Suitability of a new antimicrobial aminosterol formulation for aerosol delivery in cystic fibrosis

Kamel Alhanout, Jean Michel Brunel, Jean Christophe Dubus, Jean Marc Rolain and Véronique Andrieu*

URMITE UMR 6236, CNRS-IRD, Faculté de Médecine et de Pharmacie, 27 Boulevard Jean Moulin, 13385 Marseille cedex 05, France

*Corresponding author. Tel: +33-04-91-83-55-48; Fax: +33-04-91-78-75-75; E-mail: veronique.andrieu@univmed.fr

Received 15 April 2011; returned 12 June 2011; revised 14 August 2011; accepted 22 August 2011

Objectives: We evaluated the suitability of an aminosterol derivative (ASD), possessing interesting in vitro antimicrobial activities against various resistant pathogens involved in lung infections of cystic fibrosis patients, for aerosol drug delivery.

Methods: The suitability of 2 and 10 mg/mL ASD solutions for aerosol delivery was evaluated and compared with that of a commercial inhalable solution of tobramycin using Pari LC Plus and eFlow rapid nebulizers. Physicochemical properties of ASD solutions, including pH and osmolarity, were assessed. The particle size distribution of the aerosols was analysed using a laser diffraction method. Effects of mucin on the in vitro antibacterial activities of ASD solutions and tobramycin were assessed using the broth dilution method for MIC determination.

Results: MICs of ASD and tobramycin for Pseudomonas aeruginosa ATCC 27853 were 4 and 1 mg/L, respectively, and those for Staphylococcus aureus ATCC 25923 were 1 and 0.5 mg/L, respectively. MICs of tobramycin increased at least 4- and 16-fold for both bacteria after addition of mucin at 1 and 10 mg/mL, respectively, while MICs of ASD remained unchanged. ASD solutions should be prepared in 0.9% NaCl solution in order to produce an isotonic state, and need the addition of NaOH to give a suitable pH value for inhalation. ASD solutions were successfully nebulized using both nebulizers, as reflected by the similarity of the aerodynamic parameters to those of a commercial tobramycin solution.

Conclusions: This introductory study demonstrates the suitability of ASDs for aerosol delivery and calls for further work to evaluate such formulations using a lung-infected animal model.

Keywords: infections, nebulizers, squalamine

Introduction

Pulmonary infections are the leading cause of morbidity and mortality in cystic fibrosis (CF) patients. Though the use of antimicrobial agents improves the management of CF lung infections, toxic side effects of the drugs that are used are a major problem since high doses are needed in order to obtain efficient therapeutic concentrations in the lungs. The use of inhalable antibiotics for the treatment of lung infection of CF patients is increasingly reported, as such preparations enable the administration of a sufficient dose while minimizing adverse effects. A tobramycin solution for inhalation is used mainly for the treatment of pulmonary infection in CF patients using various nebulizers, including the Pari LC Plus jet nebulizer and the eFlow rapid mesh nebulizer. However, problems related to the emergence of multidrug-resistant bacterial and fungal species as well as drug deactivation by macromolecules present in the sputum of CF patients, such as mucin, may limit the therapeutic choice, highlighting the need to develop new antimicrobial agents via this route of administration. We have previously demonstrated that natural aminosterols, such as squalamine and its synthetic aminosterol derivatives (ASDs), possess interesting in vitro antimicrobial activities against bacterial and fungal isolates, including multidrug-resistant species, recovered from sputa of patients with CF. Moreover, these compounds show a new mechanism of antimicrobial action involving the rapid disruption of bacterial and fungal membranes. While the safety of squalamine administration to humans has been demonstrated, no toxicological data are currently available regarding synthetic ASDs. Moreover, the MICs of these compounds for relevant bacterial and fungal species remain relatively elevated, suggesting that these molecules may be used via local route of administration as aerosols. Here we evaluated the suitability of a synthetic ASD (its chemical structure is available in Alhanout et al.) for...
pulmonary delivery by aerosol inhalation. Selected physicochemical properties of aqueous solutions of 2 and 10 mg/mL ASD, including pH and osmolarity, were assessed. We also tested the inhibitory effect of porcine mucin on the in vitro activity of ASD compared with tobramycin. Finally, the suitability of ASD solutions for nebulization using different commercial nebulizer systems was assessed by analysing the particle size distribution of the aerosols generated by jet and vibrating mesh nebulizers, taking a commercially available formulation of tobramycin as a comparator.

Materials and methods

Physicochemical properties and antibacterial activity of ASD solutions

The purity of ASD has been determined to be up to 95%. The antibiotic concentration in an aerosol formulation is usually higher than its in vitro MIC value, as noted for tobramycin. Since the highest MIC value reported for ASD was 0.064 mg/mL, we decided to test a higher concentration than this MIC value in an aerosol formulation. Thus, aqueous solutions of ASD at concentrations of 2 and 10 mg/mL were used in order to test whether the ASD concentration would affect the physiochemical properties of the prepared formulations. Selected physicochemical properties of these solutions were determined, comprising pH (Radiometer Analytical, Copenhagen, Denmark) and osmolarity (Roebling Automatic, Essen, Germany). Porcine gastric mucin (PGM) has been used as a model for demonstrating a possible inhibitory effect of human mucin on antibiotic agents, including tobramycin, due to structural and functional similarities between the two types of mucins. Therefore, we compared the effect of PGM on the in vitro antibacterial activities of the prepared ASD solutions with those of tobramycin (Tobi, Novartis, Rueil-Malmaison, France). This was achieved by determining the MIC values of these compounds for Pseudomonas aeruginosa ATCC 27853 and Staphylococcus aureus ATCC 25923 using the reference broth dilution method before and after adding PGM (type II; Sigma–Aldrich, France). Since mucin concentrations in the mucus of CF patients were reported to be highly variable, ranging from 68.3 μg/mL to 47 mg/mL, we tested the effect of mucin at concentrations of 1 and 10 mg/mL in order to mimic clinical conditions.

Aerosol testing

Two nebulizers were used: a jet nebulizer, the Pari LC Plus (PARI, Germany) derived from the compressor Pari Boy SX (PARI, Germany); and a vibrating mesh nebulizer, the eFlow rapid (PARI, Germany). Particle size distribution in the aerosol was analysed with a Malvern Mastersizer S Apparatus (Malvern Instruments, UK) at a constant flow rate of 30 L/min using a 300 mm lens. The nebulizer mouthpiece was placed at a distance of 20 mm from the lens face and 23 mm from the laser beam axis (Figure 1). A fill volume of 5 mL of ASD or tobramycin solution was used for each nebulizer and aerosol testing was performed in triplicate. During each analysis, a series of three measurements were made, starting at the beginning of the nebulization process with intervals of 20 s until the automatic cessation of each device. The following aerodynamic parameters were calculated (Mastersizer S software version 2.19 for Windows): D10 or the 10% cut-off point, i.e. 10% of the particle size distribution is below this point (μm); D50 or the volume median diameter, i.e. 50% of the distribution is above and 50% is below this value (μm); D90 or the 90% cut-off point, i.e. 90% of the distribution is below this point (μm); the relative span, representative of size variation between particles and corresponding to the value of (D90–D10)/D50 and the respirable fraction (RF), i.e. the fraction of particles having a diameter <5 μm and susceptible to inhalation. Results were analysed using the two-sample t-test options in Prism 5 for Windows (GraphPad Software) and are presented as average ± SD.

Results

Unadjusted solutions of ASD demonstrated an acidic character with a pH of 5.8 ± 0.1. Adjustment of solutions in order to have a pH closer to neutrality was therefore required. This was achieved using 0.5 N NaOH, giving a pH of 6.8 ± 0.1. Moreover, ASD solutions prepared in 0.9% NaCl presented an osmolarity of 330 ± 5 mosm/L. After pH and osmolarity adjustments, the in vitro antibacterial activities of the prepared ASD formulations were evaluated. MICs of ASD solutions for P. aeruginosa and S. aureus were 4 and 1 mg/L, respectively, in the mucin-free medium. Adding mucin at 1 or 10 mg/mL did not change these MIC values. For tobramycin, MICs of 1 and 0.5 mg/L were noted for P. aeruginosa and S. aureus, respectively, in the mucin-free medium. Addition of mucin at 1 mg/mL increased the MICs of tobramycin to 4 and 2 mg/L for P. aeruginosa and S. aureus, respectively, while after addition of mucin at 10 mg/mL, MICs of 16 mg/L were noted for both P. aeruginosa and S. aureus.

Aerosol testing was conducted until the automatic cessation of each device, which was noted after 18 ± 2 and 7 ± 1.5 min for the Pari LC Plus and eFlow rapid, respectively. As shown in Table 1, aerodynamic parameters did not differ according to the ASD concentrations tested (P > 0.1). On the other hand, statistical analysis of aerodynamic parameters obtained for ASD and tobramycin solutions showed that, according to device model, the eFlow rapid nebulizer provided higher D50 and RF values than the Pari LC Plus nebulizer (P < 0.05). Interestingly, according to device model, statistical analysis demonstrated that the ASD solutions tested showed aerodynamic diameters similar to those noted for the tobramycin solution (P > 0.05).

Discussion

Pharmacokinetic studies showed that, after inhalation of 5 mL of a solution containing tobramycin at 60 mg/mL, the antibiotic was present in sputa at concentrations 10–25 times higher than its MICs for S. aureus or P. aeruginosa.
Suitability of aminosterols for aerosol delivery

Table 1. Aerodynamic parameters of nebulized ASD and tobramycin solutions using Pari LC Plus and eFlow rapid nebulizers (results are presented as average ± SD)

<table>
<thead>
<tr>
<th>Solution</th>
<th>Nebulizer</th>
<th>D_{50} (µm)a</th>
<th>D_{10} (µm)</th>
<th>D_{90} (µm)</th>
<th>Relative span</th>
<th>RF (%)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASD (2 mg/mL)</td>
<td>Pari LC Plus</td>
<td>3.09 ± 0.07</td>
<td>0.93 ± 0.29</td>
<td>9.23 ± 1.10</td>
<td>2.69 ± 0.22</td>
<td>68.07 ± 2.58</td>
</tr>
<tr>
<td></td>
<td>eFlow rapid</td>
<td>3.53 ± 0.22</td>
<td>0.86 ± 0.28</td>
<td>8.01 ± 0.84</td>
<td>2.04 ± 0.37</td>
<td>76.43 ± 5.20</td>
</tr>
<tr>
<td>ASD (10 mg/mL)</td>
<td>Pari LC Plus</td>
<td>3.12 ± 0.11</td>
<td>1.03 ± 0.47</td>
<td>9.17 ± 0.99</td>
<td>2.61 ± 0.27</td>
<td>68.07 ± 2.66</td>
</tr>
<tr>
<td></td>
<td>eFlow rapid</td>
<td>3.63 ± 0.22</td>
<td>0.92 ± 0.14</td>
<td>8.30 ± 0.50</td>
<td>2.04 ± 0.17</td>
<td>75.88 ± 4.98</td>
</tr>
<tr>
<td>Tobramycin (60 mg/mL)</td>
<td>Pari LC Plus</td>
<td>3.08 ± 0.07</td>
<td>1.01 ± 0.19</td>
<td>9.35 ± 1.10</td>
<td>2.71 ± 0.25</td>
<td>66.07 ± 4.64</td>
</tr>
<tr>
<td></td>
<td>eFlow rapid</td>
<td>3.79 ± 0.21</td>
<td>0.86 ± 0.14</td>
<td>9.01 ± 0.84</td>
<td>2.30 ± 0.31</td>
<td>76.10 ± 5.12</td>
</tr>
</tbody>
</table>

aFor each formulation, D_{50} and RF differed significantly according to nebulizer type (P<0.05), but there was no significant difference between these parameters when analysed according to the ASD and tobramycin formulations tested.

present in sputum, such as mucin, inhibit the activity of inhaled antibiotics, which was noted in the present study by using porcine mucin as a model. This inhibitory effect may explain in part the use of elevated doses of antibiotic in order to achieve desired activity. Interestingly, no inhibition by mucin was noted in the case of ASD, which is in agreement with previous data demonstrating that another group of aminosterols, namely ceragenins, are also resistant to the inhibitory effect of mucin.7 This may be due to the presence of a hydrophobic sterol core preventing interaction with glycoproteins and thus neutralizing their inhibitory effect. Solutions used for inhalation should have a pH close to neutrality and an osmolarity between 150 and 550 mosm/L in order to prevent undesired side effects, such as bronchospasm, irritation or cough.7 Thus, ASD solution should be prepared using an isotonic solution and should be adjusted by addition of NaOH in order to obtain acceptable osmolarity and pH values within the range of airway tolerability. Previously reported MICs of ASD were 2 and 4 mg/L for reference strains of S. aureus and P. aeruginosa, respectively.1 Consequently, it can be concluded that pH and osmolarity adjustments of the prepared ASD formulations did not change the in vitro antibacterial activity of this compound since the MICs obtained were comparable to the previously reported values.1 Particle size distribution is commonly analysed using the impaction method described in pharmacopoeia.10 However, a method based on laser diffraction has been increasingly reported as a suitable alternative to the impaction method, as the former was found to be reproducible, rapid and easy to use.10 Since no guidelines are currently available unifying the operational parameters of the laser diffraction technique, especially the positioning of the nebulizer mouthpiece and the pump flow rate, we adopted a previously described configuration reported to minimize interference with the laser beam without changing the quality of the aerosol cloud, leading to reproducible results.7 In agreement with previous work, the eFlow rapid nebulizer was significantly faster than the Pari LC Plus in nebulizing the same fill volume.2–4 Moreover, the values of D_{50}, D_{10} and D_{90} found in the present work are in keeping with reported data demonstrating that the Pari LC Plus nebulizer produces smaller particles than the eFlow rapid nebulizer.2–4 While nebulizers providing small D_{50} values, such as the Pari LC Plus device, are technically favoured (assuming that they would deliver a higher dose of the drug in the lungs), pharmacokinetic studies demonstrated that administering tobramycin via the eFlow rapid and the Pari LC Plus led to equivalent sputum concentrations of this antibiotic.2–4 To the best of our knowledge, a comparison of the RF of particles produced by these two devices is reported for the first time in this work, showing that the eFlow rapid nebulizer produced a higher RF than the Pari LC Plus nebulizer. Thus, the eFlow rapid nebulizer is better than the Pari LC Plus nebulizer due to its rapidity and efficiency. Overall, this introductory work demonstrates that aerosol delivery of ASDs using two different types of nebulizers is feasible from a technical point of view. Further work is needed in order to evaluate the antibacterial potential of such formulations using an in vivo lung-infected animal model.

Funding
This study was supported by the Ministry of Higher Education, Syria.

Transparency declarations
None to declare.

References

