDiego, CA). Graphs were plotted and significance *** ($P<0.001$) and ** ($P<0.01$) was established by two-way ANOVA test ($p<0.05$) for the gentamicin deposition study and one-way ANOVA test ($p<0.05$) for the metrology analysis.

3. Results

3.1. Signal processing of acoustic airflow

Two modes of acoustic airflow produced by different nebulizing systems were measured and analyzed for better understanding of the frequency components of each signal. Frequency components displayed on figure below (Fig. 2) were determined using the spectrogram of the short-time Fourier transform (STFT), which corresponds to its square modulus. On the representations of Fig. 2, the upper curve gives the signal in time, the left curve gives the modulus of the Fourier transform of the signal, and color image corresponds to the amplitude of the spectrogram (red = high, blue = low). The prevalent frequency component of the high frequency acoustic airflow is 200 Hz, which is the second harmonic of the fundamental frequency component of 100 Hz (Fig. 2). As can be observed, the signal is not purely sinusoidal and is relatively noisy, due to the resonances in the silicon tube. As for the second type of acoustic airflow, i.e. the low frequency acoustic airflow, it was observed that the signal possesses the fundamental component of 45 Hz, and it is a clean signal without noise (Fig. 2).

3.2. Particle sizing and aerosol output

The MMAD obtained were 2.75 ± 0.2 µm versus 2.25 ± 0.4 µm for the high frequency device (w versus w/o acoustic airflow respectively) and 3.3 ± 0.2 µm versus 3.3 ± 0.1 µm for the low frequency device (w versus w/o acoustic airflow respectively). Thus, results of aerosol particle size characterization have demonstrated that there is an insignificant (P= 0.4) impact of acoustic airflow on MMAD; however the MMAD of aerosol particles differs from one nebulizer to another depending on the design of the medical device (Fig. 5). Similarly, the output measurements of aerosol demonstrated insignificant decrease in output (ml) of 10% (from 0.62 ml to 0.55 ml) and of 21% (from 1.04 ml to 0.81 ml) upon superposition of the

high and low frequency acoustic airflow respectively, where the output rate of the high frequency acoustic airflow device is 0.2 ml/min versus 0.22 ml/min for the low frequency acoustic airflow device. Without acoustic airflow, about 41% of the initially introduced volume of NaF was nebulized by the low frequency acoustic airflow nebulizing system, and about 69% of this initial volume was nebulized by the high frequency acoustic airflow nebulizing system. Upon superposition of the acoustic airflow, the output fraction decreased to 37% and 54.5% in high and low frequency acoustic airflow respectively.

3.3. Radioactive gas ventilation of maxillary sinuses w or w/o acoustic airflow

Qualitative scintigraphic images of ^{81m}Kr-gas ventilation were acquired with and without the addition of acoustic airflow (Fig. 3).

Referring to Fig. 3, to the part of the figure corresponding to the ventilation of ^{81m}Kr-gas without acoustic airflow through nebulizers, we can observe that only the nasal fossa was shown on the image in addition to the nebulizer coupled to the nasal plug. As a result, without the addition of acoustic airflow and independently of the nebulizer used, maxillary sinuses were not shown on the scintigraphic images indicating that there was no penetration of the ^{81m}Kr-gas into them.

Referring to the other part of the figure corresponding to the ventilation of ^{81m}Kr-gas with acoustic airflow through the nebulizers, upon superimposing the high frequency vibrating airflow on the aerosol delivered by the low frequency acoustic airflow nebulizing system, maxillary sinuses' regions clearly appeared on the scintigraphic image. The image of the ^{81m}Kr-gas ventilation using the low frequency vibrating acoustic airflow added shows similar sinus ventilation results.

3. 4. Quantitative aerosol deposition into the maxillary sinuses

Impact of acoustic airflow on intra-sinus drug deposition is shown in Fig. 4. We have studied the effect of two different acoustic airflow modes: the high frequency and the low frequency pressure waves. The obtained results clearly demonstrate that:

- Despite the type of acoustic airflow mode being superimposed on the nebulized drug, the intra-sinus drug deposition into both the left (LMS) and right maxillary sinuses (RMS) is relatively higher in the presence of acoustic airflow than in its absence.
- Compared to aerosol deposition without acoustic airflow, the high frequency vibrating acoustic airflow superimposed onto the nebulized drug led to an increase of at least 4-fold in gentamicin deposition into the LMS and 2-fold deposition increase into the RMS (Fig. 4).
- Compared to aerosol deposition without acoustic airflow, the low frequency vibrating airflow superimposed on the drug nebulized has also demonstrated a significant increase of at least 4-fold for the RMS and 2-fold increase for the LMS (Fig. 4).
- The concentration of gentamicin deposition into the left maxillary sinus was observed to be higher than its deposition into the right maxillary sinus despite the presence or absence of acoustic airflow. This is due to the morphological difference of the ostia of the plastic nasal replica (diameter of the LMS ostium is three times higher than that of the RMS ostium), as previously explained in one the former studies (Leclerc et al., 2014a).

4. Discussion

Impact of different modes of acoustic airflow on gas sinus ventilation

The efficiency of application of the acoustic airflow to the nebulizing system for the enhancement of the radioactive ^{81m}Kr-gas ventilation through the maxillary sinuses has been clearly demonstrated. Without the application of acoustic airflow, ^{81m}Kr-gas wasn't able to penetrate the poorly ventilated organs; thus, circulating in the nasal fossa only. However, in the presence of acoustic airflow, whatever the frequency value of the acoustic airflow, clear ^{81m}Kr-gas ventilation through the MS was obtained. These results are in good accordance with the results published by Moeller et al. where the efficiency of the pulsating airflow was demonstrated by a significant increase in total ^{81m}Kr-gas activity in the MS of the nasal cast (Moeller et al., 2008). Similar conclusion has been drawn by Maniscalco et al. stating that humming leads to the enhancement in sinus nitric oxide (NO) levels when compared to silent exhalation (Maniscalco et al. 2003). In perfect coherence with these previous studies, our work clearly showed that sounding airflow leads to sinus ventilation regardless the frequency and intensity of the acoustic airflow. These factors, in addition to the geometrical dimensions of the maxillary ostia and MS, also play a significant role.

Gas ventilation is a basic requirement to obtain an effective airborne drug delivery to the site of infectious disease; such as maxillary sinuses. The benefit of acoustic airflow to enhance the gas ventilation through the maxillary sinuses can be explained using the Helmholtz resonator theory. Maniscalco et al. demonstrated that an oscillating airflow produced by phonation caused a rise in the gas exchange between the nose and the paranasal sinuses. This gas ventilation was even more important when the frequency of the acoustic airflow was close to the resonant frequency of the sinus cavity (Maniscalco et al., 2006). In fact, a large cavity of air (*e.g.* a maxillary sinus) connected to a smaller diameter neck (*e.g.* an ostium) can resonate at a natural frequency according to the Helmholtz resonator theory (Vieweg, 1863). As a

result, the ventilation of secondary spaces can be achieved by applying a resonance frequency that is related to the geometry of the resonant system. If ostia connected to the maxillary sinuses are compared to Helmholtz resonators (Fig. 6), the resonance frequency can be calculated using the formula (Fig. 6) where ν is the speed of sound, A is the cross-sectional area of the ostium, V is the volume of the maxillary sinus, and l the length of the ostium. According to the Helmholtz resonator theory, we used a simple geometric model where the maxillary ostium is approximated as a cylindrical tube and the sinus as a sphere. The diameter and length of ostium as well as the volume of sinus were determined from CT scan. The volume of the sinus was 10 cm^3 for the LMS and 14 cm^3 for the RMS. We also assessed the geometric features the short and broad ostium of the LMS (diameter: 4-5 mm, length: 4-5 mm) and the long and narrow ostium of the RMS (diameter: 3-4 mm, length: 7-8 mm). Then, applying the formula of the Helmholtz resonance, it can be assumed that the range of the theoretical resonant frequency were 150 Hz - 200 Hz for the RMS and 300 Hz - 400 Hz for the LMS.

Impact of different modes of acoustic airflow on intrasinus drug deposition

Studies of aerosol drug deposition into the maxillary sinuses under the influence of two different sound acoustic waves: the high frequency and the low frequency, performed on the plastic nasal replica, have demonstrated a significant influence of the acoustic airflow superposed on the airborne drug in the enhancement of intra-sinus drug deposition. In presence of acoustic airflow, a strong enhancement in the deposition of the nebulized gentamicin into the maxillary sinuses was observed after measuring the gentamicin concentration in the rinse liquid. Besides, a low amount of gentamicin was found at baseline in the MS w/o acoustic airflow although the major implication of ^{81m}Kr-gas results is that w/o acoustic airflow there should not be gas access to sinus cavities. We may explain this result by

the long medication delivery (above 10 min). As a result, there may be a huge drug overload in the nasal fossa. Moreover, a possible mucosa shrinking during plastination procedure may cause a slight enlargement of the ostia diameter. All things considered, although significant attention has been paid to the rinsing protocol to avoid biases, it is impossible to exclude that this medication overload may cause a slight washing of the drug from the nasal fossa into the sinus cavities.

The two types of acoustic airflow investigated in our study have improved the intra-sinus drug penetration though at different degree of deposition, specifically when considering the left and right maxillary sinuses separately. Considering the impact of each acoustic airflow independently, it can be observed that the high frequency vibrating airflow has increased the gentamicin deposition by at least 2-fold in the right maxillary sinus and by 4-fold in the left maxillary sinus. With respect to the low frequency pressure wave, it can be clearly noticed from the obtained results that the drug deposition in the right maxillary sinus was increased by 4-fold compared with the 2-fold increase in the left maxillary sinus.

We previously demonstrated that the configuration of our nasal cast mainly serves as a decongested nasal unit in a living healthy human volunteer (Durand et al. 2010). In CRS patients, ostia are in part narrowed or completely blocked. This can limits the use of our cast to extrapolate results of aerosol transport to sinuses in CRS patients. However this work aims at studying the effect of different modes of acoustic airflow on aerosol drug penetration into maxillary sinuses for different ostia geometry (short and broad versus long and narrow). In this sense, the use of the nasal replica was useful and highly relevant. Besides Moeller et al. demonstrated that pulsating airflow enhances the aerosol deposition not only in healthy volunteers and CRS patients after FESS (i.e. with an opening that connects a sinus to the nasal

cavity), but also in CRS patients prior to FESS (i.e. with narrowed or completely obstructed ostium) (Möller et al. 2013).

Referring to the results of aerosol particle sizing, it has been observed that superposition of the acoustic airflow on the nebulization left low impact on the aerosol particle size. With respect to the aerosol output, similar observation was made. Relating these observations to the results obtained in previously mentioned experiments, it might be said that the low frequency and the high frequency acoustic waves, which superposition on the nebulized aerosol left insignificant impact on the output of this aerosol.

Focusing on the Helmholtz resonator theory (Moller et al., 2014; Vieweg, 1863) where the resonance frequency of the cavity is directly proportional to the geometry of the ostium, we rely in our explanation of the difference in the degree of increase in the drug deposition inside the sinuses on the geometrical variation of both sinuses of the replica. As mentioned earlier, the left ostium is shorter and broader than the right ostium which is longer and narrower. According to the formula of the Helmholtz resonance, it can be assumed that the short and broad ostium will require higher resonance frequency than the long and narrow ostium. Similarly, we can explain a stronger influence of low frequency acoustic airflow on the enhancement of the drug deposition into the right sinus when compared to the effect of high frequency airflow. Hence, with this perspective, our experimental results are in perfect accordance with the theory standing behind the Helmholtz resonator. The geometric features can in part explain why the high frequency acoustic airflow was more efficient in enhancing the drug deposition in the LMS than in the RMS. Nevertheless, we can notice a slight gap between the theoretical resonant frequency calculated from the Helmholtz resonance theory (150 Hz - 200 Hz for the RMS and 300 Hz - 400 Hz for the LMS) compared to the frequency of the acoustic airflow used for experimental measures of intrasinus drug deposition (100-

200Hz for the high frequency acoustic airflow compared to 45Hz for the low frequency acoustic airflow). This difference may be due to the simple geometric approximation of the Helmholtz resonance theory (the ostium is approximated to a cylindrical tube and the sinus to a sphere) too far from anatomic features.

Knowing that in patients with chronic rhinosinusitis, depending on the severity of the illness, the ostium of the maxillary sinus is either partially blocked or completely obstructed. Before reaching the maxillary sinuses, the drug first passes through the ostia. Depending on the degree of obstruction of the ostium, the concentration of drug penetrated into the sinuses varies. Our study therefore has pointed to the fact that for a given diameter of an ostium, a certain mode of acoustic airflow characterized by specific frequency should be more effective in enhancement of nebulized intra-sinus drug penetration compared to other acoustic airflow modes. Hence, in the light of results obtained in our study, we can make a hypothesis that using patient specific acoustic airflow (depending on the ostia geometry), improved treatment of CRS could be achieved in the future.

However, a safe conclusion would be drawn after performing further studies on our nasal replica and clinical trials thereafter. As an example, a randomized, double-blind, placebo-controlled pilot study was performed by Mainz et al. in cystic fibrosis patients with upper airway Pseudomonas *aeruginosa* colonization. According to the results obtained, the numbers of P. *aeruginosa* colonies were reduced in four of the six patients inhaling tobramycin sinonasally as vibrating aerosols but in none of the patients inhaling placebo (Mainz et al. 2014). This pilot study showed that sinonasal inhalation of antibiotics applied as vibrating aerosols gave promise as a non-invasive method for the treatment of upper airway diseases. However, the severity of disease of each patient wasn't specified, so we cannot conclude to what extent the maxillary ostia and its sinuses were obstructed. In this case, it was shown that the application of acoustic airflow enhances the deposition of aerosolized drug into poorly-

ventilated cavities in general. It is also important to notice that our study focused on the MS but other nasal cavities are involved in CRS. Further study should be done to improve our understanding of the effect of acoustic airflow in paranasal sinuses including frontal sinus and ethmoidal sinuses.

Although our results have been obtained from a model of a healthy non-congested subject, we have clearly demonstrated the positive impact of acoustic airflow on intra-sinus drug deposition generally and specifically at various rates depending on the frequency of the acoustic airflow and the geometry of the ostium. Therefore, it is safe to say that nebulized antibiotics under the effect of acoustic airflow are able to penetrate into the maxillary sinuses, as long as there is an access from the ostia.

5. Conclusion

We have demonstrated that a significant deposition of gentamicin into the maxillary sinuses was obtained in a human plastinated nasal cast upon superposition of the acoustic airflow on the nebulized drug. However, the degree of this deposition varied with the type of acoustic airflow being superimposed, and it also differed as a function of the geometric features of the ostia and the maxillary sinuses. High frequency acoustic airflow (200 Hz) has proved to be efficient in aerosolized drug deposition in shorter and broader ostium (LMS in our nasal replica) while the low frequency acoustic airflow (45 Hz) was more efficient in drug deposition into longer and narrower ostium (RMS in our nasal replica). Referring to the variation of anatomy between the ostia of the model; specifically the diameter of the ostia, we confirmed that for a given ostium geometry and sinus volume, there is a specific frequency resulting in the optimal drug deposition. For better targeting of maxillary sinuses of different degree of obstruction, a specific acoustic airflow has to be chosen for ensuring an effective treatment.

Authors' contributions

JP, MD, LL contributed to the conception and the design of research.

LL, AEM, JP, NP, LN materially participated in the acquisition of data, research experiments analysis and interpretation of data.

LL, AEM and JP were involved with drafting article.

All authors were involved with reviewing the article.

All authors have approved the final version of this article.

Conflict of interest

Marc Durand is a scientific consultant for DTF society.

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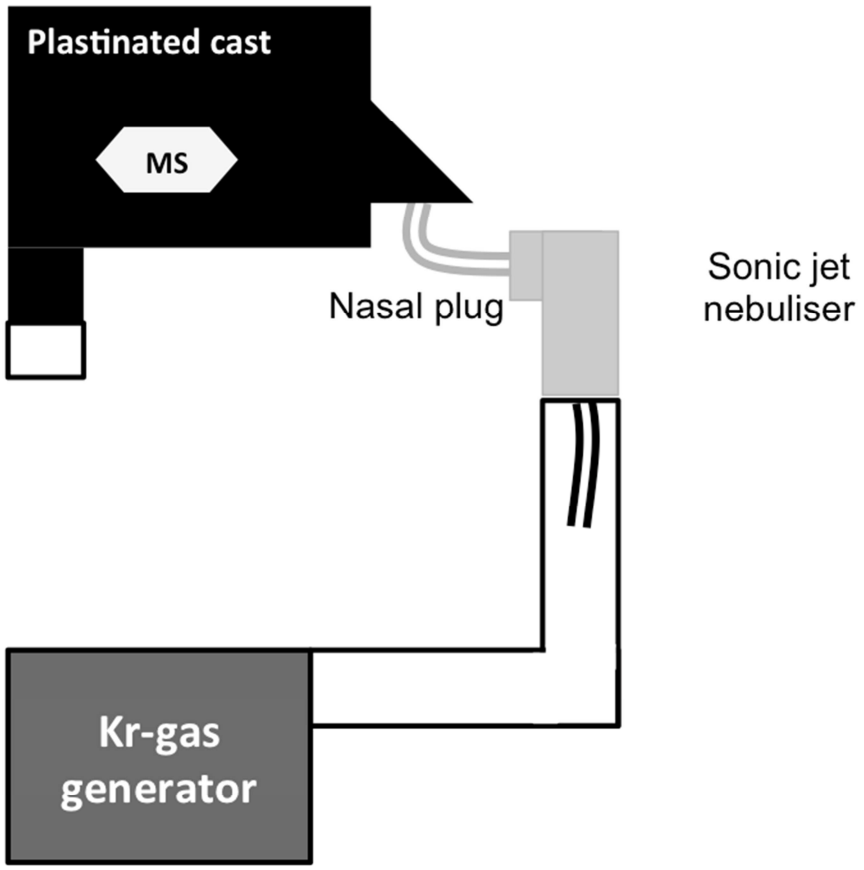

Figure 1. Experimental design of ^{81m}Kr-gas ventilation study.

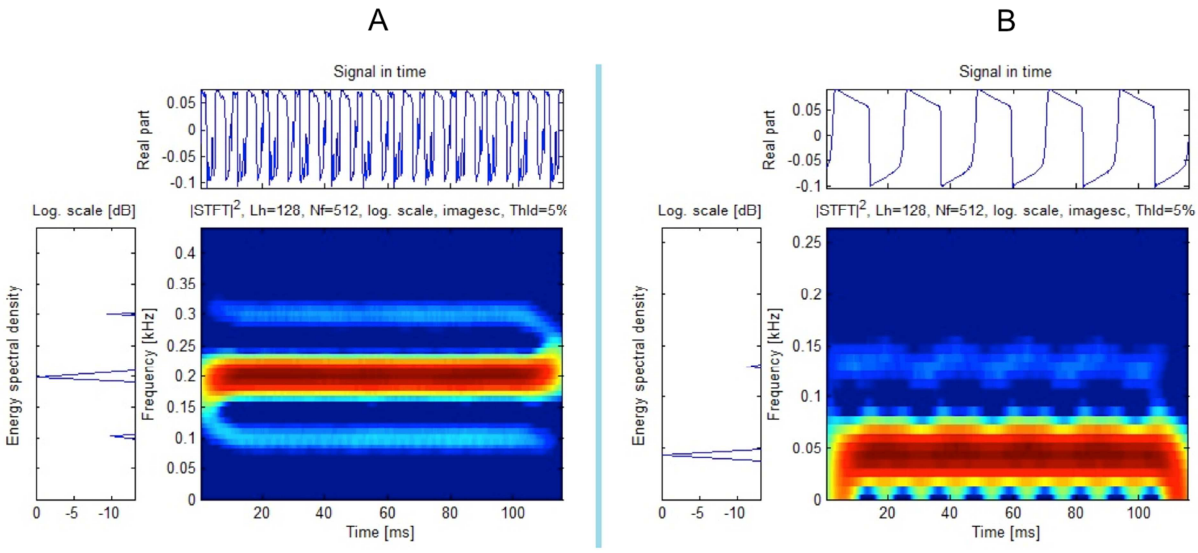

Figure 2. Spectral analysis using Short-Time Fourier Transform (STFT) of the vibrating airflow generated by the two nebulizing systems. A) High frequency nebulizing system B) Low frequency nebulizing system.

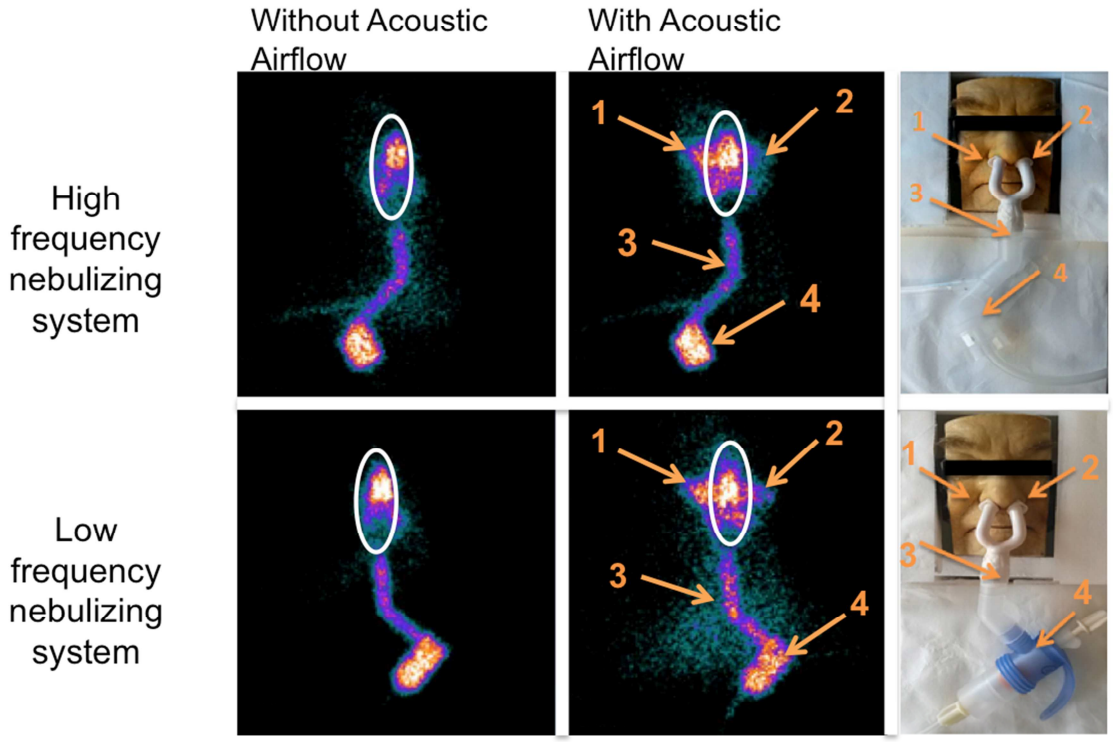

Figure 3. Anterior gamma camera images of ^{81m}Kr-gas ventilation in a plastinated cast. (1) Right Maxillary Sinus (RMS) (2) Left Maxillary Sinus (LMS) (3) Nasal plug (4) Nebulizer.

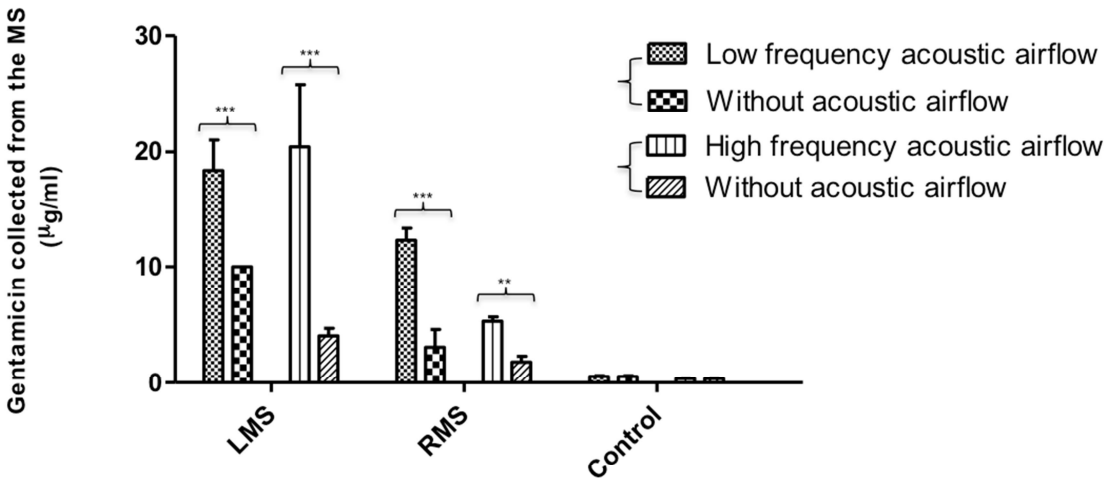

Figure 4. Impact of high frequency and low frequency acoustic airflow on nebulized gentamicin collected in the maxillary sinuses (MS) in $\mu g.ml^{-1}$. Nebulized physiological saline solution was used as the control ($p<0.05$).

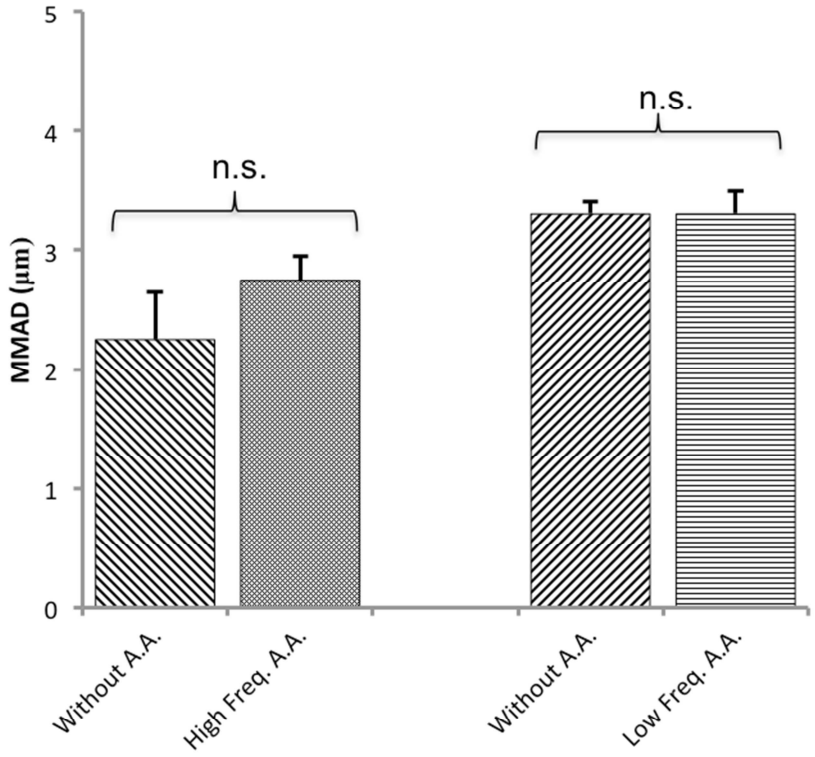

Figure 5. Impact of high frequency and low frequency acoustic airflow on the variation in MMAD (in µm) of airborne particles.

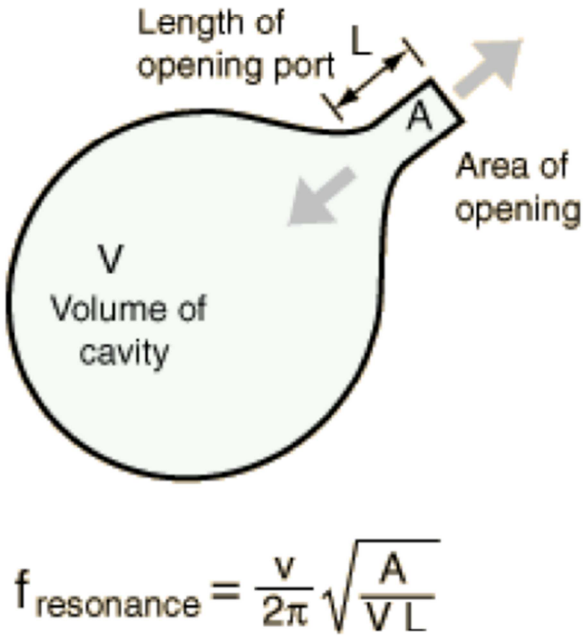

Figure 6. Helmholtz resonator design.