

Reduced Airway Inflammation and Remodeling in Parallel with Mucin 5AC Protein Expression Decreased by S-Carboxymethylcysteine, a Mucoregulant, in the Airways of Rats Exposed to Sulfur Dioxide

Sumihisa Sueyoshi Yuki Miyata Yoshihiro Masumoto Yuji Ishibashi
Shigeki Matsuzawa Naoki Harano Kiyoyuki Tsuru Shigeru Imai

Research Center, Kyorin Pharmaceutical Co., Ltd., Nogi-machi, Tochigi, Japan

Key Words

Airway inflammation · Goblet cell hyperplasia · S-carboxymethylcysteine · Alcian blue (pH 2.5)/periodic acid Schiff · Mucin 5AC immunohistochemistry

Abstract

Background: Human obstructive airway diseases are histopathologically characterized by inflammatory cell infiltration, goblet cell hyperplasia, and mucus hypersecretion in airways. We prepared a rat model of airway injury by exposure of sulfur dioxide (SO₂) and then evaluated the effects of S-carboxymethylcysteine (S-CMC), a mucoregulant. **Methods:** Rats were exposed to SO₂ gas for 44 days and orally given S-CMC at 250 mg/kg, twice daily, from 21 to 44 days of exposure for histopathological and immunohistochemical evaluation. **Results:** SO₂ exposure induced inflammatory cell infiltration and mucus cell increase in rat airways. S-CMC treatment significantly decreased this inflammatory cell infiltration in proximal and peripheral airways. Morphometrically, SO₂ exposure significantly increased the number of Alcian blue (pH 2.5)- and periodic acid Schiff (AB/PAS)-positive cells in rat airways (11.8×10^{-2} cell/nuclear profiles per micrometer basement membrane) compared to normal rat airways (1.6×10^{-2} cell/nuclear profiles per micrometer basement membrane). S-CMC treatment signifi-

cantly decreased the number of AB/PAS-positive cells (4.4×10^{-2} cell/nuclear profiles per micrometer basement membrane, $p < 0.01$ vs. SO₂-exposed rats). Immunohistochemically, SO₂ exposure increased the expression of mucin 5AC (MUC5AC) protein in the airway epithelium of rats, but S-CMC treatment inhibited the increase. **Conclusions:** The increased mucus cells and MUC5AC protein expression seem associated with SO₂-induced airway inflammation in rats. The fact that S-CMC suppresses airway inflammation and the increase in mucus cells and MUC5AC protein expression suggests that this mucoregulant may be advantageous in the treatment of inflammatory airway diseases with goblet cell hyperplasia.

Copyright © 2004 S. Karger AG, Basel

Introduction

Airway hypersecretion and inflammation are common in human obstructive airway diseases such as chronic bronchitis, asthma, and cystic fibrosis. Overproduction of mucus in the airways adversely affects mucociliary clearance, causes airway obstruction, and contributes to morbidity and mortality. Airway mucus is produced both by mucus-secreting (goblet) cells in the surface epithelium and the mucus cells in the submucosal glands. Histologi-

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2004 S. Karger AG, Basel
1018–2438/04/1344–0273\$21.00/0

Accessible online at:
www.karger.com/iaa

Correspondence to: Sumihisa Sueyoshi
Research Center, Kyorin Pharmaceutical Co., Ltd., 1848, Nogi
Nogi-machi, Shimotsuga-gun, Tochigi 329-0114 (Japan)
Tel. +81 280 57 1551, Fax +81 280 57 2336
E-Mail sumihisa.sueyoshi@mb.kyorin-pharm.co.jp

cally, human obstructive airway diseases are characterized by inflammatory cell infiltration, goblet cell hyperplasia, and submucosal gland enlargement in the airways [1–3].

Sulfur dioxide (SO₂), a well-known air pollutant, is closely associated with the development or deterioration of human obstructive airway diseases [4]. In fact, many researchers have shown inflammatory cell infiltration, mucus cell increase, and mucus hypersecretion in the airways of SO₂-exposed rats, all of which are similar to those in human obstructive airway diseases [5–8].

Recently, Rose and Gendler [9] and other teams [10, 11] demonstrated that the overexpression of mucin (*MUC*) genes correlates with goblet cell hyperplasia/metaplasia and suggested that mucin 5AC (*MUC5AC*) mRNA and protein expression in airway epithelium could be a marker of goblet cell hyperplasia. In addition, upregulation of mucin synthesis has been observed in rats when their airway epithelium is exposed to ozone [12], endotoxin [13, 14], and SO₂ or viral infection [15, 16]. Thus, there are many reports on mucin synthesis or goblet cell hyperplasia/metaplasia associated with obstructive airway diseases or animal models.

S-carboxymethylcysteine (S-CMC) has long been used for the treatment of many chronic airway diseases to reduce sputum viscosity and facilitate expectoration of secretions accumulated in the respiratory tract [17–19]. S-CMC has no direct mucolytic activity, but possesses unique ‘mucoregulating’ properties [20], whose mechanism(s) remain to be clarified, however.

Thus, the purpose of this study was to investigate a possible relationship among airway inflammation, mucus cell increase and *MUC5AC* protein expression associated with goblet cell hyperplasia in an SO₂-induced rat model of bronchitis, and then to evaluate the effects of S-CMC on these changes in this rat model.

Materials and Methods

Animals

Thirty male Wistar rats aged 6 weeks and weighing 150–160 g were purchased from Japan Laboratory Animals, Inc. (Tokyo, Japan). The animals were acclimatized for 7 days prior to the experiment. They were housed in cages (3 or 4 animals/cage) in an animal room maintained at a temperature of 23.2 ± 0.2 °C, a humidity of 48.1 ± 0.5%, and a 12-hour light-dark cycle (lighting: 7 a.m. to 7 p.m.) and fed pelleted chow (F-2; Funabashi Farm, Funabashi, Chiba, Japan) and provided with tap water ad libitum except during daily SO₂ exposure. All the animals were cared throughout the study in accordance with the institutional protocol and standard operating procedures approved by the institutional animal care/use committee.

Protocol for SO₂ Exposure

The rats were divided into two groups on the basis of their body weight. The control group consisted of 10 animals (normal, nonexposed rats). The remaining 20 rats were exposed to SO₂.

Exposures were performed in a whole body inhalation type 950-liter cubic exposure chamber system (CIS-1000G; Japan Clea, Tokyo, Japan). Anhydrous grade SO₂ (concentration 99.9%; Showa Denko K.K., Tokyo, Japan) was mixed with a clean air stream (scrubbed through high-efficiency particulate air filter) and introduced into the exposure chamber. Both the airflow (250 liters/min, 16 air changes/h) and negative pressure of 5–10 mm H₂O in the chamber were monitored continuously and, when required, controlled by an SO₂ gas generator equipped with the exposure system. The temperature and relative humidity of the room in which the chamber was installed were maintained at 17–23 °C and 20–41%, respectively, throughout daily exposures. Rats in stainless steel-wire mesh cages were placed into the chamber and exposed to 300 ppm SO₂ gas, 4 h a day, 6 days per week for 20 days. The SO₂ concentrations were measured at regular intervals, using a detection tube (Gastec Co., Ayase, Kanagawa, Japan) connected to an air supply tube placed in the breathing zone of the rats.

The 20 exposed rats were divided into two subgroups on day 21: one subgroup consisting of 10 animals which remained exposed to SO₂ and was dosed orally with the vehicle (0.3% carboxymethylcellulose sodium; Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan) for 24 days (SO₂-exposed rats); the other group consisted of 10 rats which remained exposed to SO₂ and were dosed orally with S-CMC (Nippon Rika Co., Ltd., Tokyo, Japan) at 250 mg/kg, twice a day, 6 days per week, from days 21 to 44 of SO₂ gas exposure. Single doses of S-CMC or vehicle were administered to these two subgroups on the last day (day 45 without gas exposure), i.e., 1 h before necropsy (fig. 1).

Necropsy and Tissue Preparation for Histopathological Analysis

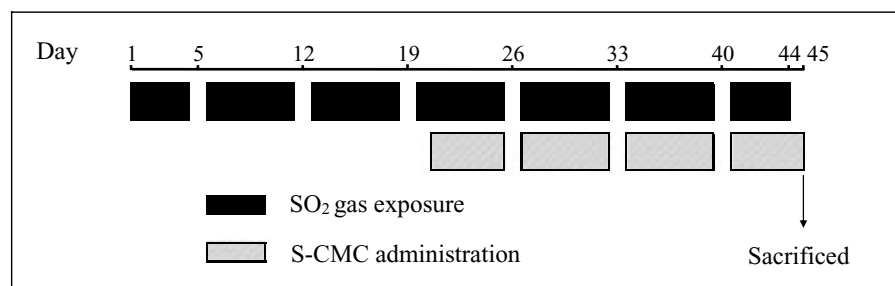
All SO₂-exposed rats were euthanized under intraperitoneal anesthesia with pentobarbital sodium (50 mg/kg; Abbott Laboratories Ltd., Chicago, Ill., USA) on day 45, or 24 h after the end of SO₂ gas exposure and the lungs removed. The left and right lungs were separated. The left lungs were fixed by immersion in 10% buffered formalin, and the right lungs were fixed by immersion in methacarn solution (methanol:chloroform:acetic acid = 6:3:1). Complete vertical serial sections, approximately 3 mm thick from the hilum, were cut from each lobe. All lung sections, oriented to include both the upper and lower lobes, were processed in the usual manner and embedded in paraffin. Tissue sections 5–6 µm in thickness were prepared and mounted on aminopropyltriethoxysilane-coated glass slides.

Paraffin sections from the formalin-fixed left lung tissues were stained with hematoxylin and eosin (HE) to evaluate the degree of inflammatory cell infiltration from proximal to peripheral airways. Separately, some formalin-fixed left lungs were stained with Alcian blue (pH 2.5)/periodic acid-Schiff (AB/PAS) to evaluate the degree of mucus cell increase. Paraffin sections from the methacarn-fixed right lung tissues were used for immunohistochemistry of *MUC5AC*.

Evaluation of Inflammatory Cell Infiltration in the Airways

Histopathological analysis of inflammatory cell infiltration in the airways was evaluated according to a modification of the method of Sadakane et al. [21] using an Olympus BH-2 light microscope (Olympus, Tokyo, Japan). The magnitude of inflammatory cell infiltration into the submucosal layer of bronchi and bronchioles was scored on a

Fig. 1. Experimental design of this study. The whole bodies of rats (except normal rats) were exposed to an atmosphere containing 300 ppm SO₂ for 4 h a day, 6 days per week for 44 days (see Materials and Methods).



semiquantitative scale: 0 = not present, 1 = minimal, 2 = mild, 3 = moderate, 4 = moderate to severe, and 5 = severe. The specimens were evaluated independently by two experienced investigators and an average score was adopted.

Morphometric Analysis of AB/PAS-Positive Cells

AB/PAS-positive cells on the luminal epithelium covering the proximal airways were analyzed morphometrically on each AB/PAS reaction slide.

Morphometric analysis was performed with a semiautomatic, computerized image analysis system consisting of a light microscope (Olympus BX-50; Olympus Corp., Tokyo, Japan) connected to a CCD camera (DXC-950; Sony, Inc., Tokyo, Japan), a color monitor, and a Power Macintosh 8500/120 computer (Apple Computer, Inc., Cupertino, Calif., USA) run by a public-domain National Institutes of Health Image program (version 1.59, the US National Institutes of Health).

The number of AB/PAS-positive cells (AB/PAS-positive cell number $\times 10^{-2}$ cell/nuclear profiles per micrometer basement membrane) was determined by counting the total number of AB/PAS-positive cells of nuclear profiles per total length of the basement membrane underlying the epithelium [22]. Ten microscopic fields per animal with a magnification of $\times 100$ were randomly sampled at equal distances apart on the basement membrane line. Data for each experimental group are expressed as the mean number of AB/PAS-positive cell/nuclear profiles per micrometer basement membrane $\pm$ SEM.

Immunohistochemistry for MUC5AC

Immunohistochemistry was carried out on deparaffinized tissue sections using the avidin-biotin complex (ABC) method (Vectastain® Elite ABC kit; Vector Laboratories, Burlingame, Calif., USA). Endogenous peroxidase activity was quenched by immersing the tissue sections in freshly prepared 1% hydrogen peroxide in methanol for 20 min, and the samples were then rehydrated in a descending ethanol series. After treatment with the Biotin Blocking System (Dako A/S, Glostrup, Denmark) for 10 min to block endogenous biotin and avidin binding, the sections were incubated with anti-MUC5AC mouse monoclonal antibody (clone 45M1, dilution 1:200; Neo Markers, Fremont, Calif., USA) at 4°C for 16 h. After incubation with the primary antibody, the sections were washed several times in phosphate-buffered saline (PBS) and incubated with biotinylated horse anti-mouse IgG for 30 min and then with avidin-biotin peroxidase complex for 30 min. After washing in PBS, the tissue-bound horseradish peroxidase activity was visualized by immersing the sections in diaminobenzidine tetrahydrochloride (Wako Pure Chemical Industries, Ltd., Osaka, Japan) containing hydrogen peroxide. Follow-

ing completion of the immunohistochemical staining procedures, the sections were counterstained with hematoxylin, dehydrated, and mounted for light microscopy.

The sections used as negative control were reacted with the mouse monoclonal IgG1 antibody (Dako A/S) instead of the primary antibody, and then treated in the same manner as above.

The immunohistochemical staining of all samples was performed in a single series to minimize series-to-series variations of staining intensity.

Evaluation of MUC5AC Protein Expression in Rat Airway Epithelium

The levels of immunohistochemical staining were estimated in two different anatomical compartments of the lung (i.e. proximal and peripheral airways). The relative number (%) of MUC5AC-positive cells (PCN value) was scored as a five-grade scale: no positive cells, 0; 1–10%, 1; more than 10–50%, 2; more than 50–90%, 3, and more than 90% positive cells, 4. The specimens were evaluated independently by two experienced investigators and an average score was adopted.

Statistical Analysis

Data were inputted in a computer spreadsheet program (Microsoft Excel; Microsoft, Redmond, Wash., USA) and all calculations were performed using a commercial statistical analysis package (SAS; version 6.12; SAS Institute Inc., Cary, N.C., USA) on a personal computer. A χ^2 test was used to analyze the histopathologic data on inflammatory cell infiltration and MUC5AC protein expression in the airways. The number of AB/PAS-positive cells morphometrically obtained is expressed as the mean $\pm$ SEM, and statistically analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. Differences were considered statistically significant if $p < 0.05$.

Results

Effect of S-CMC on SO₂-Induced Inflammatory Cell Infiltration in the Airways

The magnitude of inflammatory cell infiltration was evaluated as described in Materials and Methods. The results of this evaluation are shown in figure 2 for the effect of S-CMC on SO₂-induced airway inflammation. In SO₂-exposed rats, inflammatory cells infiltrated the peri-

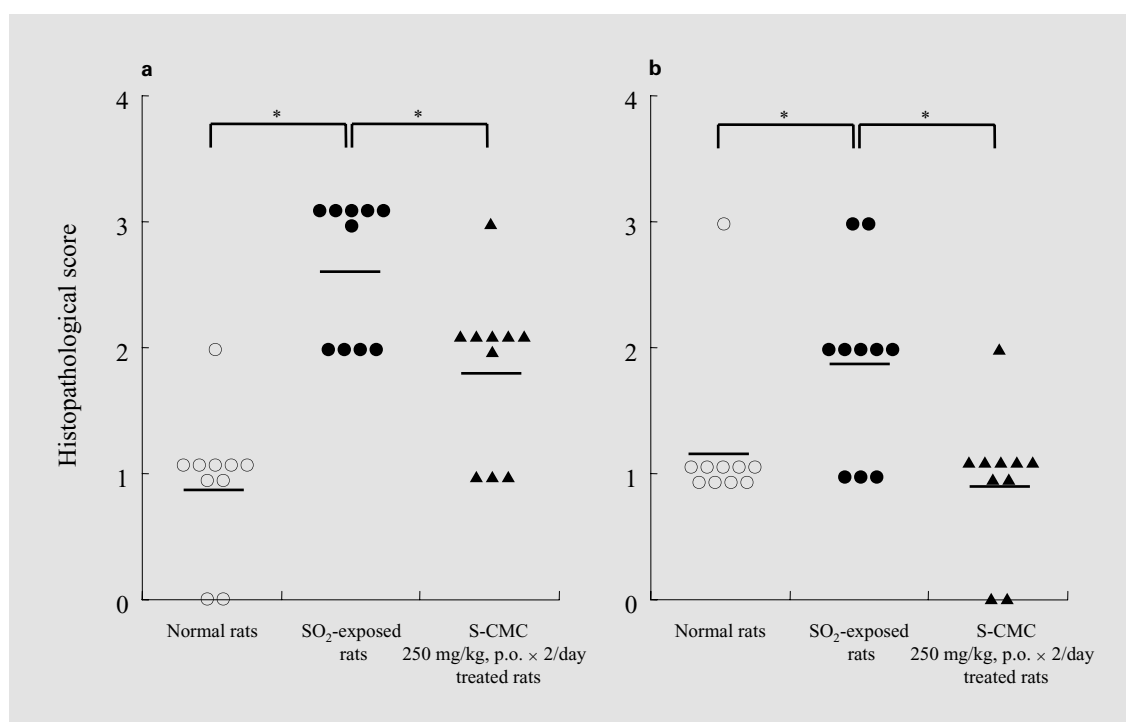


Fig. 2. Effect of S-CMC on rat airway inflammation induced by SO₂ exposure. S-CMC (250 mg/kg) was given orally, twice a day, from days 21 to 44 of SO₂ gas exposure. The magnitude of inflammatory cell infiltration in proximal (**a**) and peripheral airways (**b**) was evaluated in lung specimens stained with HE. Data are shown as individual values, and each horizontal line indicate the mean value per group. * Significantly different from the SO₂-exposed rats at $p < 0.05$ (χ^2 test).

vascular and peribronchial regions of the proximal and peripheral airways. S-CMC treatment significantly decreased the inflammatory cell infiltration in proximal and peripheral airways (fig. 2).

Effect of S-CMC on Increased Mucus Cells

The effect of S-CMC on the number of AB/PAS-positive cells in rat airways is shown in figures 3 and 4a–c.

As compared with normal rats (1.6×10^{-2} cell/nuclear profiles per micrometer basement membrane), SO₂-exposed rats showed a 7.4-fold increase in the number of AB/PAS-positive cells (11.8×10^{-2} cell/nuclear profiles per micrometer basement membrane, $p < 0.01$), which contained cytoplasm filled with AB/PAS-positive materials (fig. 4b).

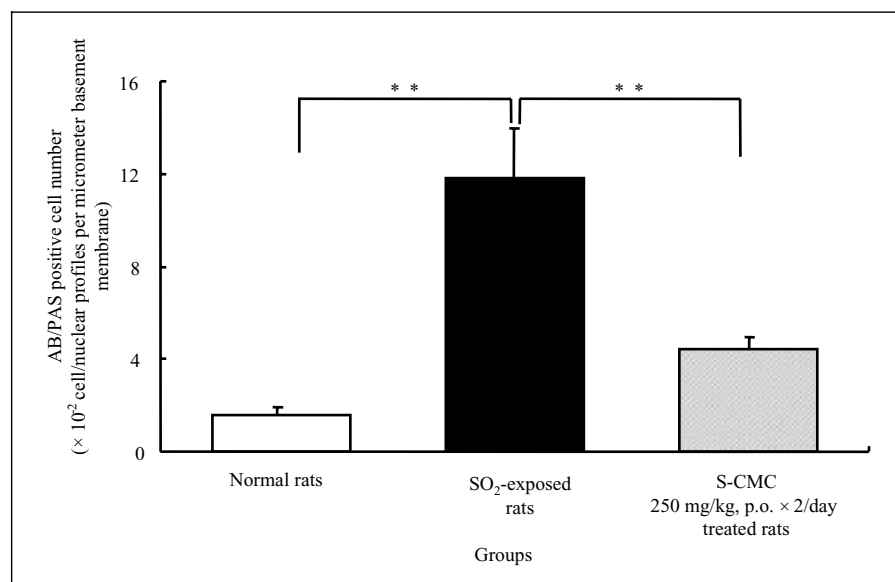
S-CMC treatment significantly decreased the number of AB/PAS-positive cells (4.4×10^{-2} cell/nuclear profiles per micrometer basement membrane, $p < 0.01$) as compared to SO₂-exposed rats. Interestingly, S-CMC treatment also reduced AB/PAS-positive material in mucus cell cytoplasm (fig. 3, 4c).

MUC5AC Localization in SO₂-Exposed Rat Airways and Inhibitory Effects of S-CMC on MUC5AC Protein Expression

We stained the specimens immunohistochemically by using monoclonal antibody to MUC5AC and compared MUC5AC protein expression in the lungs of SO₂-exposed and normal rats. The results of MUC5AC immunoreactivity and the staining scores of MUC5AC protein expression are presented in figures 4d–f and 5, respectively. The negative control stained with the mouse monoclonal IgG1 antibody gave no positive signals in airway epithelium of an adjacent section (data not shown).

MUC5AC immunoreactivity in normal rat airways was only weakly and rarely positive in the cytoplasm of epithelial cells in the proximal airways (fig. 4d). MUC5AC immunoreactivity in SO₂-exposed rat airways was strongly positive in epithelial cells in the proximal airways, though negative in alveolar epithelial cells. Epithelial staining by MUC5AC immunoreactivity was mainly localized in the intracellular organelles of the epithelial cells without staining in the nucleus (fig. 4e). Weakly posi-

Fig. 3. Effect of S-CMC on mucus cell increases in SO₂-exposed rats. S-CMC (250 mg/kg) was given orally, twice a day from days 21 to 44 of SO₂ gas exposure. AB/PAS-positive cells on the luminal epithelium covering the proximal airways were analyzed. Values are the mean $\pm$ SEM of 10 animals. ** Significantly different from the SO₂-exposed rats at $p < 0.01$ (Dunnett's test).



tive materials were also found in the surface mucus overlying the airway epithelium. In contrast, the immunoreactivity of MUC5AC was markedly decreased in S-CMC-treated rats compared with SO₂-exposed, nontreated rats (fig. 4f).

The PCN value for MUC5AC protein expression significantly increased in the proximal airways of SO₂-exposed rats as compared to those from normal rats. S-CMC treatment decreased the value for MUC5AC protein expression in the proximal airways ($p < 0.05$ vs. SO₂-exposed rats, fig. 5).

Discussion

We evaluated a possible relation among airway inflammation, mucus cell increase, and MUC5AC protein expression associated with goblet cell hyperplasia in rat model of SO₂-induced bronchitis, and then evaluated the effects of S-CMC on these changes in this rat model.

In the present study, SO₂ exposure induced mucus hypersecretion, mucus cell increase, and MUC5AC protein expression, all of which were observed in association with airway inflammation in rats. S-CMC suppressed all these changes, including SO₂-induced MUC5AC protein overexpression. All these suppressive effects may be in favor of the therapeutic properties of S-CMC as a mucoregulant without direct mucolytic activity [23] but with established anti-inflammatory properties [24, 25].

Sulfur dioxide has considerable potential for inducing histological alterations in the airways. An experimental rat model of SO₂-induced bronchitis was selected for the present study since this model is well established [5–8]. The SO₂-related airway changes described so far include increased susceptibility to cell membrane damage by many other pollutants, as well as a promoted infiltration of eosinophils into the airway epithelial layer and an increased release of proinflammatory mediators [26, 27]. These SO₂-induced alterations in rats have been suggested to histologically resemble those in human bronchitis [28]. In the present study, a 40-day exposure to 300 ppm SO₂ induced infiltration of inflammatory cells, mainly neutrophils, eosinophils, and lymphocytes/monocytes, in rat lung, and also a marked increase in mucus cells and mucus hypersecretion in large airways. All these findings are in agreement with those reported in previous studies [5, 7, 8].

Chronic airway inflammation and goblet cell hyperplasia are frequent features in chronic bronchitis, asthma, and chronic obstructive pulmonary disease. Chronic inflammation is characterized by the presence of inflammatory cells, including mast cells, neutrophils, eosinophils, macrophages, and lymphocytes, all of which release different types and quantities of cytokines and growth factors leading to aggravation of the morbidity or chronic presence of structural alterations with goblet cell hyperplasia/metaplasia. These alterations in the structure of the airway wall are defined as so-called airway remodeling [3]. Since S-CMC reduced almost all these changes in-

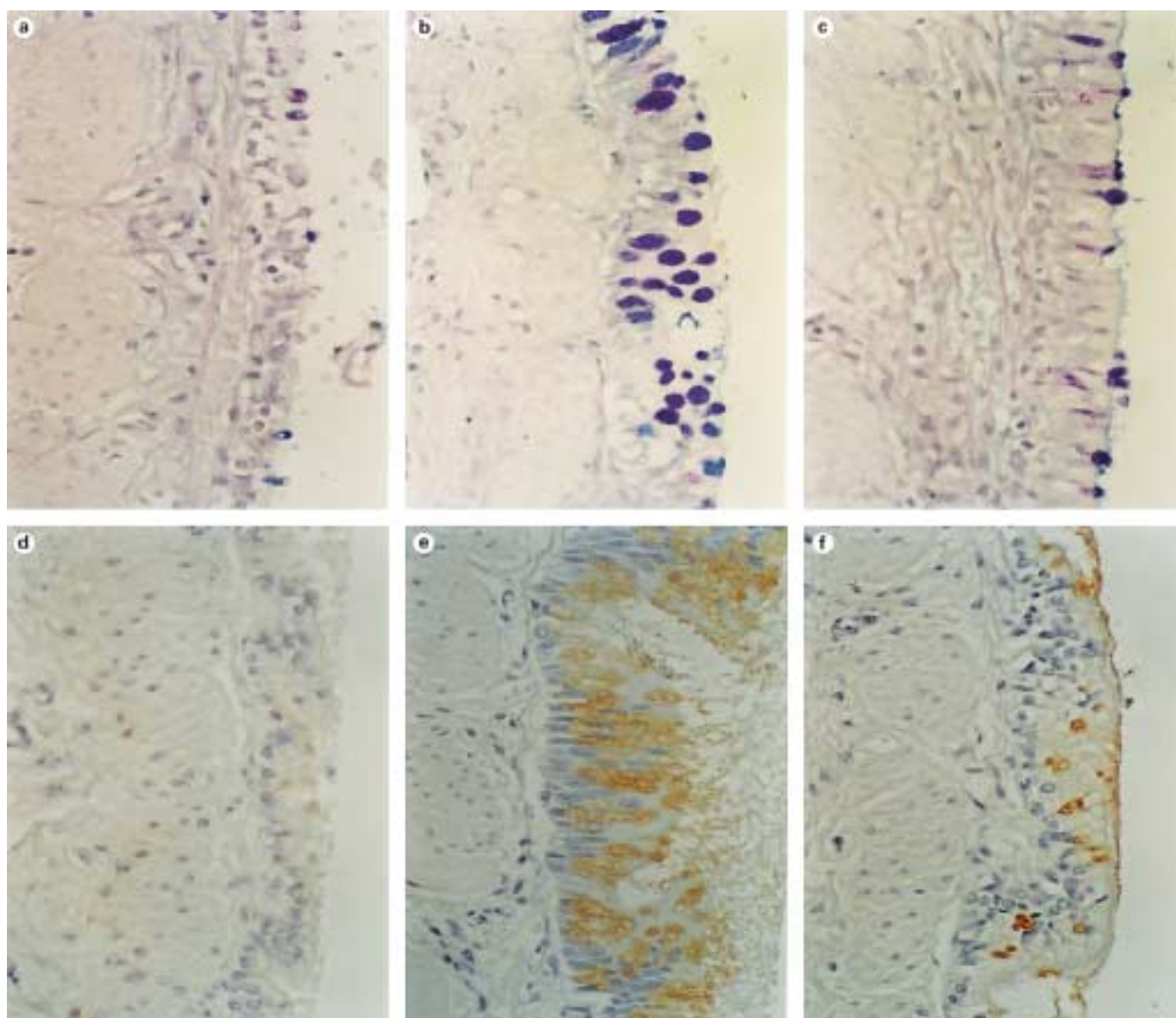


Fig. 4. Histopathology of the proximal airways from normal rats (**a**, **d**), SO₂-exposed rats (**b**, **e**) and S-CMC treated rats (**c**, **f**). Lung tissue sections were stained with AB/PAS and counterstained with hematoxylin (**a–c**). Airway epithelium from the SO₂-exposed rats (**b**) shows prominent mucus-filled goblets, which are absent in the airways of S-CMC-treated rats (**c**). Immunohistochemical staining of MUC5AC in airway epithelium is also shown for normal rats (**d**), SO₂-exposed rats (**e**) and S-CMC treated rats (**f**). MUC5AC protein

expression is rare in normal rat airways (**a**), but obviously found in SO₂-exposed rat airways (**b**). In S-CMC treated rats, the expression of MUC5AC protein is decreased (**c**). MUC5AC immunoreactivity and AB/PAS stained cells overlap each other. A combination of the AB/PAS-positive staining and MUC5AC immunoreactivity indicates the MUC5AC protein is localized to goblet cells. ABC, counterstained with hematoxylin. Original magnification $\times 100$.

duced by SO₂ in rat model of bronchitis, it may play a role in suppressing this remodeling process to some extent.

Mucin glycoproteins (mucins) play an important role in protecting the mucosa from harmful aggregation of foreign or internal particles (pathogens, irritating and other

harmful agents, leukocytes). To date, twelve different mucin genes have been identified and cloned from the respiratory tract, intestines, and reproductive organs [29]. Among them, at least eight *MUC* genes (*MUC1*, *MUC2*, *MUC4*, *MUC5AC*, *MUC5B*, *MUC7*, *MUC8* and

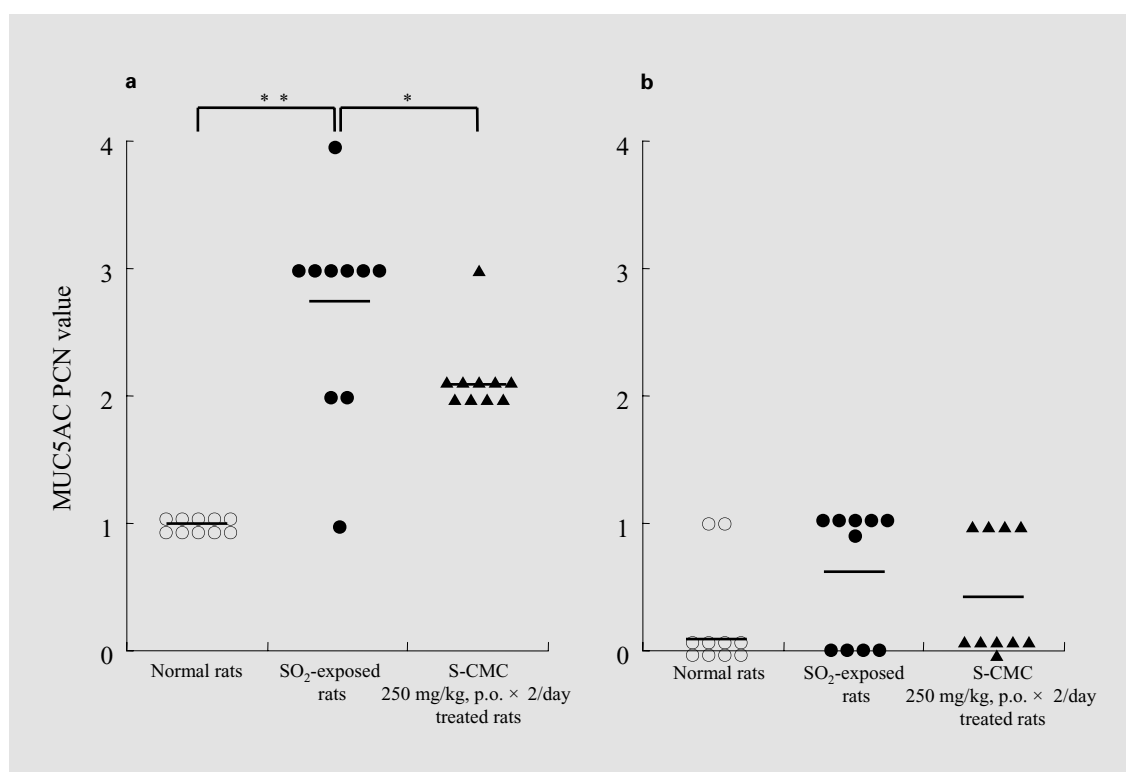


Fig. 5. Effect of S-CMC on MUC5AC protein expression induced by SO₂ exposure. S-CMC (250 mg/kg) was given orally, twice a day, from days 21 to 44 of SO₂ gas exposure. The relative number of MUC5AC-positive cells (PCN value) in proximal (**a**) and peripheral airways (**b**) was evaluated in lung specimens stained with anti-MUC5AC antibody. Data are shown as individual values, and each horizontal line indicates the mean value per group. *,** Significantly different from the SO₂-exposed rats at $p < 0.05$, $p < 0.01$, respectively (χ^2 test).

MUC13) are expressed in the airways, but vary in expression levels depending upon the types of epithelial cells within the airways [9, 30]. Recently, abnormal expression of *MUC2* and *MUC5* genes has been demonstrated in association with human respiratory diseases [10], and MUC5AC mRNA and protein expression is used as a marker of goblet cell hyperplasia/metaplasia in the human airways [11]. In the present study, S-CMC suppressed MUC5AC protein expression in parallel with reductions in mucus cell increase in a rat model of SO₂-induced bronchitis. To try and clarify SO₂-induced mucus secretion in rats, we examined whether SO₂ would induce the alterations of Clara cells involved in mucus secretion in the peripheral airway, by using immunohistochemical staining with an anti-urine protein 1 antibody. However, no increase or decrease was detected in Clara cells in the peripheral, or distal airways of SO₂-exposed rats (data not shown). Since it is generally accepted that rats exhibit few or no submucosal glands [31], a highly probable interpretation

is that mucus (goblet) cells in the surface epithelium are the major sources of luminal mucus increased by SO₂ exposure.

The cellular and molecular mechanism(s) by which S-CMC regulates MUC5AC expression is unknown. Since, however, S-CMC induced reductions in mucus cell increase, one of the inflammatory processes, reduced MUC5AC expression may be related to its anti-inflammatory properties [24]. The fact that S-CMC can alleviate inflammation-related changes in the airways of SO₂-exposed rats suggests that this drug may have the ability to favorably influence some of the airway-remodeling processes. In this connection, additional studies will be needed to investigate whether and how S-CMC would suppress remodeling (for example, hyperplasia/metaplasia) and to explain more clearly how S-CMC regulates MUC5AC expression.

In conclusion, the present findings support that the SO₂-increased mucus cells and MUC5AC protein expres-

sion are associated with airway inflammation. The results reveal that S-CMC suppresses this inflammatory cell infiltration, mucus cell increase, and mucus hypersecretion, and thus suggest that S-CMC may be advantageous for the treatment of inflammatory airway diseases with goblet cell hyperplasia.

Acknowledgments

We thank Mr. Kunio Someya for his helpful review and comments on the manuscript, and Ms. Harumi Namiki, Mr. Fumiyoshi Kobayashi, and Mr. Tamotsu Toriyabe for collecting or checking the data presented here. We are also especially grateful to the late Mr. Kenjiro Momo, former Director of our research center, for his scientific and technical advice.

References

- Aikawa T, Shimura S, Sasaki H, Ebina M, Takishima T: Marked goblet cell hyperplasia with mucus accumulation in the airways of patients who died of severe acute asthma attack. *Chest* 1992;101:916–921.
- Jeffery PK, Li D: Airway mucosa: Secretory cells, mucus and mucin genes. *Eur Respir J* 1997;10:1655–1662.
- Jeffery PK: Remodeling in asthma and chronic obstructive lung disease. *Am J Respir Crit Care Med* 2001;164:S28–S38.
- Behrendt H, Becker WM, Fritzsche C, Sliwa-Tomczok W, Tomczok J, Friedrichs KH, Ring J: Air pollution and allergy: Experimental studies on modulation of allergen release from pollen by air pollutants. *Int Arch Allergy Immunol* 1997;113:69–74.
- Lamb D, Reid L: Mitotic rates, goblet cell increase and histochemical changes in mucus in rat bronchial epithelium during exposure to sulphur dioxide. *J Pathol Bacteriol* 1968;96:97–111.
- Asmundsson T, Kilburn KH, McKenzie WN: Injury and metaplasia of airway cells due to SO₂. *Lab Invest* 1973;29:41–53.
- White R, Zoppi AL, Haroz RK, Broillet A: Sulfur dioxide induced bronchitis in rats. *Arch Toxicol* 1986;9:431–435.
- Kodavanti UP, Mebane R, Ledbetter A, Krantz T, McGee J, Jackson MC, Walsh L, Hilliard H, Chen BY, Richards J, Costa DL: Variable pulmonary responses from exposure to concentrated ambient air particles in a rat model of bronchitis. *Toxicol Sci* 2000;54:441–451.
- Rose MC, Gendler SJ: Airway mucin genes and gene products; in Rogers DF, Lethem MI (eds): *Airway Mucus: Basic Mechanisms and Clinical Perspectives*. Basel, Birkhäuser, 1997, pp 41–66.
- Alimam MZ, Piazza FM, Selby DM, Letwin N, Huang L, Rose MC: Muc-5/5ac mucin messenger RNA and protein expression is a marker of goblet cell metaplasia in murine airways. *Am J Respir Cell Mol Biol* 2000;22:253–260.
- Ordóñez CL, Khashayar R, Wong HH, Ferrando R, Wu R, Hyde DM, Hotchkiss JA, Zhang Y, Novikov A, Dolganov G, Fahy JV: Mild and moderate asthma is associated with airway goblet cell hyperplasia and abnormalities in mucin gene expression. *Am J Respir Crit Care Med* 2001;163:517–523.
- Harkema JR, Hotchkiss JA, Henderson RF: Effects of 0.12 and 0.80 ppm ozone on rat nasal and nasopharyngeal epithelial mucosubstances: Quantitative histochemistry. *Toxicol Pathol* 1989;17:525–535.
- Harkema JR, Hotchkiss JA: In vivo effects of endotoxin on nasal epithelial mucosubstances: Quantitative histochemistry. *Exp Lung Res* 1991;17:743–761.
- Steiger D, Hotchkiss J, Bajaj L, Harkema J, Basbaum C: Concurrent increases in the storage and release of mucin-like molecules by rat airway epithelial cells in response to bacterial endotoxin. *Am J Respir Cell Mol Biol* 1995;12:307–314.
- Basbaum C, Gallup M, Gum J, Kim Y, Jany B: Modification of mucin gene expression in the airways of rats exposed to sulfur dioxide. *Bio-rheology* 1990;27:485–489.
- Jany B, Gallup M, Tsuda T, Basbaum C: Mucin gene expression in rat airways following infection and irritation. *Biochem Biophys Res Commun* 1991;181:1–8.
- Edwards GF, Steel AE, Scott JK, Jordan JW: S-carboxymethylcysteine in the fluidification of sputum and treatment of chronic airway obstruction. *Chest* 1976;70:506–513.
- Puchelle E, Aug F, Polu JM: Effect of the mucoregulator S-carboxy-methyl-cysteine in patients with chronic bronchitis. *Eur J Clin Pharmacol* 1978;14:177–184.
- Poole PJ, Black PN: Oral mucolytic drugs for exacerbations of chronic obstructive pulmonary disease: Systematic review. *BMJ* 2001;322:1271–1274.
- Brown DT: Carbocysteine. *Drug Intell Clin Pharm* 1988;22:603–608.
- Sadakane K, Ichinose T, Takano H, Yanagisawa R, Sagai M, Yoshikawa T, Shibamoto T: Murine strain differences in airway inflammation induced by diesel exhaust particles and house dust mite allergen. *Int Arch Allergy Immunol* 2002;128:220–228.
- Hotchkiss JA, Harkema JR, Henderson RF: Effect of cumulative ozone exposure on ozone-induced nasal epithelial hyperplasia and secretory metaplasia in rats. *Exp Lung Res* 1991;15:589–600.
- Martin R, Litt M, Marriott C: The effect of mucolytic agents on the rheologic and transport properties of canine tracheal mucus. *Am Rev Respir Dis* 1980;121:495–500.
- Asti C, Melillo G, Caselli GF, Daffonchio L, Hernandez A, Clavenna G, Omini C: Effectiveness of carbocysteine lysine salt monohydrate on models of airway inflammation and hyper-responsiveness. *Pharmacol Res* 1995;31:387–392.
- Ishii Y, Kimura T, Morishima Y, Mochizuki M, Nomura A, Sakamoto T, Uchida Y, Sekizawa K: S-carboxymethylcysteine inhibits neutrophil activation mediated by N-formyl-methionyl-leucyl-phenylalanine. *Eur J Pharmacol* 2002;449:183–189.
- Knorst MM, Kienast K, Muller-Quernheim J, Ferlinz R: Effect of sulfur dioxide on cytokine production of human alveolar macrophages in vitro. *Arch Environ Health* 1996;51:150–156.
- Gong H Jr, Linn WS, Terrell SL, Anderson KR, Clark KW: Anti-inflammatory and lung function effects of montelukast in asthmatic volunteers exposed to sulfur dioxide. *Chest* 2001;119:402–408.
- Kodavanti UP, Jackson MC, Ledbetter AD, Starcher BC, Evansky PA, Harewood A, Winstett DW, Costa DL: The combination of elastase and sulfur dioxide exposure causes COPD-like lesions in the rat. *Chest* 2000;117:299S–302S.
- Fahy JV: Goblet cell and mucin gene abnormalities in asthma. *Chest* 2002;122:320S–326S.
- Voynow JA: What does mucin have to do with lung disease? *Paediatr Respir Rev* 2002;3:98–103.
- Plopper CG, Weir A, George JS, Tyler N, Mariassy A, Wilson D, Nishio S, Cranz D, Heidsiek J, Hyde D: Cell populations of the respiratory system: Interspecies diversity in composition, distribution, and morphology; in Mohr U, Dungworth D, Kimmeler G, Lewkowski J, McClellan R, Stöber W (eds): *Inhalation toxicology*. New York, Springer, 1988, pp 25–40.