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Antibacterial efficacy of inhaled squalamine in a rat model of chronic *Pseudomonas aeruginosa* pneumonia

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Objectives: Squalamine is a steroid extracted from sharks with proven *in vitro* antibacterial activity. We assessed its efficacy in reducing the lung bacterial load and histological lesions when given via inhalation in a rat model of chronic *Pseudomonas aeruginosa* pneumonia.

Methods: Sprague-Dawley rats were inoculated by tracheal intubation with 150 μL of a solution containing 10^8 cfu/mL of agar bead-embedded *P. aeruginosa* strain PAO1. MICs of squalamine and colistin for this strain were 2–8 and 0.5–1 mg/L, respectively. Starting the day after infection, the animals were treated twice daily with aerosolized squalamine (3 mg), colistin (160 mg) or 0.9% saline for 6 days. The bacterial load and lung histological lesions were evaluated on the seventh day.

Results: Aerosols of squalamine and colistin resulted in a significant reduction in median (IQR) pulmonary bacterial count compared with saline [10^3 (6×10^2–2×10^3), 10^3 (9×10^2–6×10^3) and 10^5 (9×10^4–2×10^5) cfu/lung, respectively; $P<0.001$ for both treated groups versus saline]. The lung weight and the lung histological severity score were significantly lower in both treated groups.

Conclusions: In a model of chronic *P. aeruginosa* pneumonia, treatment twice daily with a squalamine aerosol for 6 days leads to a significant reduction in the pulmonary bacterial count and pneumonia lesions with an efficacy comparable to that of colistin.

Keywords: animal models, anti-infectious drugs, aerosols, colonization

Introduction

The antibiotic treatment of non-community-acquired pneumonia is a challenging issue. Among these pneumonias, pulmonary infections complicating cystic fibrosis (CF) and nosocomial pneumonia are increasingly caused by multidrug-resistant (MDR) bacteria, notably by bacteria resistant to carbapenems.[1,2] The use of prolonged intravenous treatment with molecules such as colistin or aminoglycosides induces toxicity,[3] especially renal toxicity.[4] Consequently there is currently a great need for the development of new antimicrobial molecules that are active against MDR bacteria.[5] In this context, innovative molecules such as aminosterol derivatives have recently gained interest due to their effective antimicrobial activity.[6] Squalamine is a natural aminosterol extracted from *Squalus acanthias* (dogfish shark) (Figure 1).[7] This amphiphilic steroid has shown anticancer properties in addition to antibacterial[8,9] and antiviral activities.[10] Squalamine interacts with the bacterial external membrane[11] and destabilizes its structure, increasing the permeability of the bacteria to the external medium. Alhanout *et al.*[12] demonstrated its bactericidal action against Gram-negative bacteria such as *Pseudomonas aeruginosa* and Gram-positive bacteria such as *Staphylococcus aureus*. Squalamine also exhibited *in vitro* activity against clinical MDR strains isolated from patients with CF.[11] In this study, the low reported MIC of squalamine for non-mucoid strains of MDR *P. aeruginosa* (2–8 mg/L)[11] suggests its efficacy during high bacterial burden infections. Its mechanism of action is not affected by efflux pump resistance, and, to date, no bacteria has demonstrated resistance to squalamine.[12] Squalamine has also been shown to have antifungal activity against moulds isolated from CF patients.[13] This remarkable antimicrobial potential could be used for the treatment of nosocomial pneumonias and chronic lung infections in CF patients.

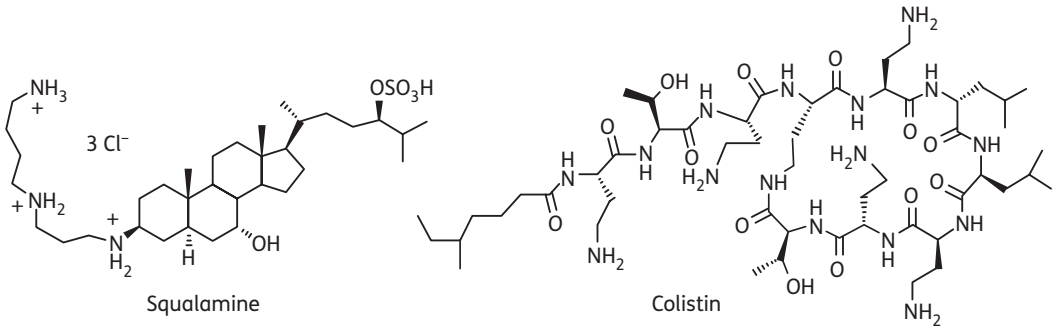

Figure 1. Structures of squalamine and colistin.

Because of the limited lung penetration of some intravenous antibiotics, inhaled antibiotics may be more effective and less toxic. In CF patients, the administration of antibiotics via inhalation has proven efficacy because of the ability to deliver high local concentrations in contact with the biofilm of the respiratory tract.[14] In patients with ventilator-associated pneumonia, recent studies have analysed the effects of inhaled antibiotics, either alone or as an adjunctive therapy to intravenous treatment.[15–17] Some resistant bacterial strains exhibit an MIC reaching 64 mg/L for squalamine,[11,12] which supports the use of inhaled local administration to reach high concentrations at the site of infection. Very recently, squalamine derivative formulations have been shown to be suitable for aerosol delivery.[18]

Therefore, the main objective of this study was to evaluate the efficacy of inhaled squalamine in reducing the lung bacterial load and histological lesions in a commonly used animal model of chronic *P. aeruginosa* lung infection using intratracheal instillation of bacteria embedded in agar beads.[19]

Methods

Preparation of bacteria

Agar bead-embedded *P. aeruginosa* (strain PAO1, ATCC reference 15692) was used for lung infection. The MICs of squalamine and colistin for this strain are 2–8 and 0.5–1 mg/L, respectively.[11] The beads were prepared according to a protocol adapted from a previously described method.[19] In brief, five colonies of *P. aeruginosa* were cultured overnight at 37°C with continuous shaking to obtain an optical density of 1 at 600 nm. Next, 2×10^{10} bacteria were pelleted by centrifugation at 4000 rpm for 10 min at 4°C, resuspended in 1 mL of PBS (pH 7.4) and added to 9 mL of 1.5% Trypticase soy agar (TSA). The mixture was pipetted forcefully into 150 mL of heavy mineral oil (Sigma-Aldrich) that had been prewarmed to 50°C, stirred rapidly with a magnetic stirring bar for 10 min at room temperature and then cooled at 4°C with continuous stirring for 20 min. The oil/agar mixture was centrifuged at 4000 rpm for 20 min to sediment the beads. The beads were then washed once with 0.5% deoxycholic acid sodium salt (SDC) in PBS, once with 0.25% SDC and four times with PBS. The beads were microscopically measured, and the number of bacteria in the beads was determined by homogenizing the suspension and plating 10-fold serial dilutions on agar plates. The inoculum for infection was prepared by diluting the bead suspension with PBS to obtain approximately 10^8 cfu/mL.

Animals

We used male Sprague-Dawley rats weighing 300–380 g (SAS Janvier, Le-Genest-St-Isle, France). During all experiments the rats were housed in a ventilated pressurized cabinet (A-BOX 160, Noroit, Rezé, France) with food and water available *ad libitum*. All experiments were performed according to the guidelines of the Ethics Committee for animal treatment. The protocol was approved by the Ethics Committee of l'Université de la Méditerranée, Marseille, France (agreement reference number 11-10042012).

Model of chronic lung infection

To assess the bacterial growth and the severity of infection without treatment, in the first experiment, 25 rats were infected and sacrificed 6 h ($n=5$), 1 day ($n=5$), 3 days ($n=5$), 7 days ($n=5$) or 14 days ($n=5$) after inoculation. Six rats were used as controls and six were inoculated with sterile agar beads.

For infection, the animals were anaesthetized using Sévoflurane® (Abbott, Rungis, France). Inoculation was performed via tracheal intubation using a 16 gauge catheter and a 1 mL syringe filled with 150 μL of a suspension of agar bead-embedded bacteria. The animals were weighed every day until sacrifice. Euthanasia was performed using an overdose of thiopental, and the lungs were removed under sterile conditions. After macroscopic observation, the right lung was weighed and then mixed in PBS using an Ultra-Turrax® homogenizer (IKA, Germany). A 10-fold serial dilution technique and TSA medium cultures were used to determine lung bacterial content. The left lung was fixed in 10% formaldehyde for histological examination.

Infection with treatment

A second experiment focused on treatment efficacy. Treatments were aerosols of colistin or squalamine. The non-treated group (controls) received aerosols of saline. Since, to our knowledge, no previous published data report the use of aerosols of colistin or squalamine for pneumonia in rats, the dose of colistin was based on data in large and small animals for *P. aeruginosa* or *Acinetobacter baumannii* acute pneumonia[20,21] using 8 and 6 mg/kg per administration, respectively. The dose of squalamine was selected to reach 0.15 mg/kg into lung parenchyma on the basis of a therapeutic effect at 10-fold the MIC (squalamine MIC for PAO1=2–8 mg/L). Previous tests on toxicity showed that 10 mg/kg of intratracheal squalamine induced no clinically and pathologically detectable toxicity (10 animals studied, data not shown). The target concentration was approximately estimated on the basis of aerosol characteristics and calculated as described below. Squalamine

was kindly provided by Professor M. Zasloff (Georgetown University, Washington, DC, USA).

Aerosols were administered using a jet nebulizer (Harvard Apparatus, Les Ulis, France) connected to an inhalation chamber containing four animals, equivalent to a 'nose-only' aerosol.[22] The airflow was 6 L/min, and each aerosol administration lasted approximately 30 min. The nebulizer was charged with 5 mL of 0.9% saline alone (control group) or with saline containing either 3 mg of squalamine or 160 mg of colistin. Aerosolized solutions were buffered to obtain a pH of 7.4. The estimated amount of squalamine and colistin inhaled by the rats was calculated from the product of the concentration of the drug in the chamber, the minute ventilation of the rats (lung volume times respiratory rate) and the exposure time.[23,24]

Twenty-four rats infected according to our model of chronic lung infection were randomly treated with aerosolized squalamine ($n=8$), colistin (Sanofi-Aventis, France, $n=8$) or saline ($n=8$). To evaluate the lung toxicity of the aerosol treatments, nine uninfected animals were treated for 6 days with aerosolized squalamine ($n=3$), colistin ($n=3$) or saline ($n=3$). Aerosol treatments were performed twice per day at 12 h intervals starting the day after infection until the seventh day of evolution, totalling 6 days of treatment. This duration was decided on the basis of lung bacterial count, which did not significantly decrease by day 7 post-infection in non-treated animals (see the Results section). On day 7, the animals were sacrificed and the lungs were removed under sterile conditions and processed for microbiological and pathological assessment.

Histological severity score (HSS)

Sections (3 μm thick) were obtained from the upper, mid and lower parts of the lungs, including the whole circumference. The sections were stained with haematoxylin and eosin. Examination was performed by a pathologist blinded to the group identity (H. L.). An HSS was calculated based on the number of bronchopneumonia lesions (0, no lesions; 1, <30 lesions/lung; 2, ≥30 lesions/lung; 3, confluent lesions of bronchopneumonia), as previously reported.[25]

Measurement of the aerosol particle size

Analysis of the distribution of the particle sizes was performed after the administration of an aerosol of squalamine, colistin or saline as in the animal experiments using a Malvern Mastersizer S apparatus (Malvern Instruments, UK) at a constant flow rate of 6 L/min with a 100 mm lens (measuring range from 0.5 to 175 μm). The nebulizer mouthpiece was placed at a distance of 20 mm from the lens face and 23 mm from the laser beam axis.

Statistical methods

Like others, this model can be characterized by variability in the extent and time course of infection, in part due to variables related to the animals. Therefore a difference of 2 logs between a treated and a non-treated group was considered relevant. Assuming such a difference, we calculated that eight animals per group were necessary to show a treatment effect, with 100% statistical power and a two-sided alpha value of 0.05. Data were expressed as the mean ± SD or the median (IQR) according to the distribution of the data. The effects of the treatments were analysed using a one-way analysis of variance or the Kruskal–Wallis test. The Student's *t*-test or the Mann–Whitney rank-sum test was used for intergroup comparisons. Data analysis was performed with SPSS for Windows (Chicago, IL, USA), version 12.0. $P \leq 0.05$ was considered statistically significant.

Results

Model of chronic lung infection

Solutions of agar beads used for the experiments contained 10^8 (6×10^7–4×10^8) cfu/mL of *P. aeruginosa*. The mean size of the agar beads was 235 ± 60 μm. Of the 25 infected animals, none died prematurely. The rats exhibited weight loss during the first 3 days post-infection (weight before infection, 362 ± 6 g; weight at day 3, 352 ± 6 g; $n=15$) and then had a normal growth curve for the 11 next days. From day 1, macroscopic observation of the infected lungs showed bilateral lesions resulting from oedema, haemorrhage and focal bronchopneumonia. The right lung weight of infected rats was significantly higher than that of uninfected rats at day 7 (1.24 ± 0.08 versus 0.56 ± 0.04 g, $P<0.01$, $n=5$ for each group) and at other timepoints (data not shown).

Bacterial growth in lung tissues from days 1 through 14 is presented in Figure 2. The growth curves exhibited a peak in the cfu/lung at day 1 [4×10^6 (6×10^5–10^7), $n=5$]. Although some animals started to clear the infection spontaneously at day 7, the median lung bacterial count remained high between day 3 [8×10^5 (6×10^5–9×10^5), $n=5$] and day 7 [3×10^5 (8×10^4–6×10^5), $n=5$], suggesting that a 7 day period following inhalation was adequate for the treatment evaluation.

The HSS was 1.2 ± 0.4 at 6 h after infection ($n=5$) and 2.7 ± 0.3 at day 1 ($n=5$), with lesions characteristic of confluent bronchopneumonia. At day 7, the HSS remained at 2.6 ± 0.2 ($n=5$) and then decreased to 0.3 ± 0.3 at day 14 ($n=5$). The animals that had received agar beads lacking bacteria had normal lung characteristics at day 1, non-specific injury at day 3 and total recovery at day 7.

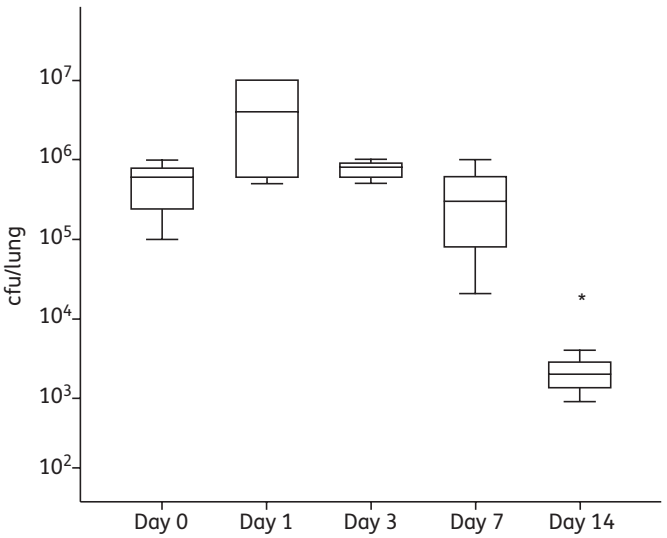

Figure 2. Time course of lung bacterial growth during the 14 days following bacterial inoculation in untreated rats ($n=5$ for each timepoint). Box plots represent the median and 25th and 75th percentiles; bars represent the 5th and 95th percentiles. *$P<0.05$ versus baseline.

Effects of aerosol treatments

The size of the aerosolized particles was 3 (1.5–5.2) μm for squalamine, 2.8 (1.6–5.5) μm for colistin and 3 (1.7–5.3) μm for saline (P=not significant between drugs). Aerosols of saline, squalamine and colistin induced no detectable clinical or histological change in uninfected rats (n=9, data not shown).

At the end of the study period (7 days post-infection), all animals were alive (n=24). The lung bacterial count was significantly lower in animals treated with squalamine or colistin when compared with saline [10^3 (6×10^2–2×10^3), 10^3 (9×10^2–6×10^3) and 10^5 (9×10^4–2×10^5) cfu/lung, respectively; $P<0.001$ for both treated groups versus control; n=8 for each group; Figure 3]. No significant difference was found between the squalamine and colistin groups.

The right lung weight was also significantly lower in animals treated with squalamine or colistin than in controls (0.84 ± 0.05, 0.98 ± 0.16 and 1.26 ± 0.07 g, respectively; $P<0.05$ for both treatment groups versus control; n=8 for each group; Figure 4). Pathological examination showed that the lesions of diffuse and confluent bronchopneumonia were markedly reduced in the treatment groups, and especially in the group receiving squalamine, in which areas of bronchopneumonia were rare and without abscess formation (Figure 5). The resulting HSS was lower in the squalamine- and colistin-treated groups than in the control group (1.4 ± 0.8, 1.2 ± 0.8 and 2.7 ± 0.5, respectively; $P<0.05$ for both treatments versus control; n=8 for each group; Figure 6).

Discussion

The results of the present study show that 6 day regimens of inhaled treatment with 3 mg of squalamine or 160 mg of colistin induced similar reductions in both the bacterial count and histological injury in a rat model of chronic lung infection with *P. aeruginosa*. This work was the first to assess the therapeutic potential of squalamine for bacterial pneumonia.

The model of lung infection using agar beads is commonly used for experiments *in vivo* in the field of CF.[19] Our results from the untreated 25 rats receiving intratracheal *P. aeruginosa* embedded in agar beads confirm the previously published reports showing a generally stable lung bacterial count up to the seventh day post-infection and 100% survival rate.[19,26] In comparison with clinical cases of hospital-acquired or ventilator-associated pneumonia, the parenchymal bacterial growth observed here, $>10^5$ cfu/mL in controls during the first 7 days, is likely to correspond to a high bacterial burden infection, since clinical cases are usually positive at 10^4 cfu/mL as sampled using bronchoalveolar lavage.[27] The biofilm created by bacteria such as *P. aeruginosa* prevents the diffusion of antibiotics administered intravenously,[28] and, in CF patients, aerosols of antibiotics have been shown to improve respiratory function.[14] Treatment with new antibiotics efficient via aerosols would be a helpful therapeutic approach, increasing the therapeutic possibilities available to date.[14] Inhaled antibiotics are also increasingly used in patients with ventilator-associated pneumonia. For example, Badia *et al.*[29] showed that inhalation of tobramycin or imipenem resulted in high antibiotic concentrations in the lower respiratory tract without systemic toxicity. In ventilated piglets with extensive pneumonia, aerosolized amikacin or ceftazidime resulted in greater lung deposition and more extensive bactericidal effects than intravenous infusion.[30] However, ventilator-associated pneumonia and/or pneumonia caused by mucoid strains represent specific pathophysiological entities, not studied here. The efficacy of aerosol therapy and especially of squalamine would need to be evaluated in this area, e.g. using ventilated animals.

Aerosolized colistin has been particularly useful since the emergence of MDR Gram-negative bacteria. Our results in rats targeting 8 mg/kg colistin in lung agree with previous findings

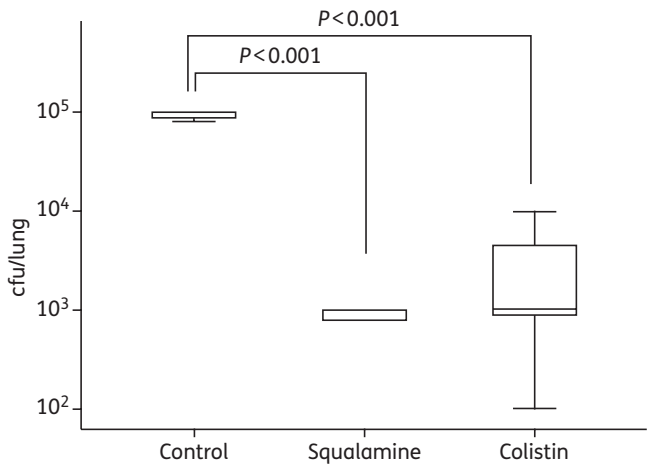

Figure 3. Lung bacterial counts after 6 days of treatment with aerosols of saline (control), squalamine or colistin (n=8 for each group). Box plots represent the median and 25th and 75th percentiles; bars represent the 5th and 95th percentiles.

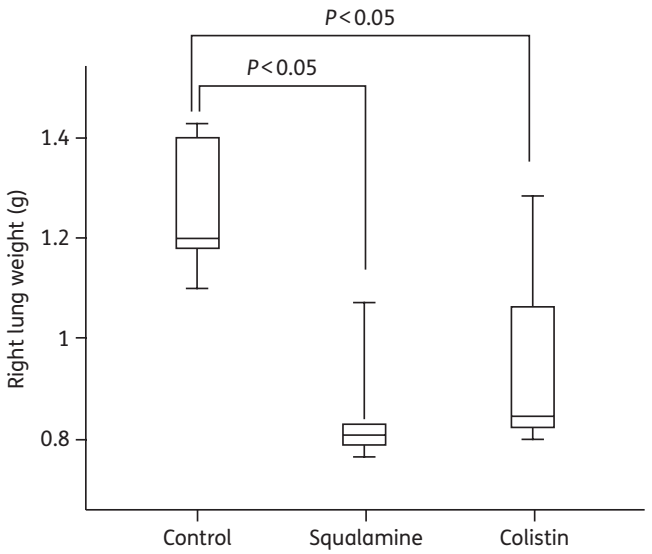

Figure 4. Right lung weight after 6 days of treatment with aerosols of saline (control), squalamine or colistin (n=8 for each group). Box plots represent the median and 25th and 75th percentiles; bars represent the 5th and 95th percentiles.

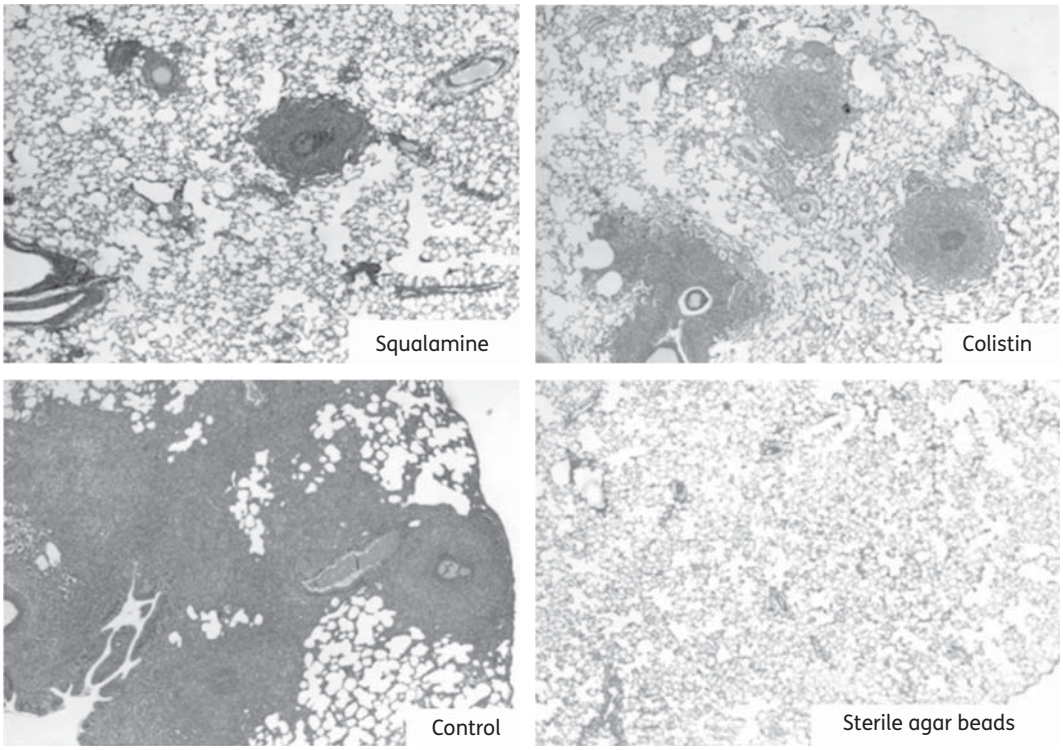

Figure 5. Lung histopathology 7 days after bacterial inoculation or inoculation with sterile agar beads. Haematoxylin/eosin-stained sections. Original magnification of pictures ×50. The control group (saline) section shows a diffuse bronchopneumonia with numerous and coalescent abscess formations. The squalamine group section shows rare areas of bronchopneumonia without coalescent abscess formations. Animals inoculated with sterile agar beads had normal lungs.

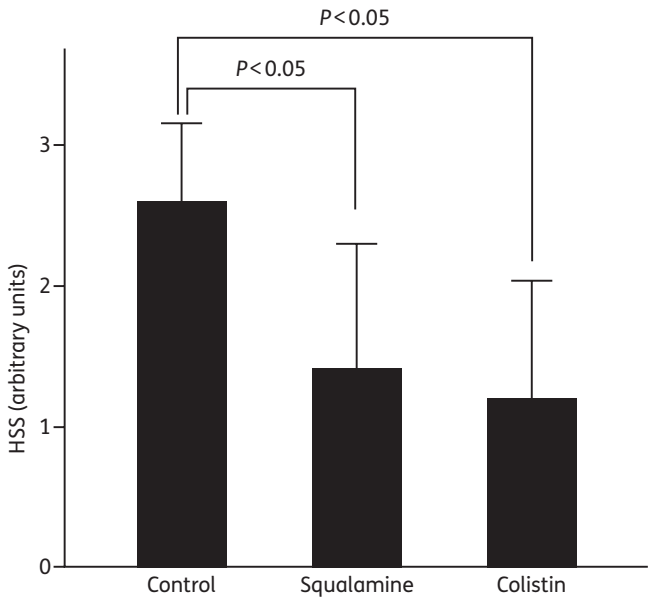

Figure 6. HSS (mean ± SD) after 6 days of treatment with aerosols of saline (control), squalamine or colistin ($n=8$ for each group).

with similar doses in infected mice with carbapenem-resistant *A. baumannii*[20] and in infected piglets with *P. aeruginosa*.[22]

The aerosolization of squalamine and its derivatives has been shown to be feasible.[18] Indeed, both jet nebulizers, such as that used in the present study, and vibrating mesh nebulizers produce particles of sufficiently small size to reach the distal airways.[18]

In uninfected animals aerosolized with squalamine, we did not detect any morbidity or mortality. The histological characteristics of lungs in this group after a 6 day aerosol treatment were normal. No previous data concerning the intrapulmonary concentration of squalamine have been published yet. However, a Phase I/IIA clinical trial in oncology patients showed the safety of this molecule after a continuous intravenous 5 day infusion of 300 mg/m^2/day.[31] Here, we did not measure the lung squalamine concentration, which represents a limitation of our study. Interestingly, according to the results of our aerosol system testing, statistical analysis demonstrated that squalamine solutions we used had aerodynamic diameters similar to those of colistin solutions. This suggests that the same proportions of aerosolized squalamine and colistin were inhaled by the animals. Otherwise, colistin and squalamine differ in their respective molecular weights, 1155 and 628 g/mol, respectively. This argues for squalamine to be effectively aerosolized and reach the lungs at least as well as colistin. In addition, squalamine, like colistin, is a positively charged molecule with a sterol core. This electro-physical property is known to favour the persistence of the molecules in the first encountered structure, here the lung. These data may favour a limited diffusion of the molecule in the blood. To determine this, we performed a test with fluorescently labelled aminosterol derivatives delivered via the tracheal route showing no blood detection of the drug,

whereas fluorescence was detected into the lung. However, lung concentration was not precisely measured, but these tests argue for the absence of plasmatic diffusion (data not shown).

Conclusions

Whereas squalamine has *in vitro* activity against Gram-positive and Gram-negative bacteria, including MDR strains, no previous study had assessed its *in vivo* efficacy against infectious pneumonia. Our results suggest that squalamine administered via inhalation can reduce the lung bacterial load and bronchopneumonia-induced histological lesions with efficacy comparable to that of colistin in animals infected with *P. aeruginosa* embedded in agar beads. Given the need to develop new antibiotics to treat antibiotic-resistant strains of *P. aeruginosa* and other bacteria, squalamine could be an effective therapeutic strategy for chronic bacterial lung infection and colonization in patients. Additional studies need to be performed in order to test its efficacy in the context of mechanical ventilation. Further studies are required to better characterize its pharmacokinetics and regional diffusion into lung parenchyma, especially when using the aerosolized route.

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Transparency declarations

The authors do not have any commercial or other association that might pose a conflict of interest.

References

1 Rice LB. Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE. *J Infect Dis* 2008; **197**: 1079–81.

2 Bassetti M, Ginocchio F, Mikulska M. New treatment options against gram-negative organisms. *Crit Care* 2011; **15**: R21.

3 Maviglia R, Nestorini R, Pennisi M. Role of old antibiotics in multidrug resistant bacterial infections. *Curr Drug Targets* 2009; **10**: 895–905.

4 Falagas ME, Kasiakou SK. Toxicity of polymyxins: a systematic review of the evidence from old and recent studies. *Crit Care* 2006; **10**: R27.

5 Livermore DM, on behalf of the British Society for Antimicrobial Chemotherapy Working Party on the Urgent Need: Regenerating Antibacterial Drug Discovery and Development. Discovery research: the scientific challenge of finding new antibiotics. *J Antimicrob Chemother* 2011; **66**: 1941–4.

6 Alhanout K, Rolain JM, Brunel JM. Squalamine as an example of a new potent antimicrobial agents class: a critical review. *Curr Med Chem* 2010; **17**: 3909–17.

7 Brunel JM, Salmi C, Loncle C *et al*. Squalamine: a polyvalent drug of the future? *Curr Cancer Drug Targets* 2005; **5**: 267–72.

8 Salmi C, Loncle C, Vidal N *et al*. Squalamine: an appropriate strategy against the emergence of multidrug resistant gram-negative bacteria? *PLoS One* 2008; **3**: e2765.

9 Djouhri-Bouktab L, Alhanout K, Andrieu V *et al*. Squalamine ointment for *Staphylococcus aureus* skin decolonization in a mouse model. *J Antimicrob Chemother* 2011; **66**: 1306–10.

10 Zasloff M, Adams AP, Beckerman B *et al*. Squalamine as a broad-spectrum systemic antiviral agent with therapeutic potential. *Proc Natl Acad Sci USA* 2011; **108**: 15978–83.

11 Alhanout K, Brunel JM, Raoult D *et al*. *In vitro* antibacterial activity of aminosterols against multidrug-resistant bacteria from patients with cystic fibrosis. *J Antimicrob Chemother* 2009; **64**: 810–4.

12 Alhanout K, Malesinki S, Vidal N *et al*. New insights into the antibacterial mechanism of action of squalamine. *J Antimicrob Chemother* 2010; **65**: 1688–93.

13 Alhanout K, Brunel JM, Ranque S *et al*. *In vitro* antifungal activity of aminosterols against moulds isolated from cystic fibrosis patients. *J Antimicrob Chemother* 2010; **65**: 1307–9.

14 Sermet-Gaudelus I, Le Cocquic Y, Ferroni A *et al*. Nebulized antibiotics in cystic fibrosis. *Paediatr Drugs* 2002; **4**: 455–67.

15 Palmer LB, Smaldone GC, Chen JJ *et al*. Aerosolized antibiotics and ventilator-associated tracheobronchitis in the intensive care unit. *Crit Care Med* 2008; **36**: 2008–13.

16 Abu-Salah T, Dhand R. Inhaled antibiotic therapy for ventilator-associated tracheobronchitis and ventilator-associated pneumonia: an update. *Adv Ther* 2011; **28**: 728–47.

17 Lu Q, Yang J, Liu Z *et al*. Nebulized ceftazidime and amikacin in ventilator-associated pneumonia caused by *Pseudomonas aeruginosa*. *Am J Respir Crit Care Med* 2011; **184**: 106–15.

18 Alhanout K, Brunel JM, Dubus JC *et al*. Suitability of a new antimicrobial aminosterol formulation for aerosol delivery in cystic fibrosis. *J Antimicrob Chemother* 2011; **66**: 2797–800.

19 Kukavica-Ibrulj I, Bragonzi A, Paroni M *et al*. In vivo growth of *Pseudomonas aeruginosa* strains PAO1 and PA14 and the hypervirulent strain LESB58 in a rat model of chronic lung infection. *J Bacteriol* 2008; **190**: 2804–13.

20 Chiang SR, Chuang YC, Tang HJ *et al*. Intratracheal colistin sulfate for BALB/c mice with early pneumonia caused by carbapenem-resistant *Acinetobacter baumannii*. *Crit Care Med* 2009; **37**: 2590–5.

21 Lu Q, Girardi C, Zhang M *et al*. Nebulized and intravenous colistin in experimental pneumonia caused by *Pseudomonas aeruginosa*. *Intensive Care Med* 2010; **36**: 1147–55.

22 Sakagami M. In vivo, in vitro and ex vivo models to assess pulmonary absorption and disposition of inhaled therapeutics for systemic delivery. *Adv Drug Deliv Rev* 2006; **58**: 1030–60.

23 Schmitt HJ, Bernard EM, Häuser M *et al*. Aerosol amphotericin B is effective for prophylaxis and therapy in a rat model of pulmonary aspergillosis. *Antimicrob Agents Chemother* 1988; **32**: 1676–9.

24 Cicogna CE, White MH, Bernard EM *et al*. Efficacy of prophylactic aerosol amphotericin B lipid complex in a rat model of pulmonary aspergillosis. *Antimicrob Agents Chemother* 1997; **41**: 259–61.

25 Marquette CH, Wermert D, Wallet F *et al*. Characterization of an animal model of ventilator-acquired pneumonia. *Chest* 1999; **115**: 200–9.

26 Cash HA, Woods DE, McCullough B *et al*. A rat model of chronic respiratory infection with *Pseudomonas aeruginosa*. *Am Rev Respir Dis* 1979; **119**: 453–9.

27 Bregeon F, Papazian L, Visconti A *et al*. Relationship of microbiologic diagnostic criteria to morbidity and mortality in patients with ventilator-associated pneumonia. *JAMA* 1997; **277**: 655–62.

28 Meers P, Neville M, Malinin V *et al*. Biofilm penetration, triggered release and *in vivo* activity of inhaled liposomal amikacin in chronic *Pseudomonas aeruginosa* lung infections. *J Antimicrob Chemother* 2008; **61**: 859–68.

29 Badia JR, Soy D, Adrover M *et al*. Disposition of instilled versus nebulized tobramycin and imipenem in ventilated intensive care unit (ICU) patients. *J Antimicrob Chemother* 2004; **54**: 508–14.

30 Goldstein I, Wallet F, Nicolas-Robin A *et al*. Lung deposition and efficiency of nebulized amikacin during *Escherichia coli* pneumonia in ventilated piglets. *Am J Respir Crit Care Med* 2002; **166**: 1375–81.

31 Herbst RS, Hammond LA, Carbone DP *et al*. A phase I/IIA trial of continuous five-day infusion of squalamine lactate (MSI-1256F) plus carboplatin and paclitaxel in patients with advanced non-small cell lung cancer. *Clin Cancer Res* 2003; **9**: 4108–15.