Bronchial Blood Flow at Anastomosis of Allografts in Relation to Immunosuppressive Drugs

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ABSTRACT: The microflow in the bronchial mucosa was measured by means of electrochemically generated hydrogen on dogs, identifying close producibility of measurement. In general anesthesia, the microflow of tracheal bifurcation was 71.5±13.23 ml/min/100g that of right main bronchus, 65.6±10.84 and that of left main bronchus, 69.0±12.51 respectively. There was no significant difference in microflow between anastomotically different sites. Basic diffusion in the bronchial mucosa was estimated as many as 17.0±4.80 ml/min/100g. The microflow at bronchial anastomosis in autografts increased on day 3 and hyperdynamic state continued on day 7 to 14. On the other hand, that in allografts treated with cyclosporine A (CyA) showed increased levels on day 5 although that treated with azathioprine demonstrated lower levels until on day 7, thereafter increased on day 10.

INTRODUCTION

Determination of microflow of the brain and digestive tract by means of electrochemically generated hydrogen has been applied in clinical use. However, little information in the field of study on microflow in the submucosa of the trachea is available1, 2).

In this study, the availability of determination of microflow of the trachea and its bifurcation is tested on dogs in terms of the reproducibility of this measurement method and diffusion volume of electrochemically generated hydrogen. Furthermore, wound healing at bronchial anastomosis was experimentally compared between lung allografts using immunosuppressive drugs of azathioprine (Aza) and cyclosporine A (CyA) in terms of microflow in the submucosal layer of the trachea to define the influence of the immunosuppressive drugs on the sites of bronchial anastomosis of lung allografts.

I Preliminary Study

1 MATERIALS AND METHODS

Mongrel dogs weighed from 8.0 to 14.5 kg were anesthetized with 35 mg/kg of nembutal sodium and intratracheally intubated and connected with Harvard respirator with respiratory rate of 14/min and tidal volume of 25 ml/kg. The counter electrode (plate type) was placed on the membranous layer of the mouth. Bronchoscopy (BF type 2 of Olympus) was also used and two electrode catheters (1.8 mm outer diameter and 2.5 mm insertion length, Fig. 1) were introduced from the channel hole. The placements of these electrodes were selected in between cartilaginous rings which was visible via bronchoscopy and stable in setting. The flow meter (RBF-1 Biomedical Sci.) and analizer
(AD-1002 Biomedical Sci.) were used in this study as shown in Fig. 1 and 2, on the condition of biochemical current of 10 μA and its time duration of 60 sec and polaro voltage of 600mV respectively.

1. Evaluation of Reproducibility
The reproducibility of microflow values by means of electrochemically generated hydrogen was evaluated with double measurements at the interval at least 5 minutes at 17 sites on the same conditions.

2. Microflow Values among the Sites of the Tracheal Bifurcation, Left and Right Main Bronchi
The measurements by means of electrochemically generated hydrogen were made at the anatomically different sites and its variation was also tested.

3. Measurement of Basic Diffusion Volume
The changes in microflow on the tracheal wall were assessed on the condition of cardiac arrest during 60 minutes after instillation of 40mEq KCL.

2 RESULTS
The relationship between time course and measured voltage was shown in Fig. 3 in a dog whose submucosal microflow of the trachea was measured by means of electrochemically generated hydrogen. It showed a plateau at 30 sec of the beginning of the measurement by the limitter of a data analizer. Rapidly decresing part on this graph contained the sites of diffusion into the surrounding tissues which was inadequate for the measurement. At the time from 180 to 230 sec after the beginning of the measurement value of 68.6 ml/min/100g was obtained. The correlation rate between two
Fig. 3. Relationship between time course and measured voltages immediately after measurement by means of electrochemically generated hydrogen.

Values obtained by double measurements were 0.9799 with a revolving equation ($Y = 1.31222 + 0.987886X$, Fig. 4). In the sites of tracheal bifurcation ($F = 71.5 \pm 13.23 \text{ ml/min/100g}$), attachment of right main bronchus ($F = 65.6 \pm 10.84 \text{ ml/min/100g}$) and attachment of left...

$F = 68.6 \text{ [ml/min/100g]}$ at interval between 18 and 23.

Fig. 4. The correlation between double values measured twice at the same site.
main bronchus \( (F = 69.0 \pm 12.51 \text{ ml/min/100g}) \), each equation was calculated without statistically significant difference \((p < 0.05)\) as shown in Fig. 5. The changes in microflow after arrest of the heart demonstrated a slow decrease and reached at 17.0 ± 4.80 ml/min/100g at 60 minutes, showing a values as false diffusion volume which was shown in Fig. 6.

II Changes in Microflow at Bronchial Anastomosis on Lung Allografts

1 MATERIALS AND METHODS

The microflow at bronchial anastomosis was assessed on dogs with auto- and allografts by means of electrochemically generated hydrogen. Lung transplantation was performed by left thoracotomy at the 5th intercostal space and operation steps of anastomosis were made in the following order, the left atrium, left pulmonary artery with continuous suture of 5-0 prolene and left main bronchus with continuous suture of 4-0 prolene. Antibiotics of sephem and/or aminogluconside were used for prevention of postoperative infection. The dogs were supplied from the Animal Center of Nagasaki University School of Medicine and divided into three groups, Au group of autotransplantation
on 10 dogs, Az group of lung allotransplantation with azathioprine 50mg/body on 9 dogs and Cy group of lung allotransplantation with cyclosporine A 20mg/kg on 8 dogs. The measurement was made with the same instruments as a preliminary study. On autografts the measurements of microflow were carried out at the four sites of the tracheal bifurcation, bronchial Anastomosis, the distal site to bronchial Anastomosis and left main bronchus at 3, 5, 7, 10, 14, 21 and 28th day after surgery. In allografts, the measurements were made at the sites of the tracheal bifurcation in a recipient preoperatively, attachment of the main bronchus postoperatively until on day 21. The dogs with loss of aeration on chest XP films and stenosis or ulcerative change at Anastomosis were excluded from this study. These values obtained were compared with the control of the tracheal bifurcation preoperatively and of attachment of left main bronchus postoperatively. Statistical comparison between the post- and preoperative values were performed using the Student’s t-test for compared variable.

2 RESULTS

1 Lung Autografts (Table 1, Fig. 7. 1-4)

There was no significant difference between the values at tracheal bifurcation until on day 7. At the 10 and 14th days, these were increased to 89.5±3.58ml/min/100g on day 10 and to 106.0±5.43ml/min/100g on day 14. At the proximal sites to anastomosis, the tissue flow were increased to 102.2±27.13ml/min/100g respectively. At the distal sites, these were reduced from 66.5±11.62 to 33.4±10.99ml/min/100g g and subsequently increased to 86.7±21.23 on day 7, 97.8±18.51 on day 10, 98.0±14.91ml/min/100 g and reduced to the normal. At attachment of the left main bronchus (in Fig. 7-4) the tissue flow was kept normal until on day 5 without significant reduction and these kept high flow of 87.9±14.41ml/min/100 g on day 7, 83.2±8.11 on day 14, 79.4±1.50 on day 28.

2. Lung Allografts with Azathioprine (Table 2, Fig. 8. 1-4)

At the tracheal bifurcation, tissue flow showed a high value of 88.5±11.36ml/min/100g. At the sites proximal to bronchial anastomosis, it was decreased to 64.5±6.59ml/min/100g on day 7. At the sites distal to bronchial anastomosis (Fig. 8-3), it was reduced from 72.1±4.96 to 30.0±8.32ml/min/100g thereafter, it was still kept reduced to 46.5±14.67 on day 3, 42.0±8.39 on day 5, 55.8±10.75 ml/min/100g on day 7. Thereafter it gradually increased on day 10. Meanwhile, at the attachment of the main bronchi (Fig. 8-4) it was reduced to 58.9±5.08 on day 5, thereafter increased on day 7 without statistical difference.

3 Lung Allografts with Cyclosporine A (Table 3, Fig. 9. 1-4)

At the site of tracheal bifurcation, the tissue flow increased to 103.9±26.59ml/min/
Table 2. The submucosal microflow at the sites of tracheal carina, left main bronchus proximal and distal to anastomosis and its attachment according to time course after left lung allotransplantation treated with Aza.

<table>
<thead>
<tr>
<th>Site</th>
<th>pre ope</th>
<th>post ope</th>
<th>p. o. 3</th>
<th>p. o. 5</th>
<th>p. o. 7</th>
<th>p. o. 10</th>
<th>p. o. 14</th>
<th>p. o. 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carina</td>
<td>73.7± 7.42</td>
<td>75.2± 9.95</td>
<td>76.2± 13.00</td>
<td>76.3± 13.30</td>
<td>88.8± 11.36**</td>
<td>77.5± 19.40</td>
<td>75.0± 18.16</td>
<td>83.1± 5.92</td>
</tr>
<tr>
<td>proximal</td>
<td>(n=9)</td>
<td>(n=7)</td>
<td>(n=7)</td>
<td>(n=7)</td>
<td>(n=6)</td>
<td>(n=6)</td>
<td>(n=6)</td>
<td>(n=4)</td>
</tr>
<tr>
<td>distal site of ana.</td>
<td>30.0± 8.32**</td>
<td>46.5±14.67**</td>
<td>42.0± 8.39**</td>
<td>55.8± 10.75*</td>
<td>63.4±13.67</td>
<td>64.9±10.22</td>
<td>66.1±24.40</td>
<td></td>
</tr>
<tr>
<td>(n=8)</td>
<td>(n=8)</td>
<td>(n=7)</td>
<td>(n=6)</td>
<td>(n=6)</td>
<td>(n=6)</td>
<td>(n=4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>left the 1st bifu</td>
<td>62.7± 6.01**</td>
<td>69.1± 6.16</td>
<td>58.9± 5.08</td>
<td>77.7± 10.83</td>
<td>66.6±14.44</td>
<td>73.2± 8.38</td>
<td>82.0±16.40</td>
<td></td>
</tr>
<tr>
<td>(n=9)</td>
<td>(n=7)</td>
<td>(n=6)</td>
<td>(n=6)</td>
<td>(n=6)</td>
<td>(n=6)</td>
<td>(n=4)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

( * p<0.05, ** p<0.01)
Fig. 8-1. Changes in microflow of tracheal carina after left lung allotransplantation treated with Aza.

Fig. 8-2. Changes in microflow in left main bronchus proximal to anastomosis after left lung allotransplantation treated with Aza.

Fig. 8-3. Changes in microflow in left main bronchus distal to anastomosis after lung allotransplantation treated with Aza.

Fig. 8-4. Changes in microflow of the attachment of left main bronchus after lung allotransplantation treated with Aza.

Table 3. The submucosal microflow at the sites of tracheal carine, left main bronchus proximal and distal to anastomosis and its attachment according to time course after left lung allotransplantation treated with CyA.

<table>
<thead>
<tr>
<th></th>
<th>pre ope</th>
<th>post ope</th>
<th>p. o. 3</th>
<th>p. o. 5</th>
<th>p. o. 7</th>
<th>p. o. 10</th>
<th>p. o. 14</th>
<th>p. o. 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carina</td>
<td>76.6 ± 9.59</td>
<td>77.0 ± 7.79</td>
<td>71.5 ± 13.18</td>
<td>103.9 ± 26.59</td>
<td>83.8 ± 11.85</td>
<td>78.5 ± 10.43</td>
<td>71.6 ± 10.65</td>
<td>87.1 ± 18.22</td>
</tr>
<tr>
<td>proximal site of ana.</td>
<td>61.6 ± 15.28 * (n=8)</td>
<td>62.9 ± 10.81 * (n=8)</td>
<td>77.7 ± 16.22 (n=7)</td>
<td>68.0 ± 9.08 (n=7)</td>
<td>77.9 ± 10.72 (n=7)</td>
<td>80.6 ± 3.39 (n=5)</td>
<td>83.4 ± 17.14 (n=5)</td>
<td></td>
</tr>
<tr>
<td>distal site of ana.</td>
<td>35.4 ± 4.95 ** (n=8)</td>
<td>49.9 ± 9.36 ** (n=8)</td>
<td>68.4 ± 11.72 (n=7)</td>
<td>68.6 ± 5.88 (n=7)</td>
<td>81.9 ± 10.44 (n=7)</td>
<td>88.1 ± 7.58 * (n=5)</td>
<td>90.2 ± 11.30 * (n=5)</td>
<td></td>
</tr>
<tr>
<td>left the 1st bifurcation</td>
<td>72.5 ± 12.55 (n=8)</td>
<td>65.6 ± 13.94 (n=8)</td>
<td>72.7 ± 16.33 (n=8)</td>
<td>67.3 ± 10.40 (n=7)</td>
<td>69.0 ± 8.43 (n=7)</td>
<td>80.4 ± 24.55 (n=7)</td>
<td>74.7 ± 10.00 (n=5)</td>
<td>72.1 ± 8.41 (n=5)</td>
</tr>
</tbody>
</table>

F = m ± S. D. : ml/min/100g

(* p<0.05, ** p<0.01)
Fig. 9-1. Changes in microflow of tracheal canine after left lung allotransplantation treated with CyA.

Fig. 9-2. Changes in microflow in left main bronchus proximal to anastomosis after left lung allotransplantation treated with CyA.

Fig. 9-3. Changes in microflow in left main bronchus distal to anastomosis after lung allotransplantation treated with CyA.

Fig. 9-4. Changes in microflow of the attachment of left main bronchus after lung allotransplantation treated with CyA.

100 g on day 5. At the sites proximal to anastomosis (Fig. 9-2) it maintained a low value of 61.6±15.28 immediately after transplantation and 62.9±10.81 ml/min/100 g on day 5, although it showed no significant change. At the sites distal to anastomosis, it reduced from 72.5/12.55 to 35.4/4.95 immediately after transplantation and to 49.9±9.36 on day 3, thereafter it gradually increased to 88.1±7.58 on day 14, 90.2±11.30 ml/min/100 g on day 21. At the sites of attachment of the left main bronchus, there was no significant changes.

DISCUSSION

The measurement of local tissue flow has been made with the aid of electrochemically generated hydrogen by means of gas inhalation. However, the main drawback to this method is that there is a fear of fire and ill effects of gas inhalation on the body, for example, suffocation by gas in small animals and also it takes considerably long time for hydrogen gas to concentration on ischemic area, in addition, the concentration of the intrachal hydrogen may contribute to make an error to measure
the tissue flow because the measured sites is very close to the sites of gas inhalation by means of hydrogen gas clearance test.

However, the measurement of local tissue flow by means of electrochemically generated hydrogen is simple and transmission of inhaled hydrogen gas is not essential to repeated measurements.

This method by using locally generated hydrogen has been modified in addition to improvement of Stosseck and Aukland’s methods. Since Koshu reported a close relationship between the values measured by Stosseck’ method and inhaled hydrogen gas clearance method, this method has been prevalent in its use. It is a major problem that tissue measured is locally limited around the introduced electrode and it is related to the inserted depth of the electrode. The electrode had a stopper at the depth of 2.5 mm so that the electrode may not be introduced more than 2.5 mm in depth. The electrode used to be obliquely introduced to the mucosal layer. In this study, the sites of electrodes introduced were bronchoscopically confirmed to anchor the mucosa. It is ascertained that the lower the current and the shorter the time, the higher a reciprocal of half-life time has become and it means relative large volume of diffusion. Damage to the tissue is caused by heat, and production of heat is ruled by Joul’s law. To minimize damage to the tissue, the measurement should be set on the condition of the current of 10 μA and current time of 60 sec. There was no damage to the tissue by measurement except for a little bleeding at the sites in which electrodes were introduced. Repeated measurement ensured close producibility which represents an equation \( Y = 1.31222 + 0.98786X \) and correlation coefficient \( r = 0.9799 \). Basic diffusion was confirmed on the condition of cardiac arrest as a value of 24.1 ± 1.4 ml/min/100 g in canine stomach, 25 ml/min/100 g in human stomach and 14.0 ml/min/100 g in human muscles. Recently Fujino reported a value of basic diffusion of 16.03 ± 4.91 ml/min/100 g in canine bronchus. In this study it was 17.0 ± 4.80 ml/min/100 g with almost the same as him. There was no difference in the microflow of the trachea among three anatomically different sites. Kobayashi reported that there was no difference in the microflow between the sites of bilateral main bronchi and attachment of bilateral diaphragmatic lobe bronchi.

Evaluation of Wound Healing at Bronchial Anastomosis in Terms of Local Microflow

1 Autotransplantation

Restoration of bronchial blood flow at bronchial anastomosis was assessed by means of electrochemically generated hydrogen. It is known that the blood flow in which the bronchial artery was interrupted at bronchial anastomosis is compensated with pulmonary bronchial collateral circulation. In lung transplantation, the bronchial bifurcation in lung grafts are well nourished as compared with those in the recipients, although microflow at distal site to anastomosis is significantly reduced. However, it was returned to the normal, accompanying a hyperdynamic state with an increase in the blood flow on day 7, 10 and 14. It is generally accepted that restoration of bronchial blood flow in lung grafts is established until on day 14. However, blood flow in the submucosa is already increased on day 3 and is pronounced on day 14.

2 Lung Allotransplantation

Immunosuppressive drugs should be prescribed on lung allografts. CyA contributes to facilitation of wound healing at bronchial anastomosis and to electromicroscopic promotion of collagen fiber as compared with autografts. Kawahara confirmed that recanalization of the interrupted bronchial artery in allografts treated with CyA is completed until on day 14 in the same manner as that in autografts although that in allografts treated with Aza is retarded. In this study, it was defined that restoration of the interrupted bronchial artery in allografts treated with CyA started on day 5, although that treated with Aza began on day 10. It is known that CyA helps lung allografts to enhance proliferation of vessels and production of fibrin by chemical mediator produced by macrophages whose activity is not depressed. It is reported that Aza enables the count of monocytes and polymuclear
leucocytes to reduce by bone marrow depression, and overdose of Aza impairs wound healing. In this study it was evident that recanalization of the interrupted bronchial artery was much more facilitated by using CyA rather than that by Aza and the use of CyA is superior to that of Aza in the respect of wound healing.

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