Study of pH stability of R-salbutamol sulfate aerosol solution and its antiasthmatic effects in guinea pigs

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Summary

Currently, all commercial available nebulized Salbutamol in China is in its racemic form. It is known that only R-salbutamol (eutomer) has therapeutic effects, while S-salbutamol (distomer) may exacerbate asthma after chronic use. Therefore, it is an unmet clinical need to develop R-salbutamol as a nebulized product that is more convenient for young and old patients. In our study, a stable aerosol solution of R-salbutamol sulfate was established, and its antiasthmatic effects were confirmed. The decomposition rate and racemization effect of the R-salbutamol sulfate solution were evaluated over a pH range from 1 to 10 (except pH = 7, 8) at 60 °C. The aerodynamic particle size of the R-salbutamol sulfate solution and commercial RS-salbutamol sulfate solution were both tested in vitro by NGI in 5 °C. Laser diffractometer was used to characterize the droplet-size distribution (DSD) of both solutions. We next conducted an in vivo animal study to document the antiasthmatic effect of R-salbutamol aerosol sulfate solution and determine the relationship to RS-salbutamol. The results showed that the R-salbutamol sulfate solution was more stable at pH 6. In vitro comparison studies indicated that there was no distribution difference between R-salbutamol sulfate solution and the commercial RS-salbutamol solution. The animal results showed that R-salbutamol was more potent than RS-salbutamol against the same dose of histamine challenge. Unlike commercial RS-salbutamol, which was acidified to a pH of 3.5 to extend bench life but may cause bronchoconstriction in asthmatic patients, the neutralized R-salbutamol solution was more suitable for clinic use.

Key words

R-salbutamol sulfate, pH stability, nebulization, particle size, antiasthma effect
Introduction

β_2-agonists are well-known bronchodilators and widely used in the clinic^1). Most β_2-agonists have at least one chiral center in their structure, which means they have pair(s) of enantiomers. Salbutamol is a short-acting beta agonist (SABA). It has been demonstrated that the bronchodilator effects of racemic salbutamol are contributed by R-salbutamol because R-salbutamol has approximately 100-fold greater binding affinity to the β_2-adrenergic receptor than S-salbutamol and a two-fold binding affinity effect over racemic salbutamol^2). Some researchers report that sustained use of salbutamol can cause a high risk of morbidity and mortality, particularly when administered as a monotherapy^3). The mechanisms attributed to the side effects are still unknown, but it has been suggested that S-salbutamol may be the main cause of the augmentation of bronchospasms and pro-inflammation reactions^4-6). It has also been demonstrated that unlike R-salbutamol, S-salbutamol may be preferentially retained in the body^7), and thus asthma may be exacerbated when the bronchodilator effects of R-salbutamol have waned, particularly the accumulation of S-salbutamol after long-term use of racemic salbutamol. Since S-salbutamol cannot meet the requirement of patients and causes side effects, there is a trend towards the therapeutic use of optical pure R-salbutamol.

The stability of salbutamol has been studied for years. Salbutamol decomposes faster in aqueous solutions at elevated temperatures^8). Sugars could accelerate the decomposition rate, depending on the pH of the solution, and this decomposition appears to proceed faster in concentrated solutions than in dilute solutions^9). It has also been demonstrated that the maximum stability of racemic salbutamol in aqueous solution occurs at a pH of approximately 3.5^10).
In China, R-Salbutamol is not commercially available, neither by oral nor inhalation routes. Our former study only evaluated the pharmacological effects of R-salbutamol sulfate solution at pH 3.5 (referred to as commercial racemic salbutamol\(^{11}\)). The decomposition performance of racemic salbutamol solution has been well studied\(^{10}\), but whether an R-salbutamol solution is as stable at pH 3.5 as racemic salbutamol remains unknown. The pH stability of R-salbutamol sulfate solutions has not been studied. Therefore, in this study, we established a novel nebulization form of R-salbutamol by systematically calculating the degradation kinetics with varying pH conditions. We also investigated the lung deposition performance of an R-salbutamol sulfate solution in vitro using an NGI and Laser diffractometer, as demanded by European pharmacopoeia guidance. The pharmacodynamics study was conducted in guinea pigs to determine the ratio relationship between R-salbutamol and RS-salbutamol.

**Materials and methods**

**Reagents**

The RS-salbutamol sulfate solution (pH = 3.5) was a commercial product, namely Daphnkechuang inhalation solution obtained in China. R-salbutamol sulfate and RS-salbutamol were kindly supplied by Key-Pharma Biomedical Inc., Dongguan. Histamine was obtained from Sigma, America. Sulfuric acid, sodium hydroxide and ethylcarbamate were purchased from Kelong Chemical Co. Ltd, Chengdu.

**pH influence**

Aliquots (2 mL) of the R-salbutamol sulfate solutions (2.5 mg/mL) were prepared in
sterilized saline in a plastic tube. Sulfuric acid or sodium hydroxide was used to adjust the solutions to different pHs (pH 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 9.0, 10.0). RS-salbutamol (5 mg/ml) solutions were adjust to the above pHs as well. The plastic tubes were sealed and placed in a preheated temperature oven (STIK, America) at 60 °C. From day 0 to 10, samples were removed from the oven once every two days, and the total impurity was determined by reversed phase high performance liquid chromatography (SHIMADZU, Japan) with UV detection using a self-control method. The percentage of residual R-salbutamol was calculated using the peak area normalization method, and the exact concentration was obtained by multiplying with the initial concentration. Next, normal phase high performance liquid chromatography (SHIMADZU, Japan) was used to verify racemization from R-salbutamol to S-salbutamol. The specific analytical method used followed a previous published study12).

**In vitro evaluation**

An R-salbutamol sulfate solution (2.5 mg/mL, pH = 6) and commercial RS-salbutamol sulfate solution (5 mg/mL, pH = 3.5), used as positive control, were tested. The nebulization device was a jet nebulizer (PARI Turbo boy, Germany).

**Next-Generation Impactor (NGI) test**

The study was conducted under the instruction of European Pharmacopoeia13). In brief, the NGI (COPLEY Scientific, UK) was placed in a refrigerator at 5 °C for 90 mins before the experiment. The flow rate during the entire procedure was fixed at 15 L/min. After nebulization for 10 mins, the amount of drug at each stage, including the throat component, was collected in deionized water and quantified using reversed phase high performance liquid
chromatography (SHIMADZU, Japan) to determine the concentration of the active particle ingredient (API). Copley Inhaler Testing Data Analysis software (CITDA, UK) was used to analyze the size distribution. Each sample was analyzed three times.

**Laser diffractometer**

The laser diffractometry study was based on a published paper in our lab\textsuperscript{14}. The particle size distribution was measured using a Malvern Spraytec (Malvern instruments, UK) with a suitable Standard Operating Procedure (SOP). The volume of two solutions was fixed at 3 mL and the total aerosol time was 5 mins. Various parameters were calculated automatically using the Spraytec software (Malvern instruments, UK) for volume-based size distribution, including the particle size below which 10 % of the spray lies (D\textsubscript{v} (10)), the particle size below which 50 % of the spray lies (D\textsubscript{v} (50)), the mass median diameter, and the particle size below which 90 % of the spray lies (D\textsubscript{v} (90)). Values presented were the average of at least three determinations.

**In vivo evaluation**

**Animals**

Healthy guinea pigs of either sex (250-300 g) were obtained from the Laboratory Animal Center of Guangdong, housed in plastic cages and maintained on a 12-h light/dark cycle at approximately 22 °C and 50 % relative humidity. Food and water were provided ad libitum. The animal study protocol was approved by the Ethics committee for animal welfare in the School of Bioscience and Bioengineering, South China University of Technology (Guangzhou, China). All guinea pigs used were tested by inhaling histamine 24 h before the experiment to ensure equal sensitivity to histamine challenge. Qualified animals were divided
into two groups, with 4 males and 4 females in each group.

**Pulmonary function measurement**

The study was conducted following the procedure used in previous research by our lab\(^1\)). In brief, urethane (1.25 g/kg, intraperitoneal injection) was used to anesthetize guinea pigs, and the tracheal cannula was intubated. The jugular vein was cannulated to administer the histamine challenge. Each guinea pig was placed in a whole-body plethysmograph (a 45 cm * 45 cm * 15 cm plastic box, homemade) in a supine position and allowed to stabilize for 10 min before baseline lung function variables were measured by histamine challenge (15 μg/mL, 0.5 mL, intravenous injection) on a PowerLab physiologic recorder system (AD Instruments Inc., Australia). The animal was subsequently treated with two doses of R-salbutamol sulfate nebulization (0.25 mg/mL * 10 sec as low dosage; 2.5 mg/mL * 2 min as high dosage) or commercial RS-salbutamol sulfate nebulization (0.5 mg/mL * 10 sec as low dosage; 5 mg/mL * 2 min as high dosage). The same histamine challenge was administered after each drug treatment to determine the bronchodilator efficacy. The effect of various concentration was determined by comparing the histamine-induced changes in airway resistance (\(R_{aw}\)) and dynamic lung compliance (\(C_{dyn}\)) before and after treatment in the same guinea pig. The respiratory rate was calculated from the flow 5 min after histamine challenge. We also calculated the relief time after every histamine challenge to verify the drug potency. The time period was set as the time at which the histamine challenge applied to the animal and the time at which the specified rise of intrathoracic pressure elapsed.

**Analytical method and statistics**

The analysis method to calculate pulmonary function parameters followed the principle
of our previous study\textsuperscript{11}. $R_{aw}$ and $C_{dyn}$ were calculated as follows:

$$R_{aw} = \frac{\Delta PR}{\Delta V}, \quad C_{dyn} = \frac{VT}{\Delta PR}$$

where $\Delta PR$ is the intrathoracic pressure (cmH\textsubscript{2}O); $\Delta V$ is the flow (cmH\textsubscript{2}O/sec); and $VT$ is the tidal volume (cmH\textsubscript{2}O).

The percentage changes in $R_{aw}$ and $C_{dyn}$ from baseline to treatment were calculated as follows:

$$R_{aw\%} = \frac{(R1-R0)}{R0} \times 100\%; \quad C_{dyn\%} = \frac{(C1-C0)}{C0} \times 100\%$$

where $R1$ or $C1$ is the maximum value of $R_{aw}$ or $C_{dyn}$ after histamine challenge; and $R0$ or $C0$ is the mean value of baseline $R_{aw}$ or $C_{dyn}$ before histamine challenge.

Data were expressed as the mean $\pm$ SEM, except when special instruction are indicated. Comparisons were made between groups using ANOVA.

**Results**

**pH influence**

The stability of the R-salbutamol sulfate solution was investigated in a plastic tube in the presence of air. As the pH of the solution decreased, the color of the solution was more obvious, from clear to light yellow to deep yellow. For the alkaline conditions, the samples not only discolored from yellow to reddish brown but also produced a flocculent precipitate. And the color changes were more serious in RS-salbutamol solutions. We found out that the decomposition rate of R-salbutamol sulfate solution fit apparent first-order kinetics. By plotting the R-salbutamol sulfate concentration vs. time, the decomposition rate constant ($k$) was obtained from the slope of the linear equation, and the correlation coefficient ($R^2$) was
determined (Table 1). The \( \lg k \) - pH profile of R-salbutamol solution and RS-salbutamol solution were displayed in Fig. 1. It had two valley points, which occurred around pH 3, pH 4 and pH 6. We further determined the racemization performance under acidic conditions. As shown in Table 2, there was little difference at day 0. The racemization ratio increased with decreased pH. Especially at pH 1 and pH 2, the percentage of S-salbutamol was nearly half at day 10, indicating that R-salbutamol sulfate was more likely to transform to the S-salbutamol form under acid conditions. The least racemization was observed at pH 6. Therefore, based on these results, pH 6 is a more stable condition for R-salbutamol solutions.

<table>
<thead>
<tr>
<th>pH</th>
<th>R-salbutamol</th>
<th>RS-salbutamol</th>
</tr>
</thead>
<tbody>
<tr>
<td>k (day(^{-1}))</td>
<td>R(^2)</td>
<td>k (day(^{-1}))</td>
</tr>
<tr>
<td>1.0</td>
<td>0.2299</td>
<td>0.9899</td>
</tr>
<tr>
<td>2.0</td>
<td>0.0079</td>
<td>0.9981</td>
</tr>
<tr>
<td>3.0</td>
<td>0.0021</td>
<td>0.9925</td>
</tr>
<tr>
<td>4.0</td>
<td>0.0033</td>
<td>0.9854</td>
</tr>
<tr>
<td>5.0</td>
<td>0.0438</td>
<td>0.9812</td>
</tr>
<tr>
<td>6.0</td>
<td>0.0325</td>
<td>0.9704</td>
</tr>
<tr>
<td>9.0</td>
<td>0.6188</td>
<td>0.9432</td>
</tr>
<tr>
<td>10.0</td>
<td>1.0093</td>
<td>0.9774</td>
</tr>
</tbody>
</table>
Fig. 1 pH - rate profile for the decomposition of R-salbutamol sulfate solutions (2.5 mg/mL) and RS-salbutamol sulfate solutions (5 mg/mL) at 60 ℃.

Table 2 Racemization profile of R-salbutamol sulfate solutions (2.5 mg/mL) at 60 ℃. Data are shown as percentage of S-salbutamol.

<table>
<thead>
<tr>
<th>pH (n = 3)</th>
<th>Day 0 (%)</th>
<th>Day 5 (%)</th>
<th>Day 10 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>0.28 ± 0.10</td>
<td>48.98 ± 2.89</td>
<td>47.17 ± 1.26</td>
</tr>
<tr>
<td>2.0</td>
<td>0.15 ± 0.09</td>
<td>23.79 ± 0.95</td>
<td>36.17 ± 1.04</td>
</tr>
<tr>
<td>3.0</td>
<td>0.27 ± 0.04</td>
<td>1.564 ± 0.27</td>
<td>6.343 ± 0.92</td>
</tr>
<tr>
<td>4.0</td>
<td>0.20 ± 0.04</td>
<td>1.496 ± 0.29</td>
<td>4.202 ± 0.33</td>
</tr>
<tr>
<td>5.0</td>
<td>0.20 ± 0.06</td>
<td>0.924 ± 0.09</td>
<td>4.372 ± 0.76</td>
</tr>
<tr>
<td>6.0</td>
<td>0.19 ± 0.09</td>
<td>0.806 ± 0.11</td>
<td>2.200 ± 0.45</td>
</tr>
</tbody>
</table>

**In vitro lung performance**

Products used for nebulization inhalation and intended for pulmonary delivery are
characterized by their particle size. We used NGI and Laser diffractometer to verify the consensus of two solutions, R-salbutamol sulfate and RS-salbutamol sulfate.

NGI

Because the NGI test focused on API distribution, the collected samples were quantitated using reversed phase high performance liquid chromatography to determine the exact mass. The data from NGI collected samples were calculated using the following equation:

\[
m = \frac{(A + 2.9063)}{7899.4} \times V \times 0.8299
\]

where \( m \) is the mass of the unknown sample (\( \mu \)g); \( A \) is the peak area of the unknown sample; and \( V \) is the volume of the unknown sample (mL).

The aerosol particle size distribution (APSD) profiles of the two solutions from stage - 1 to micro-orifice collector (MOC) are shown in Table 2. From the table, more than half of the particle sizes of the two solutions were at stage - 4 and stage - 5, meaning that half of the particle size was smaller than 4 \( \mu \)m. There was no size distribution difference between the R-salbutamol and commercial RS-salbutamol solution.
Table 3 Summary of data for R-salbutamol sulfate solution at pH 6 and RS-salbutamol sulfate solution at pH 3.5 using NGI. Mean particle distribution as % of total drug. The data are shown as the mean ± SD. MOC is the micro orifice collector.

<table>
<thead>
<tr>
<th>Stages</th>
<th>Cut-off diameter (μm)</th>
<th>R-salbutamol drug content % (n = 3)</th>
<th>RS-salbutamol drug content % (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage - 1</td>
<td>14.1</td>
<td>2.950 ± 0.58</td>
<td>3.030 ± 0.41</td>
</tr>
<tr>
<td>Stage - 2</td>
<td>8.61</td>
<td>4.640 ± 1.10</td>
<td>4.940 ± 0.99</td>
</tr>
<tr>
<td>Stage - 3</td>
<td>5.39</td>
<td>14.97 ± 1.73</td>
<td>15.57 ± 1.94</td>
</tr>
<tr>
<td>Stage - 4</td>
<td>3.30</td>
<td>26.81 ± 0.30</td>
<td>26.65 ± 0.08</td>
</tr>
<tr>
<td>Stage - 5</td>
<td>2.08</td>
<td>25.82 ± 1.36</td>
<td>25.25 ± 1.84</td>
</tr>
<tr>
<td>Stage - 6</td>
<td>1.36</td>
<td>13.70 ± 1.09</td>
<td>13.48 ± 0.84</td>
</tr>
<tr>
<td>Stage - 7</td>
<td>0.98</td>
<td>5.690 ± 0.63</td>
<td>5.580 ± 0.24</td>
</tr>
<tr>
<td>MOC</td>
<td>0.00</td>
<td>5.410 ± 0.63</td>
<td>5.480 ± 0.36</td>
</tr>
</tbody>
</table>

**Laser diffractometer**

The advantage of laser diffractometry is its online detection technique; it records real-time changes in particle size over the entire nebulization process. Various parameters characterizing the droplet size distribution were compared according to the ISO 13320 guideline. From Fig.2, we see that in R-salbutamol sulfate solution, 50 % of droplets were smaller than 3.21 ± 0.13 μm, and 90 % of droplets were smaller than 7.24 ± 0.53 μm. The distribution performance of RS-salbutamol sulfate was the same as R-salbutamol sulfate, 50 % of droplets were smaller than 3.14 ± 0.04 μm, and 90 % of droplets were smaller than 7.27 ± 0.21 μm.
Fig. 2 Droplet size-percentage profiles of R-salbutamol sulfate solution (pH = 6) and commercial RS-salbutamol sulfate solution (pH = 3.5) by laser diffractometry. Dv 10 means that 10% of the droplet volume has a diameter under that value. Dv 50 means that 50% of the droplet volume has a diameter under that value. Dv 90 means that 90% of the droplet volume has a diameter under that value. Data are shown as the mean ± SD.

**In vivo evaluation**

The injection of histamine can cause bronchoconstriction that peaks within 5 sec and acquires a more stable evoked effect. The data are shown in Fig. 3 and Table 4. There was no difference in the baseline control of the $R_{aw}$ and $C_{dyn}$ between groups. The R-salbutamol sulfate solution and commercial RS-salbutamol sulfate solution, used as a positive control, were tested. After baseline challenge, animals subsequently received a low-dose treatment of R-salbutamol sulfate nebulization. A second histamine challenge was given, and the changes $R_{aw}$ and $C_{dyn}$ slightly decreased compared to the baseline control ($R_{aw}$ 102.7 ± 14.85 vs. 425.4 ± 85.25, $C_{dyn}$ - 62.24 ± 3.571 vs. - 83.85 ± 1.633, $P < 0.05$). After the high-dose treatment, the third histamine response dramatically decreased ($R_{aw}$ 25.53 ± 7.602, $C_{dyn}$ - 25.18 ± 6.837). The equivalent doses of RS-salbutamol treatment had almost the same antiasthmatic effects.
as R-salbutamol in the low-dose group ($R_{aw}$ 140.3 ± 21.12 vs. 102.7 ± 14.85, $C_{dyn}$ - 62.09 ± 5.527 vs. - 62.24 ± 3.571, $P > 0.05$) and high-dose group ($R_{aw}$ 26.89 ± 8.11 vs. 25.53 ± 7.602, $C_{dyn}$ - 32.91 ± 4.74 vs. - 25.18 ± 6.837, $P > 0.05$). Treatment with R-salbutamol or RS-salbutamol can relieve asthma symptoms, and both displayed dose-dependent relationships. Fig.4 shows that the respiratory rate decreased significantly with R-salbutamol treatment in both doses, leading to relief of tachypnea, but in the low-dose of RS-salbutamol group it remained nearly the same. A rapid relief time is essential for a patient with respiratory distress. As shown in Fig.5, the relief time showed a trend between R-salbutamol and RS-salbutamol. In R-salbutamol, the relief time became shorter with increasing dose. However, the relief time for a high dose of RS-salbutamol was twice that of the high dose of R-salbutamol.

Fig.3 The anti-asthmatic effects of R-salbutamol sulfate or commercial RS-salbutamol sulfate solution in guinea pigs. A, Airway resistance ($R_{aw}$) induced by intravenous histamine after treatment with low dose and high dose of R-salbutamol sulfate or commercial RS-salbutamol sulfate solution, expressed as % of baseline change of $R_{aw}$ after histamine challenge. B, Dynamic lung compliance ($C_{dyn}$) induced by intravenous histamine after treatment with low dose and high dose of R-salbutamol sulfate or commercial RS-salbutamol sulfate solution, expressed as % of baseline change of $C_{dyn}$ after histamine challenge. * compared with control
in the same group, P < 0.05. ** compared with control in the same group, P < 0.01. # compared with low dose treatment in the same group, P < 0.05.

Table 4 Summary data of airway resistance ($R_{aw}$) and dynamic lung compliance ($C_{dyn}$) for R-salbutamol sulfate solution and RS-salbutamol sulfate solution against histamine challenge in guinea pigs.

<table>
<thead>
<tr>
<th>Treatment (n = 8)</th>
<th>R-salbutamol</th>
<th>RS-salbutamol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R_{aw}$ (cmH$_2$O * s/L)</td>
<td>$C_{dyn}$ (mL/cmH$_2$O)</td>
</tr>
<tr>
<td>Control</td>
<td>132.5 ± 28.3</td>
<td>- 14.00 ± 0.26</td>
</tr>
<tr>
<td>Low dose</td>
<td>33.16 ± 4.77</td>
<td>- 10.54 ± 0.57</td>
</tr>
<tr>
<td>High dose</td>
<td>8.760 ± 2.39</td>
<td>- 4.460 ± 0.19</td>
</tr>
</tbody>
</table>

Fig.4 Change in breathing rate after treatment with R-salbutamol sulfate solution or commercial RS-salbutamol solution. A, Percentage change in breath rate in low and high dose of R-salbutamol or RS-salbutamol group, respectively. * compared to low dose of RS-salbutamol, P < 0.05. ** compared to low dose of RS-salbutamol. B, The actual breathing rate in two groups after each dose. Control was used as baseline to calculate the percentage change in breathing rate. **, P < 0.01.
Fig. 5 Relief time of each treatment in low and high dose of R-salbutamol or RS-salbutamol group, respectively. * compared with high dose of RS-salbutamol, P < 0.05.

**Discussion**

In this study, we evaluated the stability of R-salbutamol sulfate solution from pH 1 to 10 (except pH = 7, 8) at 60 °C. After calculating the decomposition rate constant, we have found two valley points near pH 3 and pH 6. Because acidic condition can accelerate the transformation of R-salbutamol to S-salbutamol, we decided to use a neutral condition, and the pH was set to 6. We used NGI and Laser diffractometry to demonstrate the lung deposition performance in vitro. The aerodynamic particle size of the R-salbutamol sulfate solution was mostly located in less than 4 μm, and 50 % of particle size distribution was under 3.21 ± 0.13 μm. Guinea pigs were used to investigate the therapeutic effect. The results showed that R-salbutamol was as effective as an equimolar dose of RS-salbutamol sulfate but had a shorter relief time.

Stability studies are of great importance for an aqueous solution. Wall and Sunderland
first reported the stability of salbutamol in solution. They investigated salbutamol stability in phosphate buffers with pH 6.9 - 8.3 at 40 - 70 °C, stability decreased along with increased pH and temperature, so we excluded pH 7 and 8\textsuperscript{15}. The pH values at 9 and 10 were chosen because of the critical points of the ionization state of salbutamol, namely $pK_a = 9.3$ for the amino group and $pK_a = 10.3$ for the phenolic group\textsuperscript{16}. A previous study revealed that the alkaline form of salbutamol was less chemically and physically stable than the acidic form of salbutamol\textsuperscript{17}. To obtain a stable solution, the influence of pH on the decomposition rate of R-salbutamol sulfate was studied. Total impurity was used for the standard control of an aqueous solution. Our result showed that R-salbutamol sulfate was most stable near pH 3, in agreement with our data of RS-salbutamol solution and previous results for a salbutamol sulfate solution\textsuperscript{10}. The pH in our previous study of a R-salbutamol sulfate solution was set to 3.5\textsuperscript{11} to match commercial RS-salbutamol nebulization solutions. However, in our long-term stability study, the transformation from R-salbutamol to S-salbutamol was obvious, leading to an almost racemic solution. According to the literature, the benzylic alcohol moiety of salbutamol is subject to acid-catalyzed racemization\textsuperscript{18}. Therefore, we decided to use a neutral condition, and the pH was set to 6. The degradation profile of the R-salbutamol sulfate solution was investigated at 60 °C, as the decomposition rate at low temperature was too slow to obtain reliable data. We prepared another batch in the presence of nitrogen, but the result was similar (data not shown). To simplify the procedure, aliquots of R-salbutamol sulfate solution were tested in the presence of air.

Nebulization inhalation is a more popular delivery method because it is more adaptable to young and old people\textsuperscript{19}, who are a major portion of respiratory disease patients. Generally,
drug particles from inhalation therapy deposit in different parts of the lung: in accordance with particle sizes from large to small, these drug particles would gradually deposit in primary bronchi, bronchi, terminal bronchi and alveoli\(^{20}\). According to the European Respiratory Society, the delivery performance of nebulization inhalation is determined by the drug solution and nebulizer device\(^{21, 22}\). The size of aerosol droplets must be controlled to meet therapeutic needs. Particle sizes below 4 \(\mu\)m will more likely deposit deep in the peripheral lung than in the upper respiratory tract\(^{23}\), while particles less than 0.5 \(\mu\)m are likely to be exhaled\(^{24}\). In 2006, the European Medicines Agency (EMA) introduced regulatory guidance for nebulization inhalation\(^{25}\), and the European pharmacopoeia strongly recommended that particle size should be evaluated using NGI to determine the size distribution of the active particle ingredient (API)\(^{13}\). Therefore, it is critical to precisely evaluate the particle size of the nebulization solution to mimic the in vivo lung deposition procedure. In our study, we used two devices: NGI and a laser diffractometer. NGI yielded mass fractions of the drug by chemical detection at different aerodynamic sizes, similar to realistic impaction and deposition processes in the human respiratory tract\(^{26}\), while the laser diffractometer provided a particle size distribution that was relevant to the particle shape, density, and other physical properties\(^{27}\). Our results showed that the aerodynamic particle size of the R-salbutamol sulfate solution at pH 6 was mostly located in less than 4 \(\mu\)m, and 50 % of the particle was below 3.21 ± 0.13 \(\mu\)m. The R-salbutamol sulfate solution had the same lung decomposition performance as a commercial RS-salbutamol sulfate solution at pH 3.5. In summary, our homemade R-salbutamol sulfate solution may theoretically have the same therapeutic effect as a commercial RS-salbutamol sulfate solution.
Spirometry, a measurement of lung function, is commonly used in the clinic to evaluate the pharmacodynamics of inhaled bronchodilators. The common indexes are reported in terms of forced vital capacity (FVC) and forced expiratory volume per one second (FEV₁)\textsuperscript{28}. For animal studies, bronchoprovocation is an alternative method. Methacholine and histamine are usually used to induce bronchoconstriction, and the protective effects of the bronchodilator against them are assessed\textsuperscript{29}. A published study indicated that 0.65 mg R-salbutamol had the same therapeutic effect as 2.5 mg RS-salbutamol, suggesting that R-salbutamol produced bronchodilation comparable to that of RS-Salbutamol at a 4 : 1 dose-for-dose ratio, with a better therapeutic effect for R-salbutamol\textsuperscript{30}. However, the above phenomenon was conducted in asthmatic patients, and thus the nebulized bronchodilator was a combination absorption of gut and lung, as verified by two absorption peaks observed in plasma concentration versus time curves in patients after nebulization\textsuperscript{31}. A similar profile for R-salbutamol concentration level was confirmed in dogs that inhaled R-salbutamol through the nose\textsuperscript{32}. These results agree with the accepted hypothesis that in the first 5 min after nebulized administration, the appearance of salbutamol in plasma was due to absorption from the lung only; after that, the systemic exposure of salbutamol was due to gut absorption\textsuperscript{33}. In patients with oral charcoal-inhaled R-salbutamol, the blood concentration of R-salbutamol showed the same trend as intravenous injection\textsuperscript{34}. In our study, the antiasthma effects of R-salbutamol sulfate solution and RS-salbutamol sulfate solution were compared in guinea pigs induced by intravenous injection of histamine. Tracheal intubation was performed, and nebulization aerosol was directly delivered to the trachea to avoid the influence of gastrointestinal absorption. Our results showed that R-salbutamol sulfate was not more
effective than an equimolar dose of RS-salbutamol sulfate if directly inhaled to the trachea rather than through the nose. However, the decrease in the respiratory rate and relief time of R-salbutamol compared with RS-salbutamol may contribute to its clinical use. There are two possible explanations. Acidification of commercially available RS-salbutamol (pH 3.5) is linked with bronchoconstriction in asthmatic patients\textsuperscript{35}. It has been reported that nebulized RS-salbutamol (Ventolin, pH 3.5) causes paradoxical bronchoconstriction and lasts for up to 15 min in infants with wheezing\textsuperscript{36}. In our study, the low dose of commercial RS-salbutamol was diluted with saline before administering to the animals, and the pH after dilution should be higher (less acidic), while the high dose of commercial RS-salbutamol remained at pH 3.5. Additionally, S-salbutamol may exhibit some airway hyper-reactivity or antagonism against R-salbutamol, especially occurring with the relief time of high doses of RS-salbutamol. However, the long duration of action of R-salbutamol generally precluded detection of any ability of S-salbutamol to cause hyper-reactivity in the animal study for RS-salbutamol.

Other reports suggest the potential for significant enantioselectivity in the oral bioavailability of salbutamol, indicating that the distribution of sulfotransferase, the enzyme metabolizes salbutamol, varied significant between the lung and gut\textsuperscript{37}. It appears that R-salbutamol undergoes preferential metabolism once absorption from the gastrointestinal tract begins\textsuperscript{38}. When racemic salbutamol was administered orally, it showed ten-fold faster metabolism of R-salbutamol than that of the S-salbutamol in plasma levels, and thus the plasma concentration of R-salbutamol was two- to three-fold lower than S-salbutamol\textsuperscript{39}. No evidence for enantioselective metabolism of R-salbutamol in the lungs was found after inhaling RS-salbutamol\textsuperscript{34}. Since R-salbutamol is a preferable metabolite in the gut, it was
suitable to develop R-Salbutamol as an inhalation therapy using nebulization inhalation.

Conclusions

In summary, we have established a stable solution of R-salbutamol sulfate, and the optimal pH value was 6. The R-salbutamol sulfate solution demonstrated good performance in particle size distribution tests and exerted a dose-response relationship in its anti-asthmatic effects. Unlike commercial RS-salbutamol, which is acidified to pH 3.5 to extend bench life but may cause bronchoconstriction in asthmatic patients, the neutralized R-Salbutamol solution was more suitable for clinic use.

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Conflict of Interest

The authors declare no conflict of interest.
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