Increased Production and/or Secretion of Pulmonary Surfactant in Rats by Long Term Sulfur Dioxide Exposure

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Influence of long term SO₂ exposure on the pulmonary surfactant in rats was studied by means of chemical analysis and microscopic verification. At a time after termination of the exposure period, the general symptom in rats was similar to that of bronchitis. The content of disaturated phosphatidylcholine, a main functional component of the pulmonary surfactant, significantly increased not only in broncho-alveolar lavage fluid but also in pulmonary microsomal fraction by long term SO₂ exposure. Microscopic verification of alveolar type II cells from the bronchitic rats demonstrated the development of rough surface endoplasmic reticulums and an increase of the number of osmiophilic bodies.

The results suggest that pulmonary surfactant production and/or secretion are activated in rats with bronchitis caused by long term SO₂ exposure.

Keywords — pulmonary surfactant; sulfur dioxide; phosphatidylcholine; broncho-alveolar lavage fluid

Introduction

The pulmonary surfactant lowers surface tension at the air-cell interface, thereby preventing alveolar collapse. Recently, the following actions relating to the defense mechanisms in the overall respiratory tract have been attended: 1) a lubricating action on the respiratory tract mucosa surface, 2) a potentiating action on the phagocytosis of alveolar macrophage and 3) a protecting action on the mucociliary transport function. It also may have beneficial effects in expectoration. Our recent study has revealed that some expectorants produced the action through the stimulation of pulmonary surfactant secretion.

In addition, there have been reports linking respiratory diseases to dysfunction of the surfactant system of the lung: the respiratory distress syndrome of newborns, pneumonia and alveolar proteinosis.

Disaturated phosphatidylcholine (DSPC), the functional main component of pulmonary surfactant, is known to be present in relatively constant amounts in the intra- and extra-cellular pools in the normal condition, implying the existence of regulatory mechanisms maintaining the content. The content and the ratio between the two pools, however, may be altered by pulmonary damages and/or diseases. However, study on the relationship of the surfactant system to pulmonary diseases with respiratory tract obstruction such as bronchitis is needed.

In the present study, we investigated the influence of a long term SO₂ exposure, which is known to produce bronchitis, on the pulmonary surfactant system in rats, addressing the contents of DSPC in broncho-alveolar lavage fluid (BALF) and in the microsome fraction as well as the morphological change in the alveolar type II cells.

Materials and Methods

**Animals** — Male Wistar strain rats weighing 180—200 g, were used and allowed free access to solid diets and water.

**SO₂ Exposure** — SO₂ exposure was carried out using the apparatus described previously. The following protocol for SO₂ exposure was employed to facilitate preparation of chronically bronchitic rats: 100—200 ppm for the 1st week, 200—300 ppm for the 2nd week, and 300—400 ppm for the 3rd to 10th weeks; 2 h a day
and 5 d a week.

**Broncho-Alveolar Lavage** — Animals were anesthetized with intraperitoneal urethane (1.25 g/kg). The trachea was cannulated and the inferior vena was cut. The lungs were gently perfused, with physiological saline (37 °C) through the pulmonary artery and then removed. The lungs were gently lavaged twice with 10 ml of physiological saline (37 °C). The lavage solution was centrifuged at 400 × g for 10 min to remove cellular components, and then subjected to phospholipid analysis.

**Preparation of Pulmonary Microsomal Fraction** — The lavaged lungs were homogenized in a 9-fold volume of ice cold 0.15% KCl solution to wet tissue weight and centrifuged at 600 × g for 10 min. The supernatant was centrifuged at 13500 × g for 30 min. The resultant supernatant was further centrifuged at 105000 × g for 60 min to isolate the microsomal fraction.

**Lipid Analysis** — DSPC was isolated according to the method of Mason *et al.* 14) Phosphatidylcholine (PC) and DSPC were both quantitated by means of PL kit-k (Nippon Shoji Co., Ltd.). The average recovery of DSPC after the entire lipid analysis was approximately 72%.

**Histomorphological Studies** — For electron microscopic observation, the following procedures were taken. Blocks of lung tissue (1 mm³) from control or exposed animals were prefixed in 4% glutaraldehyde phosphate-buffer solution (pH 7.3) and postfixed in osmium tetroxide solution. The fixed specimens were embedded in epoxye resin, sliced, and stained with uranyl acetate and lead citrate.

**Blood Gases** — PaO₂ and PaCO₂ of blood gas were measured, as an index of the ventilation ability, using a pH-Blood gas analyzer (IL 1304, ALLIED I.L.). Blood samples were obtained from the aorta after termination of the SO₂ exposure period.

For statistical comparisons, Student’s *t*-test for unpaired experiments was used.

**Results**

After termination of the exposure period, the rats showed hypotrophy and emphysema in lung tissues, and accumulation of sputa in the tracheo-bronchus. Furthermore, a gain in body weight was suppressed to 76% of the control animals. The ability of ventilation was clearly lowered in the exposed rats, as indicated by the change in blood gas parameters: for control rats, PaO₂ 96 ± 6 torr, PaCO₂ 39 ± 9 torr; for the exposed rats, PaO₂ 60 ± 5 torr, PaCO₂ 55 ± 6 torr (mean ± S.E., n = 6). Thus, rats were confirmed to have a bronchitis at a time after termination of the exposure period.

PC and DSPC content in BALF of the SO₂-exposed rats were both significantly higher compared with those of control rats (Table I). The DSPC content was 3.74 ± 1.02 mg/lung, 70% higher than that in BALF of the control rats. The ratio of DSPC to PC, however, was not significantly different between the two groups, although tending to be higher in exposed group.

In the microsomal fraction, only DSPC content increased, *i.e.* 2.01 ± 0.08 mg/lung in the SO₂-exposed animals and 1.50 ± 0.09 mg/lung in the control animals (Table II). Since the microsomal PC content was not changed by SO₂ exposure, the ratio of DSPC to PC content significantly increased by SO₂ exposure.

**Table I.** PC and DSPC Contents in BALFs from SO₂-Exposed and Control Rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>SO₂ exposure</th>
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</thead>
<tbody>
<tr>
<td>PC (mg/lung)</td>
<td>2.92 ±0.33</td>
<td>4.67 ±0.08²</td>
</tr>
<tr>
<td>DSPC (mg/lung)</td>
<td>2.19 ±0.37</td>
<td>3.74 ±1.02²</td>
</tr>
<tr>
<td>DSPC/PC (%)</td>
<td>75.08 ±2.22</td>
<td>80.14 ±2.07</td>
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Each value is mean ± S.E. of six experiments. *a*) Significantly different from control (*p* <0.05).

**Table II.** PC and DSPC Contents in Microsomal Fraction of the Lung from SO₂-Exposed and Control Rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>SO₂ exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC (mg/lung)</td>
<td>2.87 ±0.25</td>
<td>2.86 ±0.10</td>
</tr>
<tr>
<td>DSPC (mg/lung)</td>
<td>1.50 ±0.09</td>
<td>2.01 ±0.08³</td>
</tr>
<tr>
<td>DSPC/PC (%)</td>
<td>52.65 ±2.34</td>
<td>70.14 ±4.13³</td>
</tr>
<tr>
<td>DSPC</td>
<td>1.46 ±0.19</td>
<td>1.86 ±0.24</td>
</tr>
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Each value is mean ± S.E. of six experiments. *a*) Significantly different from control (*p* <0.05).
the contamination of PC originated from the lung tissue and/or blood. In this experiment, we quantified PC and DSPC contents to determine the ratio of DSPC to PC. It is well known that more than 70% of the PC molecules in the pulmonary surfactant contain two saturated fatty acyl constituents, and then the degree of saturation of PC in the surfactant is much higher than that in PC of the lung tissue or blood.\textsuperscript{15,16} The ratio of DSPC to PC in BALF of normal rats determined here was similar to that in the pulmonary surfactant.\textsuperscript{13} Accordingly, most of the DSPC and PC obtained here originated from the pulmonary surfactant.

Upon SO\textsubscript{2} exposure, the walls of the bronchi are increasingly penetrated by inflammatory infiltrates and plasma constituents, and the epithelial cells are damaged.\textsuperscript{17} Therefore, increases in DSPC and PC contents and the ratio of DSPC to PC in BALF of SO\textsubscript{2} exposed rats might be due not only to the stimulated secretion but also to the disturbed transportation of the surfactant from alveoli to the trachea due to airway obstruction. In fact, we have reported that DSPC content in the respiratory tract fluid transported to the trachea was reduced in the bronchitic rabbits produced by SO\textsubscript{2} exposure.\textsuperscript{5}

However, DSPC contents in the microsomal fraction increased by SO\textsubscript{2} exposure. Furthermore, histological examination revealed that rough surface endoplasmic reticulums and osmiophilic bodies which synthesize and store the pulmonary surfactant increased in alveolar type II cells of SO\textsubscript{2}-exposed rats. These results indicate that pulmonary surfactant production and following secretion are activated in bronchitic animals by a long term SO\textsubscript{2} exposure.

Although the definite mechanism of the increase of pulmonary surfactant by SO\textsubscript{2} exposure has not been investigated, the pulmonary surfactant increases noted in the present study are likely to be partly through the action of leucotrienes released from activated leucocytes or alveolar macrophages in inflammation.

Our preliminary study demonstrated that leucotriene B\textsubscript{4} and C\textsubscript{4}, stimulated pulmonary surfactant secretion in the isolated alveolar type II cells,\textsuperscript{18} and lipid peroxides prominently increased in BALF of SO\textsubscript{2}-exposed rats.

The ratio of DSPC content in BALF to that in microsomal fraction was also increased by SO\textsubscript{2} exposure.

Morphological changes of alveolar type II cells are shown in Fig. 1. The following features were noted in alveolar type II cells from the exposed rats; 1) an increase in number of alveolar type II cells, 2) the development of rough surface endoplasmic reticulums, 3) an increase of osmiophilic bodies in number.

Discussion

The present data have demonstrated that a long term SO\textsubscript{2} exposure increased the content of pulmonary surfactant phospholipids in BALF and microsomal fractions of the lung in rats.

When the secretion of pulmonary surfactant is evaluated by only the alteration of PC contents in BALF, the evaluation may be disturbed by

Fig. 1. Transmission Electron Micrograph of Type II Alveolar Cells from Control (a) and SO\textsubscript{2}-Exposed (b) Rats
In the cells from SO\textsubscript{2}-exposed rats, large osmiophilic bodies are found and rough surfaced endoplasmic reticulums developed are noted.
Denaturation of the surfactant associated protein by lipid peroxidation reaction may be another explanation for the excessive secretion of surfactant phospholipids, since the surfactant associated protein such as surfactant apoprotein A suppresses the secretion of surfactant phospholipids on alveolar type II cells.  

Several investigators have reported about the relationship between pulmonary surfactant production and diseases. Dethloff et al. reported that intratracheal injection of silica increased the contents of surfactant phospholipids in both the extra- and intra-cellular compartments of the lung. The different points from our results are that the ratio of the extracellular surfactant content to the intracellular one was reduced by silica injection, and that both PC and DSPC contents in the intracellular compartment increased in silica exposure. Thus, the mechanism of increases in PC and DSPC contents caused by SO$_2$ exposure seems to differ from that of silica-induced increases.

At present, it is difficult to refer to the relationship between the excessive production of the pulmonary surfactant and the airway obstruction, although a remark can be made. SO$_2$ exposure causes the airway obstruction accompanied by impairments of the mucociliary transport and the increased mucus production. Rensch et al. reported that the particle transport was reduced in surfactant films from chronic bronchitis rats caused by SO$_2$ exposure. The quantitative and qualitative changes in the surfactant components as described above must also cause the disturbance of the airway clearance function of the surfactant. Therefore, the reduced physiological function of the surfactant associated with inflammation might be a possible factor for the airway obstruction caused by SO$_2$ exposure.

References

18) A. M. Gillfillan and S. A. Rooney: Leukotrienes stimulate phosphatidylcholine secretion in cultured type II
