Ion-pairs – a novel formulation strategy to alter drug disposition in the lungs

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Summary

The lungs are an attractive route for the administration of both systemically and locally acting medicines for numerous reasons, including their large surface area and relative lack of metabolic activity for many drug molecules. Inhaled medicine administration does, however, also have its challenges. A major issue for pulmonary drug delivery is the rapid elimination of the active compound for the lungs, meaning that many medicines must be administered multiple times a day, leading to poor patient compliance. An interesting strategy for altering the post-deposition fate of drug delivered to the lungs by inhalation is the use of ion-pairs. The formation of drug ion-pairs offers an opportunity to alter the biopharmaceutical properties of inhaled compounds without altering their structure or pharmacological activity. In order for a drug to form an ion-pair an excess of counter ion must be used. Existing medicines that contain counter ions, such as salbutamol sulfate, do not take advantage of their ion-pairing potential. In this study the ion-pairing between salbutamol and 3 counter ions (sulfate, gluconate and octanoate) was investigated using Fourier Transformed infra-red spectroscopy, and it was found that the salbutamol gluconate complex is stronger than that of salbutamol sulfate with binding constants of 2.270 and 1.569, respectively. Salbutamol octanoate binding could not be quantified due to the complex nature of the ion-pair formed. The results of this study illustrate how ion-pairs can be formed and characterised in preparation for future pharmacokinetic assessment in the lungs.

Introduction

Drug delivery to the lungs remains constrained by the rapid elimination of the administered active pharmaceutical ingredient (API) by the natural clearance mechanisms of the lungs¹. Several strategies to retain drugs in the lungs have been reported, but these have had limited success. Nanoparticles, liposomes and other carrier systems have all been investigated; however there are issues with each of these formulation approaches²,³,⁴.

Formulation as ion-pairs has been shown to modulate the pharmacokinetics of charged drug molecules in both orally and transdermally applied medicines⁵,⁶,⁷, but this strategy has yet to be investigated for use in the lungs. An ion-pair is two oppositely charged molecules that are held together through non-covalent interactions, such as the electrostatic interaction of opposite charges and hydrogen bonds⁸. In order to form an ion-pair in solution the counter ion must be present in excess to drive the majority of the drug molecule into association with the counter ion⁹. As the ion-pairs distribute in the body, they dissociate to yield free drug and counter ion which are eliminated individually⁹. The ion-pairing strategy therefore represents a novel way to alter the physicochemical properties of a drug, such as solubility or partitioning behaviour, without actually altering the molecular structure or the pharmacological activity of the API¹⁰.

In this study, the formation of drug ion-pairs was investigated using salbutamol (Figure 1) as a model ionised drug. The aim of these experiments was to show that salbutamol ion-pairs form in the presence of excess of the counter ions sulfate, gluconate and octanoate (Figure 2) and verify that the counter ions selected do not adversely affect the respiratory epithelium at concentrations to be delivered by inhaled formulations.

Methods

Fourier Transformed infra-red spectroscopy

A universal transmission cell system with CaF₂ windows and 25 µm mylar spacer was used for the Fourier Transformed infra-red spectroscopy (FTIR) measurements. All samples were made up in D₂O and the pH was adjusted to 7.4 (± 0.2) with 1 M HCl or NaOH in D₂O as needed. Salbutamol peaks were assigned according to the literature. An increasing concentration of counter ion (sodium sulfate, sodium gluconate, or sodium octanoate) was titrated into a fixed concentration of salbutamol in D₂O and any changes in the salbutamol FTIR peaks were noted. The spectra were recorded within the range of 4000 – 500 cm⁻¹. The resolution was set at 4 cm⁻¹ and 32 scans were performed for each measurement. All spectra were baseline corrected and differentially substracted with the spectra of the baseline corrected blank solutions. Any changes in the peaks of the salbutamol spectra upon addition of counter ion were noted. These changes in peaks were used to create a binding curve of % salbutamol bound vs. –log free counter ion, where possible, and the binding constant was calculated as the 50% value on this curve.
**Cell culture**

Calu-3 human bronchial cells were seeded into a 96-well plate at a density of $1 \times 10^5$ cells/mL. After 24 h incubation they were treated with a range of concentrations of counter ion in cell culture medium. The negative control for this assay was cell culture medium, the positive control was 1% v/v triton-X solution in cell culture medium. After a further 24 h incubation the cells were exposed to 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) for 4 h. Any formazan crystals formed as a result of MTT metabolism were solubilised in 10% w/v sodium dodecyl sulfate (SDS) in 50:50 DMF:H$_2$O. A UV reader at a wavelength of 570 nm was used to quantify the percentage of viable cells, a background reading was taken at 650 nm. The half maximal inhibitory concentration ($IC_{50}$) and no effect values were obtained from the graph of cell viability vs. counter ion concentration.

**Results and Discussion**

The most significant change in the FTIR spectrum upon addition of sulfate to salbutamol was an increase in the N-H peak height at 1596 cm$^{-1}$ hence the sulfate binding was characterised by calculating a peak height ratio between the 1596 cm$^{-1}$ and the 1617 cm$^{-1}$ peak (C=C) which did not change. Addition of gluconate produced a pronounced increase in the 1617 cm$^{-1}$ peak, which was compared to the inert CH$_3$ peak found at 1385 cm$^{-1}$. The octanoate caused an increase in the CH$_3$ peak at 1385 cm$^{-1}$ compared with the C=C peak at 1617 cm$^{-1}$. From these observations binding constants (pK) of 1.569 and 2.270 were found for the sulfate and gluconate, respectively (Figure 3). A binding curve could not be drawn for salbutamol octanoate as it appears that the relationship between the drug and the counter ion exceeded the 1:1 complex that might be expected on the basis of the charges of the molecules. Although evidence of binding between the two species was observed, this method was not suitable to calculate a binding constant. The concentration at which changes in the FTIR spectra developed indicated that the octanoate binding event was stronger compared to that of the sulfate or gluconate (Figure 4). The increased pK value for salbutamol gluconate shows that the 2 species bind more strongly than salbutamol and sulfate, this could be attributed to many factors including the presence of many more hydrogen bonding sites on the gluconate molecule which have the potential to form additional attachments to the salbutamol host.

The MTT results gave $IC_{50}$ values for sodium sulfate, gluconate and octanoate of 14.5, 38.3, and 1.7 mg/mL, respectively (Figure 5, Table 1). There was no obvious relationship between $IC_{50}$ and the log P of the counter ion. The results did show, however, that all of these compounds are suitable for use in an ion-paired inhaled formulation, as the $IC_{50}$ values exceed any likely concentration post-deposition in the lungs, even if they were to be used at a 1:20 ratio of drug:counter ion. The $IC_{50}$ value of each of the counter ions did not exceed values found for excipients that are licensed for use in the lungs.

![Figure 1. The structure of salbutamol at pH 7.4](image1.png)

![Figure 2. From left to right, the structures of the sulfate, gluconate and octanoate counter ions at pH 7.4](image2.png)
Figure 3. FTIR binding affinity plot for salbutamol sulfate (2:1) in D$_2$O at pH 7.4 (±0.2) (closed circle). Data is shown as the % of salbutamol bound vs the negative free sulfate concentration, and salbutamol gluconate (1:1) in D$_2$O at pH 7.4 (±0.2) (red triangle). Data is shown as the % of salbutamol bound vs the negative free gluconate concentration.

Figure 4. The relationship between counter ion concentration and % salbutamol bound for sulfate (closed circle), gluconate (red triangle) and octanoate (yellow square).

Figure 5. The relationship between the concentration of counter ion and the Calu-3 cell viability for sodium sulfate (closed circle), sodium gluconate (red triangle), and sodium octanoate (green square). Data is shown as an average ± SEM.
Table 1. The IC_{50} values and no effect values of each counter ion, plus their logP values

<table>
<thead>
<tr>
<th>Counter ion</th>
<th>IC_{50} (mg/mL)</th>
<th>No effect value (mg/mL)</th>
<th>logP</th>
</tr>
</thead>
<tbody>
<tr>
<td>sulfate</td>
<td>14.5</td>
<td>2.9</td>
<td>-2.43</td>
</tr>
<tr>
<td>gluconate</td>
<td>38.3</td>
<td>5.0</td>
<td>-1.87</td>
</tr>
<tr>
<td>octanoate</td>
<td>1.7</td>
<td>0.1</td>
<td>2.67</td>
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</table>

Conclusion

This study has shown that salbutamol forms ion-pairs with sulfate, gluconate and octanoate in solution when the counter ion is present in excess. The binding constants were calculated for salbutamol sulfate and gluconate. However, salbutamol octanoate formed a complex that did not accord with 1:1 stoichiometry that was expected and further investigation into the salbutamol-octanoate complex is required.

The MTT data indicated that all of the counter ions studied are likely to be safe for use in an inhaled medicine that used an ion-pairing formulation strategy. The IC_{50} values for all 3 counter ions were high enough for them to be used in excess in a formulation in order to drive ion-pairing and maintain the molecular complex post-deposition in the lungs for sufficient time to modify drug disposition.

Although this study has shown ion-pairing that has potential to modulate drug disposition in the lungs, the research is at an early stage and much remains to be investigated and understood in order to apply this formulation strategy to an inhaled medicine.

References