Treatment of canine asthma by high selective vagotomy

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Objectives: This study evaluated the effect of high selective bilateral vagotomy of hilus pulmonis with video-assisted thoracoscopic on asthma.

Methods: Eight dogs with skin sensitive to Ascaris suum antigens were randomly divided into groups A and B. Asthma was induced by aerosol inhalation of A suum antigens. Respiratory rate and peak airway pressure were significantly increased \( P < 0.05 \) in both groups. Dynamic compliance was dramatically increased \( P < 0.05 \) in both groups. Two days later, bilateral vagotomy of hilus pulmonis under thorascopic guidance was performed on dogs in group A; dogs in group B underwent bilateral sham vagotomy plus thoracoscopy. Five days after treatment, all dogs had rechallenge with a second aerosol inhalation.

Results: Dogs in group A did not show typical asthmatic symptoms, and no significant changes were found in respiratory rate, peak airway pressure, and dynamic compliance \( P > 0.05 \). Dogs in group B still had typical symptoms, and respiratory rate and peak airway pressure were increased and dynamic compliance decreased significantly \( P < 0.05 \) for all. Significant differences in respiratory rate, peak airway pressure, and dynamic compliance were observed between groups. Moreover, inflammatory cells in the lungs and broncoalveolar lavage fluid of group A were dramatically reduced relative to group B \( P < 0.05 \). There were no significant changes in heart rate and mean arterial pressure after vagotomy, indicating that vagotomy did not affect the cardiac plexus of vagus.

Conclusions: High selective bilateral vagotomy of hilus pulmonis with thoracoscope can effectively control asthma in dogs. (J Thorac Cardiovasc Surg 2014;148:683-9)

Asthma is a condition characterized by chronic inflammation, bronchial hyper-responsiveness, and reversible airway obstruction and was reported to affect 300 million people. In some parts of the world, about 30% of people with asthma are children, and the asthma-related medical care cost is more than that of AIDS and tuberculosis combined. In fact, from 1957 to 1964, some studies showed that asthma could be brought under control after vagotomy. Vagotomy, however, could not be used as a routine treatment for asthma because traditional thoracotomy was associated with a number of severe complications, protracted hospital stay, and uncertainty of vagal excision site. With the development of video-assisted thoracoscopic surgery, the risk of thoracotomy was substantially reduced, thus rendering surgical blockade of vagal pathway clinically feasible.

In this study, we bilaterally cut off branches of hilus pulmonis of asthmatic dogs under thoracoscopic guidance to observe the effect on controlling asthma in an attempt to provide a new treatment alternative for asthma.

MATERIALS AND METHODS

Animals

All animal studies were approved and conducted in strict accordance with the guidelines of the institutional animal care and use committee.

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Domestic male dogs were acquired and housed in the Animal Center of Medical College of Sun Yat-sen University. Eight dogs, weighing 15 kg on average and with positive *Ascaris suum* antigen skin tests, were assigned to 2 groups of 4 dogs each. Group A underwent high selective bilateral vagotomy of the hilus pulmonis (HSVHP) with video-assisted thoracoscopic surgery and group B underwent sham bilateral vagotomy with video-assisted thoracoscopic surgery.

### Antigen Sensitization and Challenge

As previously described, all the dogs were subjected to *A. suum* antigen skin tests, and 8 dogs were found to have positive test results. The dogs with positive results (Figure 1, A) were first challenged with aerosol inhalation of *A. suum* antigen (1%, for 5 minutes) by tracheal intubation. After the operation, the dogs were challenged with a second aerosol inhalation of *A. suum* antigen (1%, for 5 minutes).

### Surgical Procedures

Two days after the first challenges, dogs were kept on an overnight fast. The next morning, the animals were anesthetized with a ketamine (20 mg/kg) and xylazine (2 mg/kg) solution, and anesthesia was maintained with inhalational isoflurane. The dogs were intubated and continuously ventilated, with a tidal volume of 200 to 250 mL. Single-lung ventilation was achieved with a bronchial blocker (Cook Medical Inc, Bloomington, Ind). The *PaO₂* and heart rate (HR) were monitored by pulse oximetry (Surgivet; Smiths Medical PM, Waukesha, Wis).

For the right-sided operation, the dogs were secured in the left lateral decubitus position. The first port was placed in the right fifth intercostal space, 4 cm from the spinal processes, and the second port was placed in the right seventh intercostal space on the midaxillary line. The second port was used for the camera. After the camera was introduced, the surgical instruments were inserted under direct vision through the first port. Video-assisted thoracoscopic surgery was performed with the LTF-240 scope (Olympus Optical Co Ltd; Tokyo, Japan). Topographically, on the right side, the vagus nerve gives off 2 to 3 branches above the azygos vein arch to the carina tracheae. The main trunk continuously descends behind the root of the lung and supplies 7 to 8 branches along the posterior surface of the right bronchia, forming the vagal pulmonary plexus. We dissociated vagus nerve along the trachea, azygos vein arch, and right main bronchus and then cut off the pulmonary plexus in group A dogs (Figure 1, B), whereas the group B dogs only underwent sham thoracoscopic surgery, with the vagus nerve left intact.

For the left-sided surgery, the dogs were placed in the right lateral decubitus position. Another 2 ports were placed in the corresponding places of the left side for introducing instruments and camera. At the inferior border of the aortic arch, the main trunk of the vagus nerve gives off the left recurrent laryngeal nerve and 1 to 2 cardiac branches, and behind the root of lung the main trunk it descends along the posterior surface of the left bronchus, providing 4 to 5 branches to form the vagal pulmonary plexus (Figure 1, C). In group A, we dissociated vagus nerve along trachea, aortic arch, and esophagus (Figure 1, D) and then cut off pulmonary plexus on group A while sham thoracoscopic surgery was performed in group B (Figure 1, E).

### Lung Function and Hemodynamic Index

Respiratory rate (RR), peak airway pressure (Ppeak), dynamic compliance (Cdyn), tidal volume (VT), HR, and mean arterial pressure (MAP) were recorded 5 minutes before and 30 minutes after each challenge. Pulmonary inflation pressure was measured through a sidearm of the tracheal cannula. Bronchoconstriction was measured in terms of the increase in the pulmonary inflation pressure above the basal inflation pressure produced by the ventilator.

### Arterial Blood Gas Analysis and Proinflammatory Serum Markers

Arterial blood was collected from the femoral artery. *PaO₂*, *PaCO₂*, *IgE*, and interleukin 4 (IL-4) were determined 5 minutes before and after each challenge.

### Bronchoalveolar Lavage Analysis

After the second challenge, the lungs were lavaged with 5 10-mL aliquots of warm phosphate-buffered saline solution containing 100–μmol/L isoproterenol (Sigma-Aldrich Corporation, St Louis, Mo) in situ through the tracheal cannula. Recovered lavage fluid was centrifuged, cells were resuspended in phosphate-buffered saline solution, and total cells were counted with a hemocytometer. Aliquots of the cell suspension were centrifuged, put onto glass slides, and stained for differential analysis.

### Statistical Analysis

Data were expressed as mean ± SD and analyzed with the SPSS statistical software package (version 11.5; IBM Corporation, Armonk, NY). Differences between groups were assessed with the Student t test.

### RESULTS

#### Successful Establishment of Canine Asthmatic Model

No significant differences were found between the groups 5 minutes before the first challenge with *A. suum* antigen (*P > .05*) in indices of lung function and arterial blood gas analysis (Figure 2).

After the first challenge, all dogs showed typical symptoms of asthma, such as tachypnea, breathlessness, chest tightness, and tongue cyanosis. Reinforced breath sounds and wheezing rale could be heard during the attack. In addition, 5 minutes after the challenge, dogs in both groups exhibited a significant increase in RR (Figure 2, A), with the RR of group A increasing from 12 ± 5.2 to 33 ± 7.6 breaths/min (*P < .05*) and the RR of group B rising from 10 ± 3.6 to 40 ± 9.0 breaths/min (*P < .05*). Similar changes in Ppeak were observed in both groups (Figure 2, B), with the Ppeak of group A increasing from 8 ± 2.8 to 19 ± 3.6 cm H₂O (*P < .05*) and the Ppeak of group B rising from 8 ± 3.2 to 18 ± 4.5 cm H₂O (*P < .05*). Cdyn was dramatically reduced in both groups (Figure 2, C), with Cdyn in group A decreasing from 38.5 ± 9.5 to 11.1 ± 5.0 mL/cm H₂O (*P < .05*) and Cdyn in group B dropping from 37 ± 4.1 to 9.8 ± 3.4 mL/cm H₂O (*P < .05*). VT in both groups showed a descending trend (Figure 2, D), with VT in group A decreasing from 266 ± 99.6 to 208 ± 91.9 mL (*P > .05*) and VT in group B decreasing from 200 ± 12.3 to 138 ± 15.2 mL (*P < .05*).
dropping from 317 ± 120.0 to 193 ± 97.8 mL ($P > .05$). $\text{PaO}_2$ in both groups was substantially reduced (Figure 2, E), $\text{PaO}_2$ in group A dropped from 96 ± 13.5 to 65 ± 22.3 mm Hg ($P > .05$) and $\text{PaO}_2$ in group B dropped from 78 ± 10.3 to 60 ± 7.6 mm Hg ($P < .05$), whereas no significant difference was found in $\text{PaCO}_2$ (Figure 2, F), with $\text{PaCO}_2$ in group A rising nonsignificantly from 34 ± 3.4 to 41.3 ± 7.8 mm Hg ($P > .05$), and $\text{PaCO}_2$ in group B rising nonsignificantly from 37.2 ± 8.4 to 37.7 ± 8.5 mm Hg ($P > .05$).

The symptoms of dogs in both groups were relieved and relevant indices returned to normal about 30 minutes after the first challenges (Figure 2, A-D). The RR of group A decreased to 19 ± 7.3 breaths/min, and the RR of group B to 7 ± 4.5 breaths/min; the Ppeak of group A was 13 ± 3.7 cm H$_2$O, and the Ppeak of group B 11 ± 5.0 cm H$_2$O; the Cdyn of group A was restored to 18.2 ± 8.5 mL/cm H$_2$O and the Cdyn of group B to 25.0 ± 11.1 mL/cm H$_2$O; the VT of group A was back to 245 ± 109.8 mL/cm H$_2$O and the VT of group B to 235 ± 115.1 mL.

Relieving Effect of HSVHP on Airway Hyperresponsiveness

The dogs of group A underwent HSVHP under thoracoscopic guidance, whereas the animals of group B were subjected to a sham thoracoscopic surgery. Five days later, the second challenge was delivered in both groups. Five minutes before the second challenge, no differences were found in indices of lung function and blood gas analysis between the groups (Figure 3).

Five minutes after the second challenge, dogs in group A showed no asthma-related symptoms, whereas the dogs of group B still had typical asthmatic symptoms. In group A, no significant change in RR was observed, whereas the dogs in group B exhibited a substantial increase in RR (Figure 3, A), with the RR of group B increasing from 12 ± 4.2 to 40 ± 12.9 breaths/min ($P < .05$) and the RR of group A increasing only nonsignificantly from 10 ± 4.9 to 12 ± 4.2 breaths/min ($P > .05$). There was a significant difference in RR between the groups ($P < .05$). The Ppeak in group A showed no significant change, whereas a great increase in Ppeak was seen in group B (Figure 3, B), with the Ppeak of group B increasing from 7 ± 3.6 to 18 ± 5.1 cm H$_2$O ($P < .05$) and the Ppeak of group A increasing only nonsignificantly from 8 ± 3.1 to 10 ± 3.1 cm H$_2$O ($P > .05$) and a significant difference between the groups ($P < .05$) observed in Ppeak. The Cdyn in group A dropped slightly, whereas in group B a significant decline was found (Figure 3, C), with the Cdyn of group A decreasing from...
36.7 ± 8.5 to 34.7 ± 9.5 mL/cm H2O (P > .05) and the Cdyn of group B decreasing from 38.6 ± 6.3 to 10.8 ± 5.3 mL/cm H2O (P < .05). There was also a significant difference in Cdyn between the groups (P < .05). The VT in group B decreased from 301 ± 71.2 to 206 ± 89.9 mL (P > .05), whereas the VT of group A was increased from 275 ± 81.5 to 320 ± 82.5 mL (P > .05; Figure 3, D). For PaO2, no significant change was found in group A, whereas a dramatic drop was seen in group B (Figure 3, E), with the PaO2 of group B decreasing from 84 ± 15.6 to 58 ± 11.6 mm Hg (P < .05) and the PaO2 of group A dropping from 81 ± 13.7 to 73 ± 13.8 mm Hg (P > .05). PaCO2 showed no significant change in either group (Figure 3, F), with the PaCO2 of group A dropping from 34.9 ± 4.5 to 34.2 ± 5.5 mm Hg (P > .05) and the PaCO2 of group B rising from 37.1 ± 4.0 to 42.4 ± 9.2 mm Hg (P > .05).

The indices were restored to normal about 30 minutes after the second challenge (Figure 3, A-D). The RR of group A declined to 13 ± 3.8 breaths/min, and the RR of group B to 19 ± 5.5 breaths/min; the Ppeak of group A was 10 ± 3.9 cm H2O, and the Ppeak of group B 12 ± 3.4 cm H2O; the Cdyn of group A returned to 35.5 ± 7.7 mL/cm H2O, and the Cdyn of group B to 21 ± 9.1 mL/cm H2O; the VT of group A went back to 21 ± 9.1 mL/cm H2O, and the VT of group B to 270 ± 87.3 mL.

Reduced Airway Inflammation After HSVHP

After the second challenge, dogs in group A showed a dramatic decrease in inflammatory cells, including neutrophils, macrophages, and eosinophils, in the lungs and bronchoalveolar lavage fluid relative to group B (Table 1). In group A, the total cells, macrophages, neutrophils, lymphocytes, and eosinophils were 6.1 ± 2.8, 3.6 ± 1.9, 1.8 ± 0.9, 0.6 ± 0.4, and 0.01 ± 0.02 10^6 cells/mL, whereas in group B, they were 36.9 ± 9.6, 26.2 ± 8.0, 8.2 ± 2.0, 1.3 ± 0.5, and 0.51 ± 0.44 10^6 cells/mL. The differences between the groups in the numbers of total cells, macrophages, neutrophils, lymphocytes, and eosinophils were statistically significant (P < .05).

Impact of HSVHP on Circulating Proinflammatory Markers

After the first challenge, IL-4 in group A in plasma increased from 0.396 ± 0.279 to 0.413 ± 0.325 ng/L (P > .05), and in group B it increased from 0.428 ± 0.195 to 0.444 ± 0.266 ng/L (P > .05; Table 2). Significant increases...
in IgE were observed in both groups (Table 2). IgE in group A increased from 0.856 ± 0.328 to 1.232 ± 0.434 IU/mL (P < .05), whereas IgE in group B increased from 1.040 ± 0.127 to 1.514 ± 0.106 IU/mL (P < .05).

After the second challenge, the IL-4 of group A increased from 0.412 ± 0.311 to 0.514 ± 0.450 ng/L (P > .05), whereas the IL-4 of group B increased from 0.370 ± 0.236 to 0.581 ± 0.357 ng/L (P > .05) (Table 2). A significant increase in IgE was observed in group B, with the IgE of group B increased from 0.660 ± 0.093 to 0.972 ± 0.238 IU/mL (P < .05). The IgE of group A was elevated from 0.586 ± 0.109 to 0.994 ± 0.282 IU/mL (P > .05), and there was no significant difference in IgE between the groups (Table 2).

### DISCUSSION

After the operation, dogs in group A showed digestive symptoms including vomiting, with normal health restored gradually 4 days later, whereas in group B no similar symptoms were observed. The vomiting may have been

<p>| TABLE 1. The numbers of total cells and inflammatory cells in bronchoalveolar lavage fluid |
|-----------------------------------------------|----------------|----------------|</p>
<table>
<thead>
<tr>
<th>Types of cells</th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cells</td>
<td>6.1 ± 2.8</td>
<td>36.9 ± 9.6</td>
</tr>
<tr>
<td>Macrophages</td>
<td>3.6 ± 1.9</td>
<td>26.2 ± 8.0</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>1.8 ± 0.9</td>
<td>8.2 ± 2.0</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>0.6 ± 0.4</td>
<td>1.3 ± 0.5</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0.01 ± 0.02</td>
<td>0.51 ± 0.44</td>
</tr>
<tr>
<td>Others</td>
<td>0.13 ± 0.25</td>
<td>0.69 ± 0.86</td>
</tr>
</tbody>
</table>

All figures represent mean ± SD and are in 10⁶ cells/mL. P < .05 for all comparisons of numbers of inflammatory cells between groups A and B.
due to the intraoperative stimulation of the vagal trunk. No bronchorrhea was found in either group. On the other hand, in group A bronchial submucous glands secreted less mucus because of blockade of the parasympathetic nerve pathway. No pneumothorax developed in either group, and no significant changes in HR and MAP were observed in group A after the operation relative to preoperative values, suggesting that the vagal cardiac plexus was functionally normal.

Airway hyperresponsiveness is the most striking feature of asthma. In this study, the most commonly used indicators of pulmonary function (RR, Ppeak, Cdyn, and Vr){ref}[16-15] were used for the evaluation of airway hyperresponsiveness. Airway hyperresponsiveness was induced by the first aerosol inhalation of A suum antigen in both groups. After the second challenge, no significant changes were observed in RR, Ppeak, and Cdyn in group A, whereas dramatic changes in RR, Ppeak, and Cdyn could still be observed in group B. The results show that HSVHP protected the dogs in group A from airway hyperresponsiveness.

Non-specific chronic inflammation of the airway has been seen as the pathologic basis of asthma. Some investigations have suggested that the hyperreactivity of the vagus nerve, in conjunction with certain immunologic mechanisms, may produce proinflammatory chemical mediators. In our study, the dogs treated by HSVHP showed dramatic decreases in inflammatory cells in the lungs and bronchoalveolar lavage; however, HSVHP had no impact on serum proinflammatory markers. After the second challenge, IL-4 in both groups increased, as it did after the first challenge. Although the serum IgE of group A did not increase after the second challenge as significantly as did the IgE of group B, an increasing trend was observed and the underlying mechanism warrants further investigation.

According to our results, the HSVHP worked the same way as anticholinergic inhalers do. Both suppress asthma by blocking the airway vagal pathway. HSVHP, however, specifically inhibited the bronchial vagal reaction, so that side effects such as dry mouth, which is especially common in asthmatic patients receiving long-acting anticholinergic agents, were avoided. Recently, bronchial thermoplasty was reported to use heat generated by radiofrequency energy to ablate smooth muscles and thereby relieve bronchoconstriction. Consistent with this notion, HSVHP controls bronchoconstriction by inhibiting enhanced vagal tone that induces excessive contraction of airway smooth muscles. Moreover, anatomic investigation demonstrated that human vagal branches in the hilus pulmonis consists of anterior branches, posterior branches, and superior branches that were only found in some people. The bilateral vagotomy is feasible in human beings, which suggests that it could become a new choice for the treatment of asthma.

This study also had some limitations. The totally sedated dogs were challenged through tracheal intubation, and airway indices were also detected via tracheal intubation, which may have affected the results of the determination of airway parameters. In fact, it is very difficult to induce asthma and measure airway indicators in awake dogs without intubation. In our future studies, we will evaluate the effect of HSVHP in terms of airway parameters on awake patients with asthma without intubation.

References