Disturbance of the normal mucociliary clearance due to hyperproduction of mucus and modification of its physicochemical characteristics is a common finding in airway diseases. Drugs that affect airway secretion have been proposed and used to cleanse the respiratory tract for many centuries and in many countries. On the basis of the mechanism of their actions, the mucoactive drugs were classified into several groups [1]. Some mucoactive drugs have direct effects on the production or composition of airway secretions, resulting in increased effectiveness of mucociliary clearance. The other mucoactive drugs do not have a specific action on mucus, but have beneficial effects on airway structure and function, which lead to correction of pathophysiological mechanisms that result in abnormal secretions.

However, since many drugs have overlapping effects, it is difficult to classify these drugs into groups based on their major actions. For example, it is well known that ambroxol stimulates the formation and release of pulmonary surfactant by alveolar type II cells [2]. However, recent reports indicate that ambroxol as well as pulmonary surfactant by alveolar type II cells [2]. How-ever, recent reports indicate that ambroxol as well as new cysteine derivatives. On the basis of these findings, we believe that the efforts to seek for compatible actions between glucocorticoids and oriental medicines may provide new opportunities for development of ideal mucoactive drugs with specified actions, i.e. suppression of gene expression.

In the light of this idea, we must use specific experimental models to simulate pharmacological events in airway inflammation. Nowadays, the development of new techniques has made it possible to identify and measure the mucus components, to measure the rheological parameters more accurately, and to evaluate mucociliary clearance precisely in animals and humans. Therefore, with modifications of methods, we have evaluated mucoactive drugs from various points of view in order to reflect actions in inflammatory states over two decades. Here, we introduce the methods we have used to study many of the parameters involved in airway clearance, and we describe some of the mucoactive drugs that we have studied recently.
Screening systems for mucoactive drugs

In vivo screening systems for airway secretion in inflammation

There are several experimental models of airway inflammation. Amongst these, we used a sulphur dioxide (SO₂) exposed model as an in vivo screening system for airway secretion in inflammation, because SO₂ mainly causes airway epithelial damage similar to features of bronchitis. There are two convenient methods to study airway secretion: the Perry and Boyd method [14, 15], and the bronchoalveolar lavage method.

The Perry and Boyd method has been used extensively to study the effects of sympathetic or parasympathetic agents and the effects of many kinds of mucoactive drugs. In our previous studies, the mucous production of rabbits with subacute bronchitis induced by a long-term exposure (5 weeks to 3 months) to SO₂ (50–300 parts per million (ppm)) has been determined with this method. We determined sugar, protein and phospholipid contents in airway secretions from normal and bronchitic rabbits and evaluated the effects of various mucoactive drugs on airway secretion [16–21]. The majority of sugars in mucins are composed of fucose, galactose, N-acetylgalactosamine, N-acetylglucoasamine and N-acetylneuraminic acid. As shown in table 1, we found that the sugars were increased in airway secretions of rabbits exposed to SO₂. The finding that there was a large increase in galactose and N-acetylglucoasamine is similar to that observed in the sputum of bronchitic patients. The finding suggests that airway secretions from rabbits exposed to SO₂ are composed of long chains of sugars in mucins, resulting in secretions with a viscous nature, similar to the sputa of bronchitic patients. The reason is that these two sugars are major components of the elongated sugars in mucins. Figure 1 shows the basis of the sugar structure of mucin.

Bronchoalveolar lavage methods were also employed to study airway secretions. The advantage of this method is that many components can be examined quantitatively and qualitatively. For example, we examined the influence of long-term exposure to SO₂ on the pulmonary surfactant by this method [21]. Recently, in order to investigate mucin production in pathological states, we made monoclonal antibodies (4H6, 2D11) against the mucins from bronchoalveolar lavages of hamsters with bronchitis caused by SO₂ exposure [22]. In the immunohistochemical studies, the antibodies recognized the mucins secreted into the lumen, but not those stored in goblet cells or submucosal gland mucous cells (fig. 2). The enzyme-linked immunosorbent assay (ELISA) method has shown that the antibodies react with some mucins from hamster intestine and swine stomach, and bronchoalveolar lavages of rats, guinea-pigs, dogs and humans. However, the antibodies did not recognize bovine submaxillary gland mucins or proteoglycans (fig. 3 and table 2). Therefore, ELISA, using the antibodies, could be applied to quantify airway mucin production in future mucoactive drug screening studies.

<table>
<thead>
<tr>
<th>Animals</th>
<th>Fucose</th>
<th>Galactose</th>
<th>GalNAc</th>
<th>GlcNAc</th>
<th>NANA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>13.0±2.4</td>
<td>45.4±2.9</td>
<td>68.3±3.7</td>
<td>41.8±1.5</td>
<td>18.6±1.6</td>
</tr>
<tr>
<td>Bronchitic</td>
<td>30.9±7.6</td>
<td>122.0±31.0</td>
<td>259.3±74.9</td>
<td>83.8±10.2</td>
<td>34.5±2.5</td>
</tr>
</tbody>
</table>

GalNAc: N-acetylgalactosamine; GlcNAc: N-acetylglucoasamine; NANA: N-acetylneuraminic acid.

Table 1. – Sugar components of airway secretions in normal and bronchitic rabbits

Fig. 1. – Schematic representation of the structure of O-linked carbohydrate. GalNAc: N-acetylgalactosamine; Gal: galactose; GlcNAc: N-acetylglucoasamine; Fuc: fucose; NeuNAc: N-acetylneuraminic acid; Ser/Thr: serine or threonine.

Fig. 2. – Immunohistochemical staining of hamster trachea with monoclonal antibody 4H6: a) 4H6 reacted against tracheal surface secretions in a normal hamster; b) tracheal submucosal gland secretions in a bronchitic hamster haematoxylin counterstain. Internal scale bar = 50 μm. (Reproduced with permission from [22]).
secretion in hamster tracheal epithelial cells (table 3). Recently, we have shown that protein kinase C was involved in HMWG secretion in hamster tracheal epithelial cells in culture [30].

Table 3. – Effects of pharmacological agents on the secretion of high molecular weight glycoconjugates (HMWG) in primary culture of hamster tracheal epithelial (HTE) cells.

<table>
<thead>
<tr>
<th>Neurotransmitters</th>
<th>HMWG secretion % control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control water</td>
<td>100.0±3.6</td>
</tr>
<tr>
<td>ATP (2 mM)</td>
<td>236.5±13.6*</td>
</tr>
<tr>
<td>PMA (10 nM)</td>
<td>226.1±9.1*</td>
</tr>
<tr>
<td>Norepinephrine (10 µM)</td>
<td>73.2±3.6*</td>
</tr>
<tr>
<td>Isoproterenol (10 µM)</td>
<td>66.4±4.1*</td>
</tr>
<tr>
<td>Forskolin (1 µM)</td>
<td>61.3±4.6*</td>
</tr>
<tr>
<td>8-Br-cAMP (10 µM)</td>
<td>97.1±7.9*</td>
</tr>
</tbody>
</table>

HTE cells were incubated with pharmacological agents for 30 min. Each value represents the mean±SEM of four samples. *: p<0.05 versus control. ATP: adenosine triphosphate; PMA: phorbol myristate acetate; cAMP: cyclic adenosine monophosphate.

Table 2. – Cross-reactivities of monoclonal antibody 4H6

<table>
<thead>
<tr>
<th>Species</th>
<th>Sources</th>
<th>ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamster</td>
<td>BALF</td>
<td>+</td>
</tr>
<tr>
<td>Rat</td>
<td>BALF</td>
<td>+</td>
</tr>
<tr>
<td>Guinea-pig</td>
<td>BALF</td>
<td>+</td>
</tr>
<tr>
<td>Dog</td>
<td>BALF</td>
<td>+</td>
</tr>
<tr>
<td>Pig</td>
<td>Stomach</td>
<td>+</td>
</tr>
<tr>
<td>Cow</td>
<td>Submaxillary gland</td>
<td>-</td>
</tr>
<tr>
<td>Human</td>
<td>BALF</td>
<td>+</td>
</tr>
</tbody>
</table>

+: positive reaction; -: negative reaction. ELISA: enzyme linked immunosorbent assay; BALF: bronchoalveolar lavage fluid; ILF: intestinal lavage fluid; Stomach: porcine gastric mucin; Submaxillary gland: bovine submaxillary gland mucin. Porcine gastric mucin and bovine submaxillary gland mucin were obtained from commercial sources. (Reproduced with permission from [22]).

In vitro screening systems for airway secretion in inflammation

For drug evaluation, we used two kinds of in vitro model to evaluate mucoactive drugs. To investigate mucous secretions as a major component of the gel layer of airway secretions, we have used culture systems of hamster tracheal epithelial cells or human pulmonary mucus-secreting cells. We indicated that neutrophil elastase may be a crucial mediator to induce mucous secretion in inflammation.

Pulmonary surfactant, which is composed of phospholipids and apoproteins, is mainly produced in alveolar type II cells, lowers the surface tension at the air-liquid interface in the lung and provides alveolar stability. De Sanctis et al. [33] clearly demonstrated that, in addition to the vital role, surfactants were also important in airway mucociliary clearance. Several studies have suggested that the presence of phospholipids in the airways may modify the clearance of mucus [34–39]. The existence of surfactant films has been demonstrated in the airways of several species by electron microscopy [36, 40], and by in situ surface tension measurement [36, 41]. We also confirmed a protective effect of surface active phospholipids on an acid-inducing inhibition of mucociliary transport in pigeons [42].

To simulate an inflammatory state, we have used co-culture systems of mucous cells with polymorphonuclear leucocytes (PMNLs) activated by several stimuli [31, 32]. Abnormal and excessive mucous secretion is a characteristic feature of many chronic inflammatory lung diseases, accompanied by the influx of PMNLs into the airway and release of substance P (SP) from the peripheral endings of primary sensory neurons. We examined whether PMNLs activated by SP (10 mM) can affect the secretion of HMWG from cultured hamster tracheal epithelial cells. We measured both the released and the cell-associated HMWG. SP-activated PMNLs (106 cells·mL−1) reduced the amount of cell-associated HMWG to 76% of the control level, but did not affect the amount of the released HMWG. The reduction of the amount of cell-associated HMWG was inhibited by ONO-5046, a specific elastase inhibitor (fig. 4). In addition, the HMWG was digested by the activated PMNLs. These findings suggested that SP stimulated the release of cell-associated HMWG, which was then degraded by elastase released from PMNLs activated by SP. As suggested in many reports, we indicated that neutrophil elastase may be a crucial mediator to induce mucous secretion in inflammation.
CURRENT STATUS OF MUCUS DRUG DEVELOPMENT

To simulate inflammatory states, we have used co-culture systems of alveolar type II cells with activated PMNLs or eosinophils [47, 48]. Activated PMNLs and eosinophils in airway epithelium are thought to be involved in the pathogenesis of many airway diseases. PMNLs or eosinophils activated by opsonized zymosan caused a significant increase in phosphatidylcholine secretion. Pretreatment of the culture with the combination of superoxide dismutase and catalase reduced the increase in phosphatidylcholine secretion (data on activated eosinophils shown in figures 6 and 7). These results suggested that activated PMNLs and eosinophils stimulated the secretion of pulmonary surfactant, and that the stimulation was partly mediated by oxygen radicals. These systems may be useful to assay the antioxidant effects of mucoactive drugs, although it remains unclear whether the increased pulmonary surfactant plays a defensive role in inflammation.

Mucociliary transport in inflammation

Mucociliary clearance is an important pulmonary defense mechanism that serves to remove inhaled substances from the lung [49]. The mucociliary function is depressed by a variety of water-soluble atmospheric pollutants such as SO₂ and nitrogen dioxide (NO₂) [49]. The techniques for in vivo measurement of mucous transport rates involve the placement of an optically, radiographically, or scintigraphically detectable solid or liquid marker on the mucosa [50].

Table 4. – Effect of β-adrenoceptor antagonists on β-adrenoceptor agonist-induced secretion of [3H]phosphatidylcholine in rat alveolar type II cells

<table>
<thead>
<tr>
<th>Antagonists</th>
<th>Isoprenaline (0.1 µM)</th>
<th>Dobutamine (0.1 µM)</th>
<th>Procaterol (0.1 µM)</th>
<th>Basal secretion</th>
<th>No antagonist</th>
<th>Propranolol (0.1 µM)</th>
<th>Atenolol (1.0 µM)</th>
<th>ICI 118,551 (0.1 µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.45±0.05</td>
<td>0.39±0.04</td>
<td>0.50±0.07</td>
<td>1.22±0.08</td>
<td>0.96±0.06</td>
<td>1.24±0.11</td>
<td>1.00±0.09</td>
<td>0.56±0.05*</td>
</tr>
<tr>
<td></td>
<td>(100)</td>
<td>(100)</td>
<td>(100)</td>
<td>(100)</td>
<td>(100)</td>
<td>(100)</td>
<td>(74.1)</td>
<td>(29.8)</td>
</tr>
<tr>
<td>Propranolol</td>
<td>0.78±0.08*</td>
<td>0.54±0.03*</td>
<td>0.65±0.08*</td>
<td>0.72±0.07</td>
<td>0.86±0.04</td>
<td>0.61±0.16*</td>
<td>0.68±0.18*</td>
<td>(63.6)</td>
</tr>
<tr>
<td>(0.1 µM)</td>
<td>(42.9)</td>
<td>(26.3)</td>
<td>(20.3)</td>
<td>(71.4)</td>
<td>(29.8)</td>
<td>(14.9)</td>
<td>(29.9)</td>
<td>(82.5)</td>
</tr>
<tr>
<td>Atenolol</td>
<td>1.00±0.09</td>
<td>0.56±0.05*</td>
<td>1.32±0.06*</td>
<td>0.94±0.07</td>
<td>0.86±0.04</td>
<td>0.61±0.16*</td>
<td>0.68±0.18*</td>
<td>(63.6)</td>
</tr>
<tr>
<td>(1.0 µM)</td>
<td>(71.4)</td>
<td>(29.8)</td>
<td>(114)</td>
<td>(71.4)</td>
<td>(29.8)</td>
<td>(114)</td>
<td>(29.9)</td>
<td>(82.5)</td>
</tr>
<tr>
<td>ICI 118,551</td>
<td>0.94±0.07</td>
<td>0.86±0.04</td>
<td>0.61±0.16*</td>
<td>0.68±0.18*</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>(0.1 µM)</td>
<td>(63.6)</td>
<td>(82.5)</td>
<td>(14.9)</td>
<td>(63.6)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Secretion is expressed as the amount of [3H]phosphatidylcholine in the medium as a percentage of that in cells plus medium at the end of the incubation period. The percentage change in the secretion attenuated by antagonists was obtained from the comparison with the effect of agonists alone, as shown in parentheses. Antagonists were added 5 min before the addition of agonists. The data are presented as mean±SEM from four to six experiments and were analysed statistically with Duncan’s multiple-range test. *: p<0.05 as compared to control group values. ND: not done.

Fig. 4. – Effect of substance P (SP)-activated polymorphonuclear leukocytes (PMNLs) on: a) secretion of [3H] high molecular weight glycoconjugates (HMWG); and b) amount of the cell-associated [3H] HMWG incubated in cultured hamster tracheal epithelial cells. Cultures were incubated with water, SP (10 µm), PMNLs (10⁶ cells·mL⁻¹), or SP-activated PMNLs (10⁸ cells·mL⁻¹, 10⁹ cells·mL⁻¹ or 10¹⁰ cells·mL⁻¹) for 30 min, or were pretreated with ONO-5046 (ONO; 10 mM) for 1 min before the addition of SP-activated PMNLs (10⁹ cells·mL⁻¹). Each value represents the mean±SEM of four samples. #: p<0.05 versus control (control, or PMNLs alone); *: p<0.05 versus SP-activated PMNLs (10⁶ cells·mL⁻¹). (Reproduced with permission from [31]).

Table 4. – Effect of β-adrenoceptor antagonists on β-adrenoceptor agonist-induced secretion of [3H]phosphatidylcholine in rat alveolar type II cells

Fig. 5. – Northern blot analysis of β₁- and β₂-adrenoceptor messenger ribonucleic acid (mRNA) in rat alveolar type II cells. Two micrograms of poly (A) ribonucleic acid (RNA) from primary cultured alveolar type II cells were electrophoresed on formaldehyde-agarose gel, transferred to a nylon membrane. The blot was hybridized with [13P]dCTP-labelled β₁- (β₁-AR), β₂-adrenoceptor (β₂-AR) or β₂-actin complementary deoxyribonucleic acid (cDNA) probes. The probes for adrenoceptors were as follows: β₁-AR, Pst I fragment (1.3 kb) of hamster cDNA; β₂-AR, Sma I - Hind III fragment (1.3 kb) of hamster cDNA. The membrane was washed, and autoradiographed at -70°C for 2–4 days.

(45) and confirmed that both β₁- and β₂-adrenoceptor genes were expressed in rat alveolar type II cells. Four micrograms of poly (A) ribonucleic acid (RNA) from primary cultured alveolar type II cells were electrophoresed on formaldehyde-agarose gel, transferred to a nylon membrane. The blot was hybridized with [13P]dCTP-labelled β₁- (β₁-AR), β₂-adrenoceptor (β₂-AR) or β₂-actin complementary deoxyribonucleic acid (cDNA) probes. The probes for adrenoceptors were as follows: β₁-AR, Pst I fragment (1.3 kb) of hamster cDNA; β₂-AR, Sma I - Hind III fragment (1.3 kb) of hamster cDNA. The membrane was washed, and autoradiographed at -70°C for 2–4 days.

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Mucociliary transport in inflammation

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To simulate inflammatory states, we have used pigeons and quails for the evaluation of drugs on mucociliary transport. A major reason for the use of birds is based on histological findings and biochemical study of bronchoalveolar lavages. As shown in figure 8, the histological features are similar to airway inflammatory states, because there are many proliferated goblet cells and submucosal glands in tracheal epithelium. Furthermore, in bronchoalveolar lavages of birds, an extremely high content of fucose, a typical sugar in mucins, was found (table 5). So far, we have examined the effects of many mucoactive drugs on mucociliary clearance in birds [16, 19, 42, 51–58]. For example, we found that inhalation of bromhexine, classified as a mucolytic agent, increased mucociliary transport in quails (fig. 9). Recently, we investigated the effect of leukotriene D4 (LTD4) on mucociliary transport in quails [58]. As shown in figure 10, a topical application of LTD4 (0.2–2 ng) to tracheal mucosa resulted in a dose-dependent increase in mucociliary transport 5 or 10 min after the application. Forty minutes after the application of 2 ng of LTD4, the mucociliary transport was decreased to about 84% of that in

Fig. 6. – a) Phosphatidylcholine secretion from rat alveolar type II cells in response to the number of activated eosinophils by opsonized zymosan (100 mg·mL−1). Data are the mean±SEM of five experiments. b) Time course of phosphatidylcholine secretion stimulated by activated eosinophils (10⁵ cells·mL−1) by opsonized zymosan (100 mg·mL−1) in alveolar type II cells. [3H]phosphatidylcholine secretion is expressed as an amount of [3H]phosphatidylcholine in the medium as the percentage of that in cells plus medium at the end of incubation period. Data are the mean±SEM of four experiments. (Reproduced with permission from [48]).

Fig. 7. – Effects of several inhibitors on phosphatidylcholine secretion induced by activated eosinophils. Inhibitors of eosinophil products were added 10 min before eosinophils (10⁵ cells·mL−1) and opsonized zymosan (100 mg·mL−1) were applied and the incubation was continued for 90 min. As inhibitors of eosinophil products, superoxide dismutase (SOD; 2 units·mL−1), catalase (1000 units·mL−1), TCV309 (1 mM, PAF receptor antagonist) and ONO1078 (1 mM, leukotriene D4 (LTD4) receptor antagonist) were used. Relative secretion activity was expressed as a percentage of the values for activated eosinophils without inhibitors. Each value represents the mean±SEM of five experiments. **: p<0.01 vs no inhibitor. (Reproduced with permission from [48]).

Fig. 8. – Haematoxilin eosin section of tracheal tissues in: a) quail; and b) pigeon. Internal scale bar = 50 µm.

Table 5. – Biochemical components of bronchoalveolar lavages of quail, pigeon and rat

<table>
<thead>
<tr>
<th>Components</th>
<th>Quail</th>
<th>Pigeon</th>
<th>Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein mg·mL⁻¹</td>
<td>25.4±3.8</td>
<td>1.4±0.2</td>
<td>26.5±1.6</td>
</tr>
<tr>
<td>Fucose mg·mL⁻¹</td>
<td>182.7±21.6</td>
<td>7.0±0.4</td>
<td>155.9±37.9</td>
</tr>
<tr>
<td>NANA mg·mL⁻¹</td>
<td>30.9±4.1</td>
<td>30.9±5.1</td>
<td>46.2±8.6</td>
</tr>
<tr>
<td>PC mg·mL⁻¹</td>
<td>25.4±3.8</td>
<td>1.4±0.2</td>
<td>26.5±1.6</td>
</tr>
<tr>
<td>DSPC mg·mL⁻¹</td>
<td>30.9±4.1</td>
<td>30.9±5.1</td>
<td>46.2±8.6</td>
</tr>
</tbody>
</table>

NANA: N-acetyleneuraminic acid; PC: phosphatidylcholine; DSPC: disaturated phosphatidylcholine.
CURRENT STATUS OF MUCUS DRUG DEVELOPMENT

Inhibitory effect of ONO-1078, a leukotriene D<sub>4</sub> (LTD<sub>4</sub>) receptor antagonist, on LTD<sub>4</sub>-induced changes in mucociliary transport (MCT). Values are expressed as a percentage of pre-application values. Each value represents the mean±SEM of six animals. ●: control; ■: 0.2% bromhexine. **: p<0.01, statistically significant difference from the control value (Dunnett’s t-test).

The transient increase and the subsequent decrease were blocked by ONO-1078 (0.03–3 mg·kg<sup>-1</sup>, i.m.), a specific LTD<sub>4</sub> receptor antagonist. These results suggested that LTD<sub>4</sub> had a biphasic effect on tracheal mucociliary transport through leukotriene receptors. Moreover, we found that topical application of histamine (1 pmol) to tracheal mucosa markedly decreased mucociliary transport 5 or 10 min after the application. The inhibitory effect was prevented by the anti-allergic drug ketotifen (fig. 11).

In preliminary experiments, we found that sphingomyelin-rich lipid fractions from sputa suppressed mucociliary transport in the trachea [59]. Although the mechanism of action remains unclear, the finding suggests that we have to consider composition of phospholipids in sputa as inhibitory factors of mucociliary clearance.

Current mucoactive drugs

We have evaluated several mucoactive drugs over two decades. Of these, we introduce here the following drugs with anti-inflammatory properties: sodium aceneuramate; glucocorticoids; traditional Chinese medicines; and new cysteine derivatives.

Sodium aceneuramate (N-acetylenuraminic acid (NANA) sodium salt)

Although the participation of sialic acids, mainly NANA, in many biological and pathological processes has been well documented [60], there are only a few studies concerning the significance of the sialic acid in the airway [61]. Using a selected ion monitoring technique, we found that the sputa of bronchitic rabbits contained much higher levels of both free and bound NANA than the airway secretions of normal rabbits [62, 63]. In addition, we found that NANA concentrations in the sputa of patients with chronic bronchitis (free: 15–204 mg·mL<sup>-1</sup>; bound: 276–1298 mg·mL<sup>-1</sup>) were also apparently higher than those in the bronchoalveolar lavages of healthy subjects. Furthermore, we have reported that an inhalation of NANA repaired inflammation in the airway (fig. 12), and caused bronchitic rabbits to produce sputa with a low viscosity, similar to normal airway secretions [20, 63]. In addition, we found that NANA concentrations in the sputa of patients with chronic bronchitis (free: 15–204 mg·mL<sup>-1</sup>; bound: 276–1298 mg·mL<sup>-1</sup>) were also apparently higher than those in the bronchoalveolar lavages of healthy subjects. Furthermore, we have reported that an inhalation of NANA repaired inflammation in the airway (fig. 12), and caused bronchitic rabbits to produce sputa with a low viscosity, similar to normal airway secretions [20, 63]. Furthermore, NANA, but not lactose, protected the mucociliary transport impaired by cigarette smoke in a dose-dependent manner (fig. 13) [54]. The results suggest that NANA may participate in the defense mechanism in the airway against irritant gases. In addition, we studied the in vivo anti-allergic effect of NANA in guinea-pigs passively sensitized with anti-ovalbumin rabbit serum [64]. NANA inhibited bronchial...
anaphylaxis and the release of histamine into bronchoalveolar lavages (fig. 14). NANA dose-dependently attenuated heterologous passive cutaneous anaphylaxis and haemorrhaging in the passive Arthus reaction (fig. 15). Interestingly, NANA did not inhibit the release of

Fig. 12. – Haematoxylin eosin section of tracheal tissues of bronchitic rabbits repeatedly administered: a) N-acetylneuraminic acid (NANA); or b) saline for 3 weeks. Internal scale bar = 50 µm. (Reproduced with permission from [20]).

Fig. 13. – Effects of N-acetylneuraminic acid (NANA) and lactose (50 mg·mL⁻¹) on mucociliary transport (MCT) impaired by cigarette smoke exposure. The time course of percentage changes in the MCT rate after cigarette smoke exposure, which was obtained from comparison with the mean value of the MCT rate during 20 min before the treatment, is shown. Vertical bars indicate standard errors. The statistical analysis between NANA (■)- and distilled water (●)- or lactose (▲)-treated groups was performed using the unpaired t-test. **, ***: p<0.01, p<0.001). (Reproduced with permission from [54]).

Fig. 14. – Effect of N-acetylneuraminic acid (NANA) on bronchial anaphylaxis provoked by inhalation of ovalbumin: a) 1 mg·mL⁻¹ for 10 min; b) 5 mg·mL⁻¹ for 3 min; c) 10 mg·mL⁻¹ for 1.5 min and treatment with three antagonists, in passively sensitized guinea-pigs. The three antagonists were: 2.5 mg·kg⁻¹ pyrilamine; 1 mg·kg⁻¹ propranolol; and 1 mg·kg⁻¹ atropine, i.v. NANA (20 mg·kg⁻¹) or saline (1 mL·kg⁻¹) was administered, i.v., 30 min before antigen exposure. The time of onset of dyspnoea and mortality were determined with the tidal volume measured by body plethysmography. If a guinea-pig did not die within the observation period (10 or 3 min without treatment or 21.5 min with treatment), the onset time was taken as the time of termination of the observation. In the guinea-pigs treated with three antagonists (c) death occurred in 100% of controls and 14.3% of NANA treated animals. *: p<0.05, statistically significant difference from the control values (Student’s t-test). Vertical bars represent the mean±SEM. (Reproduced with permission from [64]).

Fig. 15. – Effect of N-acetylneuraminic (NANA) on the passive Arthus reaction. NANA or saline was administered i.v., 30 min before the antigenic challenge. The doses of the antigen were 0.025 (▲) and 0.05 mg·site⁻¹ (■). Data are presented as mean±SEM for six experiments. *: p<0.05, statistically significant difference from the control values (Dunnett’s t-test). Reproduced with permission from [64].
Glucocorticoids

Glucocorticoids are first-choice drugs in the management of the inflammatory process seen in asthma. Although current pharmacological approaches to airway mucus production are limited, glucocorticoids seem to be the most effective among a few useful drugs. However, there are a few studies documenting the benefits of glucocorticoid therapy on the mucociliary clearance and the excessive production of airway mucus. Systemic administration of glucocorticoids ameliorated bronchial obstruction and facilitated expectoration in patients with asthma and chronic bronchitis, although they did not alter sputum viscosity [67]. Direct exposure of the bronchial mucosa to prednisolone resulted in mild cilioexcitation [68], whereas the topical application of beclomethasone had no effect on mucociliary transport in conscious sheep [67]. Thus, the contribution of glucocorticoids to mucociliary transport remains unclear. We previously examined the effect of corticosterone on tracheal mucociliary transport in pigeons (fig. 16) [56]. Corticosterone (5.0 mg·kg⁻¹) significantly increased the mucociliary transport rate. Metyrapone, an adrenal 11-b steroid hydroxylase inhibitor, significantly increased the mucociliary transport rate. Metyrapone and corticosterone, 1 mg·kg⁻¹. The value is presented as mean±SEM from six experiments. *: p<0.05 versus control group. (Reproduced with permission from [56]).

chronic bronchitis [87–90], and because MUC-5 was cloned from the human airway [83]. In Northern blot analysis, dexamethasone (10⁻⁶–10⁻⁷ M) attenuated steady-state messenger ribonucleic acid (mRNA) levels of MUC-2 and MUC-5 mucin genes (data not shown). Thus, we concluded that dexamethasone suppressed the basal production of HMWG and decreased steady-state mRNA levels of mucin genes in airway mucus-producing cancer cells.

Traditional Chinese medicines

There is an increasing usage of traditional Chinese herbal medicines in clinics and hospitals, because they tend to have moderate side-effects and sometimes produce remarkable efficacy. In order to renormalize overall defects in airway disorders, Chinese medicines may be adequate drugs, because the medicines are composed of various herbs with weak, but ubiquitous pharmacological activities.

Qing-Fei-Tang. This preparation of 16 herbs was described in "Wang Bin Hui Chun", the medical literature published in 1587 in China. Qing-Fei-Tang has been clinically applied in the treatment of chronic obstructive pulmonary diseases with severe cough and sputum. Qing-Fei-Tang was also effective for the treatment of the bronchitis with an asthmatic attack-like symptom [91]. In this patient, the abnormally elevated chemiluminescence of oxygen radicals in leucocytes was normalized as symptoms improved during administration of Qing-Fei-Tang for 5 weeks. Our previous study showed that Qing-Fei-Tang inhibited the release of slow-reacting substance of anaphylaxis from passively sensitized guinea-pig lung after antigen challenge [59]. Qing-Fei-Tang also suppressed the chemiluminescence of oxygen radicals, when healthy human leucocytes were stimulated...
by opsonized zymosan. In normal rabbits, *Qing-Fei-Tang* increased the output volume and fatty acid content in airway secretions. In the bronchitic rabbits, administration of *Qing-Fei-Tang* for 6 weeks restored the decreased amount of saturated fatty acid in the sputa, and histological examinations revealed an amelioration of the inflammation of lung tissues. In pigeons, *Qing-Fei-Tang* facilitated tracheal mucociliary transport. Accordingly, *Qing-Fei-Tang* seems to exert effectiveness via its multiple mechanisms.

**Mai-men-Dong-Tang.** As another Chinese traditional medicine, we have investigated *Mai-men-Dong-Tang,* consisting of 6 herbs, *Ophiopogonis tuber,* *Pinelliae tuber,* *Zizyphi fructus,* *Glycyrrhizae radix,* *Ginseng radix* and *Oryzae fructus.* *Mai-men-Dong-Tang* has been used for the treatment of bronchitis and pharyngitis accompanying severe dry cough. We found that unlike codeine, *Mai-men-Dong-Tang* had a notable antitussive activity against the cough associated with bronchitis and the cough increased by angiotensin-converting enzyme inhibitors [92]. Recently, we found that, in alveolar type II cells, *Mai-men-Dong-Tang* attenuated phosphatidylcholine secretion increased by oxygen radicals from activated PMNLs. In addition, we found that *Mai-men-Dong-Tang* by itself, slightly stimulated phosphatidylcholine secretion (fig. 17) and increased $\beta_1$-adrenoceptor gene expression in rat alveolar type II cells. The mechanism of action remains unclear, but the effect may contribute to its effectiveness in the treatment of airway diseases.

**New cysteine derivatives**

We are developing a new cysteine derivative, S-(3-hydroxypropyl)-L-cysteine (SS320A), as a new mucoactive drug [93]. In rabbits, SS320A significantly increased pulmonary secretion of the marker dye, indicating bronchoregulatogagogue activity. In addition, SS320A increased the volume of airway secretions in normal rabbits collected by the Perry and Boyd method. SS320A (10^{-2} M) did not influence the rheological properties of the pig gastric mucin *in vitro.* SS320A (500 mg·kg·day, p.o., 2 weeks) restored the decreased content of free sialic acid in bronchoalveolar lavages in animals with bronchitis induced by long term SO$_2$ exposure. SS320A inhibited the hyperplasia of goblet cells in airway epithelium caused by isoproterenol (0.05 mg·kg, i.p.). SS320A (500 mg·kg$^{-1}$, p.o.) did not affect the normal tracheal mucociliary transport in quails, while inhalation of SS320A dose-dependently restored the mucociliary transport impaired by cigarette smoke exposure. The results suggest that SS320A possesses mucoactive and mucoregulatory activity.

Erdosteine, (di-S-(2-(N-3-(2-oxotetrahydrothienyl)acetamido)-thioglycolic acid; under development in Japan as KW-9144) is a novel thiol derivative with mucoalytic, mucomodulatory and free radical scavenging properties, and without gastric adverse effects, unlike other cysteine derivatives [94]. We found that erdosteine (600 mg·kg$^{-1}$, p.o.) significantly promoted mucociliary transport in quails and suppressed capsaicin-induced cough reflex [95].

Several studies reported two new cysteine derivatives, S-carboxymethylcysteine-lysine salt [96, 97], N-acetyl-cysteine i-lysacine (Nacystelyn) [98], for water-soluble pharmaceutical forms. Because of the lysine, the compounds are better tolerated by the gastroenteric tract than other mucoactive drugs. This allows the administration of the drug at higher doses, resulting in more effectiveness of the drugs in clinical use. We are also investigating the cysteine derivatives in our systems.

**Future directions**

Various parameters (chemical properties, physical properties, mucous production, surfactant phospholipid production, and mucociliary clearance) are considered to be important for the dynamics and the mobilization of airway secretions. Pharmacological investigation, with appropriate techniques, of the ability of an agent to modify these parameters can provide useful information about its mechanism of action. However, since these parameters are strictly interconnected, it is very complicated to elucidate the precise mechanism of action of mucoactive drugs. This means that the goal of the treatment cannot always be achieved by the modification of a single parameter, but should, more realistically, be aimed at a renormalization of several parameters. On the basis of this idea, it will be taken for granted that glucocorticoids are ideal mucoactive drugs, because they possess various pharmacological effects in the lung. From pharmacological points of view, a traditional Chinese medicine can be classified as a glucocorticoid-like drug because Chinese medicines consist of many kinds of active components that have various pharmacological effects.

As one future course of research, we believe that the efforts to seek compatible actions between glucocorticoids and oriental medicines may lead us to a new opportunity for development of ideal mucoactive drugs with specified actions, *i.e.* suppression of gene expression.

**References**

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