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Carrageenans can regulate the pulmonary absorption of antiasthmatic drugs and their retention in the rat lung tissues without any membrane damage

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Abstract

Effects of various viscous vehicles on pulmonary absorption of antiasthmatic drugs were examined by an in situ pulmonary absorption experiment. Theophylline and fluticasone propionate were used as antiasthmatic drugs. The serum concentration–time profile of theophylline without viscous vehicles was similar to that following the intravenous injection, indicating that pulmonary absorption of theophylline was rapid and absolute. The serum concentration of theophylline was not controlled in the presence of 5% gelatin or 2% sodium alginate. However, 1% iota-carrageenan could control and regulate the serum concentration of theophylline. In the pharmacokinetic analysis, the C_{max} values of theophylline significantly decreased, and its T_{max} values increased in the presence of 1% and 2% iota-carrageenan, 1% kappa-carrageenan, and 2% sodium alginate compared with the control. The MRT and MAT values of theophylline with 1% iota-carrageenan were significantly higher than those without viscous vehicles. The local concentration of theophylline in the lung at 1 h after intratracheal administration increased five-fold with 1% iota-carrageenan compared with the control. On the other hand, the pulmonary absorption of fluticasone propionate was controlled and regulated in the presence of 0.5% kappa-carrageenan. Additionally, the pulmonary inflammation after the exposure of carrageenans administered to the lung was evaluated in rats. Iota- and kappa-carrageenans did not cause local serious

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damage and inflammation to the pulmonary tissue. Therefore, these findings indicated that the carrageenans were effective to regulate the absorption rate of antiasthmatic drugs including theophylline and fluticasone propionate.

Keywords: Pulmonary absorption; Viscosity; Carrageenan; Mucoadhesion; Controlled release; Pulmonary inflammation

1. Introduction

The large surface area of the alveolar epithelium and the short distance of the air-blood pathway are unique features of the lung that can facilitate systemic delivery alternative for delivering therapeutic proteins and peptides (O'Hagan and Illum, 1990; Agu et al., 2001). Actually, pulmonary absorption of these proteins and peptide drugs which are poorly absorbed from the gastrointestinal tract, was observed (Enna and Schanker, 1972a; Wigley et al., 1971). Moreover, the bioavailability of these drugs was significantly improved by co-administration with various adjuvants such as absorption enhancers and protease inhibitors (Okumura et al., 1992; Komada et al., 1994; Kobayashi et al., 1994; Morita et al., 1994; Yamamoto et al., 1994, 1997, 2001; Todo et al., 2001). However, rapid pulmonary absorption of these peptides, especially insulin may cause hypoglycemic events and other serious side effects. Consequently, the control of pulmonary absorption rate of drugs is very important for therapeutic effects and safety of the drugs.

On the other hand, recently, chronic obstructive pulmonary disease (COPD) is a major public health problem and it is the fourth leading cause of chronic morbidity and mortality throughout the world (WHO, 2000). Moreover, nocturnal and early morning wheezing is very common in asthmatics. The clinical importance of this symptom is underlined by the fact that the majority of deaths from asthma and many episodes of ventilatory arrest occurred in the early morning (Barnes et al., 1980; Kiyokawa et al., 1999). Therefore, the local acting drugs including antiasthmatic agents, bronchodilators and expectorants should be localized for a long period in the lung tissues. Nevertheless, few studies have been examined to control and regulate the absorption rate of such drugs after intrapulmonary administration (Morimoto et al., 2001).

In a previous study, effect of various viscous vehicles on the pulmonary absorption of water-soluble drug, 5(6)-carboxyfluorescein (CF) was examined by an in situ pulmonary absorption experiment. Consequently, viscous vehicles, especially 5% gelatin and 1% PVA, were effective to control the pulmonary absorption of CF, a water-soluble drug with low molecular weight and they might be useful to increase the local concentration of drugs in the lung (Yamamoto et al., 2004).

Carrageenan is a generic term for naturally occurring polysaccharides that fill the void spaces in the cellulose backbone structures in certain species of red seaweed. The use of carrageenan extends back to ancient times and carrageenans are widely utilized due to their excellent physical functional properties such as thickening, gelling, and stabilizing abilities in the current food industry (Ikeda, 2003). Moreover, carrageenans are used as an excipient for the pharmaceutical formulation. However, few studies have been reported the effect of carrageenans on the control and regulation of pulmonary absorption of drugs, especially antiasthmatic drugs.

In this study, theophylline and fluticasone propionate were chosen as antiasthmatic drugs, and the effects of various viscous vehicles including gelatin, iota-, kappa-carrageenan, sodium alginate on the absorption of the drugs after intrapulmonary administration were examined in rats. Additionally, the pulmonary inflammation after the exposure of carrageenans administered to the lung was evaluated in rats.

2. Materials and methods

2.1. Materials

Theophylline was purchased from Shiratori Pharmaceutical Co. (Chiba, Japan). Fluticasone propionate was purchased from Sicor S.p.A. (Perugia, Italia). Gelatin and sodium alginate were obtained from Nacalai Tesque Inc. (Kyoto, Japan) and Wako Pure Chemical Industries, Ltd. (Osaka, Japan), respectively. Iota-, lambda- and kappa-carrageenans were purchased

from Sigma-Aldrich Chemical Co. (St. Louis, MO). All other chemicals were of analytical grade.

2.2. Preparation of drug solution

Dosing solution containing theophylline or fluticasone propionate was prepared in isotonic phosphate buffer (PBS) at pH 7.4 to yield a final concentration of 1 mg/mL or 0.5 mg/mL, respectively. The dosing solutions were added with various viscous vehicles such as gelatin, iota- and kappa-carrageenan, sodium alginate at a concentration of 1–5% (w/v). The solutions for the evaluation of pulmonary inflammation containing only carrageenans were prepared in PBS at a concentration of 1% (w/v).

2.3. Absorption studies

Absorption of theophylline or fluticasone propionate from rat lung was investigated according to the methods outlined Enna and Schanker (1972b). Male Wistar rats (Japan SLC, Inc., Hamamatsu, Japan), weighing 200–250 g, were anaesthetized with an intraperitoneal injection of sodium pentobarbital (32 mg/kg). All the animals were fasted for 16 h before the experiments but they were allowed free access to water. After the animal had been secured on its back on animal board, the trachea was exposed through a longitudinal incision along the ventral aspect of the neck. The trachea was then cut transversely, halfway through, between the forth and fifth tracheal rings caudal to the thyroid cartilage. A section of polyethylene tubing (i.d., 1.5 mm, o.d., 2.5 mm) 2.5 cm in length, which served as a tracheal cannula, was inserted through the tracheal incision caudally for a distance of 0.6 cm so that 1.9 cm of the cannula protruded from the trachea. The incision in the skin was then closed with wound clips after drawing the skin up close to the sides of the cannula. Animal body temperatures were maintained at $37 \pm 1\,^{\circ}C$ with a 40 W incandescent heat lamp and the use of a reflector suspended over the animal at a distance of about 25 cm during the experiment.

One hundred microliters of drug solution at a temperature of $37\,^{\circ}C$ were injected into the lungs through the obtuse needle of a calibrated 250 μL syringe (Microliter® no. 725, Hamilton Co.). For the injection, the needle was inserted through the tracheal cannula to a depth of 2.5 cm below the tracheal incision. Then, at this distance of insertion, the tip of the syringe needle was located 1–2 mm above the bifurcation of the trachea. Then, the solution was injected over a period of 1–2 s, with the rat being maintained at an angle of 80°. Immediately thereafter, the tubing was withdrawn completely and 45 s after administration the animal was returned to an angle of 10°.

The remaining percents of theophylline in the lung tissues at 60 min after intratracheal administration were calculated by determination of theophylline concentrations in homogenates of the lung tissues. The homogenates were prepared as follows. At 60 min after intratracheal administration of drug solutions, the animals were exsanguinated, the diaphragm was punctured, and the chest was opened. The lung was washed by injecting 10 mL PBS into pulmonary artery. The lungs were dissected free and removed immediately from thorax. The isolated lungs were homogenized in total volume 10 mL PBS at $4\,^{\circ}C$ using polytron homogenizer.

In certain experiment, theophylline solution in PBS was intravenously administered into the caudal vein by bolus injection in order to calculate the absolute bioavailability and MAT. For determination of the drugs concentration in serum, 250 μL blood samples were taken from jugular vein periodically after dosing, centrifuged at $1800 \times g$ for 10 min, and the serum was collected. All biological samples were stored at $-30\,^{\circ}C$ prior to analysis.

2.4. Analytical methods of theophylline

The theophylline concentrations in serum and lung tissue homogenate were determined on the column switching high-performance liquid chromatography (Shimadzu LC-10) drawing upon the reported method (Kizu et al., 1999). In brief, 100 μL of methanol containing β-hydroxyethyltheophylline as internal standard was added to each 100 μL of serum or homogenate and mixed. After being allowed to stay at room temperature for about 15 min, 100 μL of purified water was added and the resulting solution was centrifuged at 14,000 rpm for 5 min. The supernatant was filtrated through ULTRAFREE-MC 0.22 μm filter (MILLIPORE) and 100 μL of the filtrate was injected onto an TSK-Precolumn BSA-ODS (4.6 mm × 35 mm, TOSOH, Japan). The mobile phase for pretreatment was 10-fold diluted PBS and the flow rate was

1.0 mL/min. The fraction containing theophylline was transferred from the precolumn to the analytical column, YMC-Pack ODS-AM 303 column (4.6 mm × 250 mm, YMC, Japan) by a mobile phase containing 20 mM sodium acetate buffer (pH 4.8)/acetonitrile/methanol (900:50:50) at a flow rate of 1.0 mL/min delivered by switching the electric six-port valve. The detection was performed with UV set at 273 nm.

2.5. Analytical methods of fluticasone propionate

The concentrations of fluticasone propionate in serum were quantified by a validated the high-performance liquid chromatography/mass spectrometry (HPLC/MS) method following appropriate sample preparation. To each 50 μL of serum, 20 μL of budesonide solution as internal standard was added and mixed. The resulting solution was extracted with 2 mL of *n*-hexane/ethyl acetate (2:1) at room temperature, then the organic solvent was evaporated to dryness under reduced pressure. The residue was extracted with 1 mL of *n*-hexane and 2 mL of acetonitrile for 10 min at room temperature, and then acetonitrile was evaporated to dryness under reduced pressure. The residue was dissolved in 150 μL of a 1:1 mixture of 2% acetic acid solution and methanol. A total sample volume of 100 μL was injected into HPLC/MS system. The HPLC was performed isocratically at ambient temperature using a CAPCELL PAK C18 MGII (5 μm, 2 mm × 150 mm, Shiseido Fine Chemicals, Japan). The mobile phase consisted of methanol–water–acetic acid (75:25:1). The flow-rate was 0.2 mL/min and the HPLC system was Waters 2690 Separations Module. The mass spectrometer was a Thermo Quest TSQ 7000 triple quadrupole mass spectrometer equipped with an atmospheric pressure chemical ionization (APCI). The heated capillary temperature was set to 150 °C, and the vaporizer temperature was set to 350 °C. During selected ion monitoring (SIM) operation the instrument was set to record the monitoring ion (*m*/*z* 501.4) of fluticasone propionate and the monitoring ion (*m*/*z* 431.3) of budesonide as internal standard.

2.6. Pharmacokinetic analyses

The pharmacokinetic analyses were obtained using the previously reported method (Yamamoto et al., 2004). In brief, the peak serum concentration (C_{max}) and the peak serum concentration time (T_{max}) were obtained from the serum concentration–time curve from individual animals. The serum concentration profiles of theophylline and fluticasone propionate were analyzed based on the statistical moment theory. Moment parameters for the serum concentration profiles (the area under the serum concentration–time curve (AUC) and mean residence time (MRT) were calculated by numerical integration using a linear trapezoidal formula and extrapolation to infinite time based on a monoexponential equation (Yamaoka et al., 1978). Mean serum absorption time (MAT) was calculated from the following equation

$$\mathrm{MAT} = \mathrm{MRT_{it}} - \mathrm{MRT_{iv}} \quad (1)$$

where $\mathrm{MRT_{it}}$ and $\mathrm{MRT_{iv}}$ are the mean residence time for intratracheal and intravenous administration, respectively.

2.7. Evaluation of pulmonary inflammation

The solutions containing carrageenans at a concentration of 1% were directly administered to trachea of male Wistar rats anaesthetized with an inhalation of isoflurane (ca. 0.1%) according to the method of Ho and Furst (1973). Five or 24 h later, the animal was bled from the abdominal aorta under pentobarbital anesthesia. After perfusion of the lung with physiological saline via the pulmonary artery, bronchoalveolar lavage (BAL) was performed with PBS (4 mL × 4). The recovered fluids were centrifuged at 200 × *g* for 7 min at 4 °C. The concentrations of protein in the supernatant were determined using an assay kit DC protein assay kit (Bio-Rad Laboratories) using bovine serum albumin (BSA) as a standard. The activity of lactate dehydrogenase (LDH) was determined using an assay kit LDH CII (Wako Pure Chemical Industries, Ltd.). The number of leukocytes obtained after the centrifugation of BAL fluids (BALF) was counted on a hematocytometer. The differential cell counts were determined by Diff-Quik staining. BAL was also adapted to rat with treatment of PBS for control.

2.8. Statistical significance

Results were expressed as the mean ± S.E. and statistical significance was performed by the Student's or

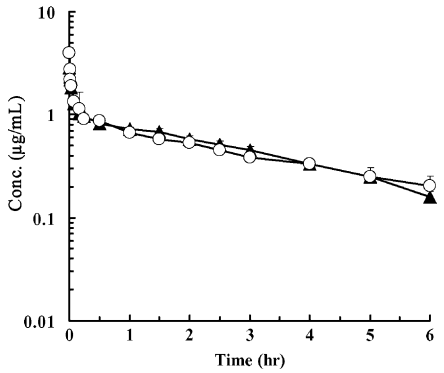

Fig. 1. Serum concentration–time profiles after intratracheal or intravenous administration of theophylline without viscous vehicles in rats. Results are expressed as the mean ± S.E. of four rats. Keys: (◯) intratracheal, (▲) intravenous.

Welch's *t*-test with the minimum levels of significance with $P < 0.05$, or Dunnett's test for multiple comparison.

3. Results

Fig. 1 shows the concentration–time profiles of theophylline after intravenous or intratracheal administration without any viscous vehicle in rats. As shown in this figure, the pulmonary absorption of theophylline was extremely rapid and its profile was same as that after intravenous administration. The absolute bioavailability of theophylline after intratracheal administration without any viscous vehicle was 104% and therefore, it was suggested that theophylline could be absorbed from the lung completely.

Fig. 2 shows the concentration–time profiles of theophylline after intratracheal administration with various viscous vehicles in rats. As shown in Fig. 2, the concentration–time profile of theophylline in the presence of 5% gelatin was similar to the control without any viscous vehicle. C_{max} value decreased in the presence of 2% sodium alginate, but the serum concentrations could not be prolonged. In the presence of 1% iota-carrageenan, C_{max} value of theophylline decreased and the serum concentration of theophylline was prolonged as compared with the control. C_{max} values decreased in the presence of 2% iota-carrageenan, 1% kappa-carrageenan, but the serum concentrations could not be prolonged.

Table 1 summarized the pharmacokinetic parameters of theophylline, which were calculated from the results of Figs. 1 and 2. As shown in Table 1, each C_{max} value significantly decreased in the presence of various viscous vehicles except for 5% gelatin, and T_{max} value increased with the viscous vehicles compared with the control. The MRT and MAT values of theophylline significantly increased with 1% iota-carrageenan. Therefore, these findings suggested that iota-carrageenan was effective to regulate the absorption rate of theophylline.

Fig. 3 shows the effect of two viscous vehicles on the remaining percent of theophylline in the lung tissue at 60 min after intratracheal administration. As shown

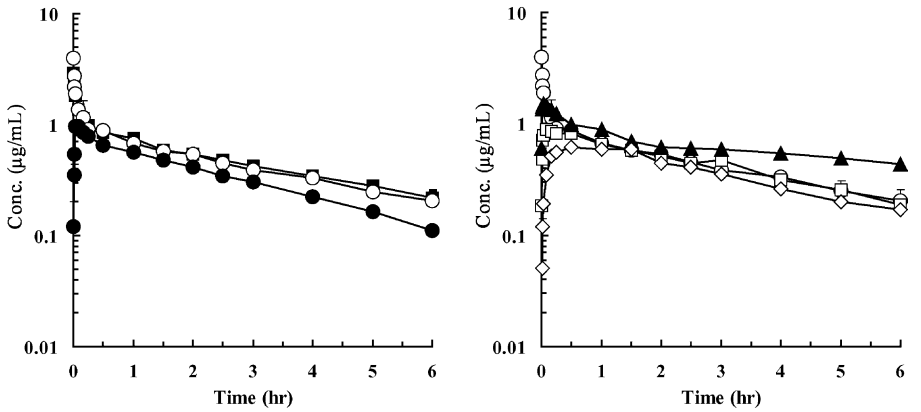

Fig. 2. Serum concentration–time profiles after intratracheal administration of theophylline with various viscous vehicles in rats. Results are expressed as the mean ± S.E. of four rats. Keys: (◯) control, (■) 5% gelatin, (●) 2% sodium alginate, (▲) 1% iota-carrageenan, (◊) 2% iota-carrageenan, (□) 1% kappa-carrageenan.

Table 1
Pharmacokinetic parameters of theophylline after intratracheal administration in the presence of various viscous vehicles

Viscous vehicles	C_{max} (μg/mL)	T_{max} (min)	AUC (μg min/mL)	MRT (min)	MAT (min)
Control	3.96 ± 0.55	0.25 ± 0	240.9 ± 43.2	266.7 ± 65.1	78.7
Gelatin (5%)	3.16 ± 0.52	0.5 ± 0.2	247.0 ± 27.7	269.9 ± 37.4	81.9
Iota-carrageenan (1%)	1.59 ± 0.04***	1.4 ± 0.4	499.5 ± 55.2***	572.3 ± 92.1**	384.3
Iota-carrageenan (2%)	0.63 ± 0.03***	33.8 ± 9.4***	177.0 ± 8.6	260.0 ± 20.8	72.0
Kappa-carrageenan (1%)	0.91 ± 0.05***	5.5 ± 1.7	213.6 ± 24.9	242.7 ± 47.0	54.7
Sodium alginate (2%)	0.99 ± 0.52***	3.5 ± 0.9	146.3 ± 5.7	178.7 ± 11.5	−9.3

Results are expressed as the mean ± S.E. of three to four rats.
** $P < 0.01$, compared with the control.
*** $P < 0.001$, compared with the control.

in this figure, the remaining percent of theophylline was significantly increased in the presence of 1% iota-carrageenan. However, 5% gelatin did not increase the remaining percent of theophylline in the lung tissue at 60 min.

Furthermore, we examined the effect of carrageenans on the pulmonary absorption of fluticasone propionate as a clinically used inhaled corticosteroid. The concentration–time profiles of fluticasone propionate after intratracheal administration with carrageenans in rats were presented in Fig. 4 and the pharmacokinetic parameters of fluticasone propionate were summarized in Table 2. C_{max} value of fluticasone propionate decreased in the presence of 1% iota-carrageenan which regulated the pulmonary absorption of theophylline, but MRT did not significantly increase with the vehicle. On the other hand, the serum concentration of fluticasone propionate was prolonged and, MRT increased in the presence of 0.5% kappa-carrageenan compared with the control.

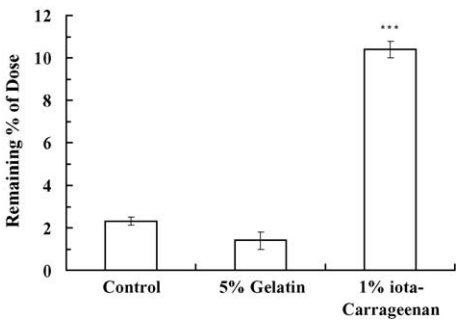

Fig. 3. Effect of two viscous vehicles on the remaining percent of theophylline in the lung tissue at 60 min after intratracheal administration. Results are expressed as the mean ± S.E. of four rats. *** $P < 0.001$, compared with the control.

Fig. 5 shows the number of sorted leukocytes recovered into BALF after intratracheal administration of three types of 1% carrageenans. The number of sorted leukocytes at 5 h after intratracheal administration of carrageenans was similar to PBS, but the number of mononuclear cells at 24 h slightly increased and that of neutrophils extremely increased by intratracheal administration of lambda-carrageenan compared with PBS. The number of mononuclear cells and neutrophils increased with iota-carrageenan at 24 h compared with PBS, but the numbers of sorted leukocytes with kappa-carrageenan were similar to PBS.

Amount of total protein and the activity of LDH in BALF are shown in Fig. 6. The total amount of protein significantly increased in the presence of lambda-

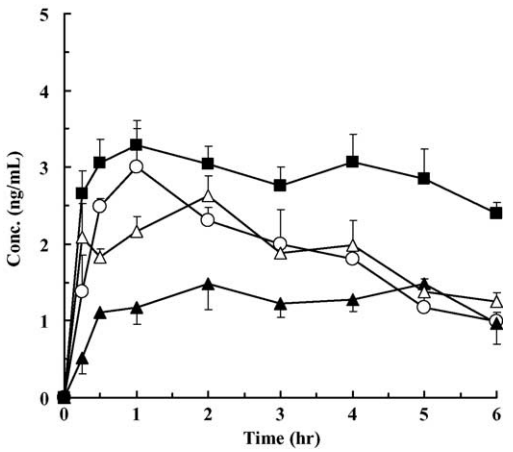

Fig. 4. Serum concentration–time profiles after intratracheal administration of fluticasone propionate with carraggenans in rats. Results are expressed as the mean ± S.E. of four rats. Keys: (◯) control, (▲) 1% iota-carrageenan, (△) 0.5% iotas-carrageenan, (■) 0.5% kappa-carrageenan.

Table 2
Pharmacokinetic parameters of fluticasone propionate after intratracheal administration in the presence of carrageenans

Viscous vehicles	C_{max} (ng/mL)	T_{max} (min)	AUC_{∞} (ng min/mL)	MRT (min)
Control	3.12 ± 0.45	52.5 ± 7.5	870.6 ± 75.8	244.8 ± 6.8
Iota-carrageenan (0.5%)	2.84 ± 0.31	93.8 ± 26.3	953.3 ± 92.4	283.2 ± 26.8
Iota-carrageenan (1%)	1.75 ± 0.24*	210.0 ± 71.4	676.8 ± 136.0	298.8 ± 52.5
Kappa-carrageenan (0.5%)	3.62 ± 0.09	225.0 ± 56.8	1555.5 ± 39.0***	313.8 ± 9.3**

Results are expressed as the mean ± S.E. of three to four rats.
* $P < 0.05$, compared with the control.
** $P < 0.01$, compared with the control.
*** $P < 0.001$, compared with the control.

carrageenan and was not significantly increased in the presence of iota- and kappa-carrageenans at 5 and 24 h after intratracheal administration. The activity of LDH significantly increased only with lambda-carrageenan at 24 h.

4. Discussion

The pulmonary absorption of drugs was generally influenced by various physicochemical and biological factors. The physicochemical factors include molecular size of drugs (Enna and Schanker, 1972a), lipophilicity of drugs (Enna and Schanker, 1972b), pH in drug solution (Arakawa and Kitazawa, 1987), various additives (Ohtani et al., 1991; Morita et al., 1993), etc. In previous study, we demonstrated that the pulmonary absorption of CF with low molecular weight and a highly water-soluble characteristic could be regulated in rats by using the various viscous vehicles. Therefore, these vehicles may be useful to retain the local acting drugs to the lung tissues and to control the absorption rate of drugs from the lung to the systemic circulation (Yamamoto et al., 2004). In this study, we examined the effects of various viscous vehicles on the pulmonary absorption of theophylline and fluticasone propionate as antiasthmatic agents in rats.

The pulmonary absorption of theophylline occurred rapidly and absolutely in rats (Fig. 1). It was reported that xanthine derivatives could be rapidly absorbed from the lung in rats, and the 1-min absorption value of theophylline was about 90% (Arakawa and Ki-

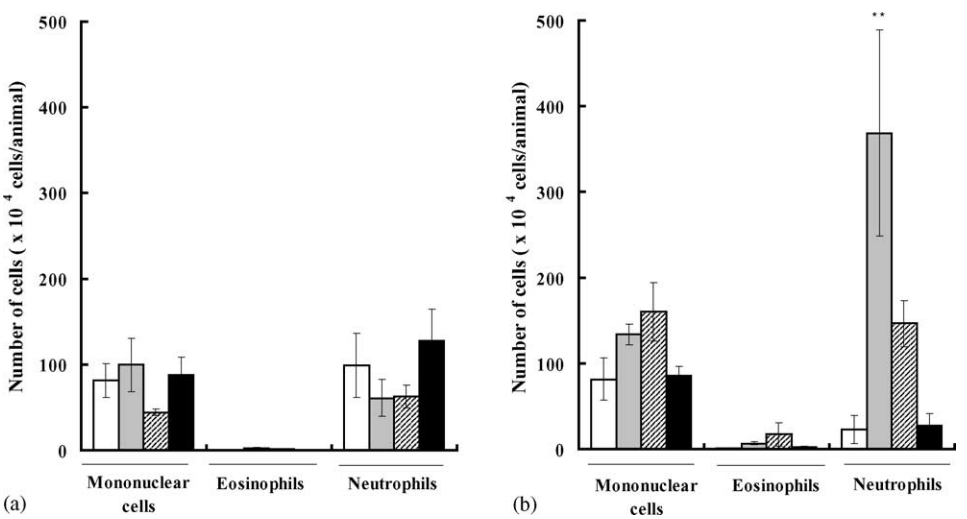

Fig. 5. Differential leukocytes in bronchoalveolar lavage fluid at 5 h (a) or 24 h (b) after exposure of PBS (open bars), lambda- (gray bars), iota- (hatched bars), kappa- (filled bars) carrageenans administered to lung. Results are expressed as the mean ± S.E. of four rats. ** $P < 0.01$, compared with the control.

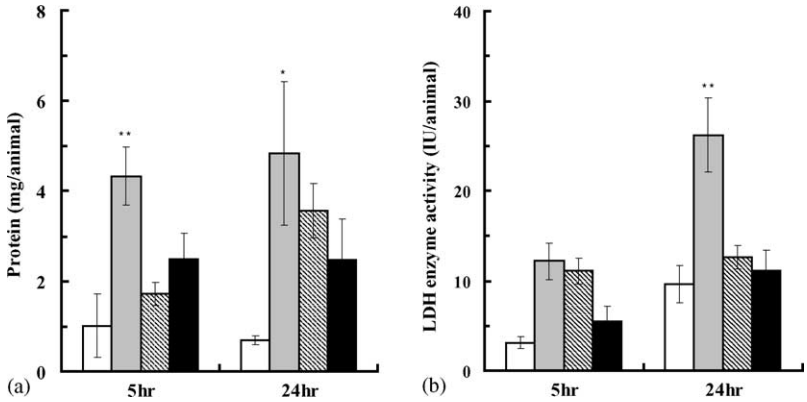

Fig. 6. Levels of total protein (a) and LDH enzyme activity (b) in bronchoalveolar lavage fluid at 5 h or 24 h after exposure of PBS (open bars), lambda- (gray bars), iota- (hatched bars), kappa- (filled bars) carrageenans administered to lung. Results are expressed as the mean ± S.E. of four rats. $^{*}P < 0.05$ and $^{**}P < 0.01$, compared with the control.

tazawa, 1987). The present study indicated that the pulmonary absorption of theophylline was significantly regulate in the presence of 1% iota-carrageenan (Fig. 2, Table 1). However, 5% gelatin, which could control the pulmonary absorption of CF, 2% sodium alginate, 1% kappa-carrageenan and 2% iota-carrageenan could not regulate the pulmonary absorption of theophylline (Fig. 2, Table 1). Therefore, it was suggested that the controlling the pulmonary absorption of theophylline by various viscous vehicles would depend on the concentrations and types of these vehicles.

Moreover, we examined the effect of carrageenans on the pulmonary absorption of fluticasone propionate in rats. Fluticasone propionate is commonly prescribed inhaled corticosteroid used for the local treatment of inflammatory diseases in the airways, e.g. asthma and rhinitis. The pulmonary absorption rate of fluticasone propionate in the absence of carrageenans was relatively lower than that of theophylline in rats. The pulmonary absorption of fluticasone propionate could be regulated in the presence of 0.5% kappa-carrageenan and could not be controlled in the presence of 1% iota-carrageenan, which could regulate that of theophylline (Fig. 4). Kappa-carrageenan forms the elastic gel compared to iota-carrageenan. Therefore, kappa-carrageenan can potently regulate the pulmonary absorption of fluticasone propionate with relatively lower pulmonary absorption rate, and may excessively reduce the pulmonary absorption of theophylline with high pulmonary absorption rate. Adachi and Wada (1994) reported that fluticasone propionate dry powder in single dose 400 μg was administered to healthy volunteers and the plasma concentration of fluticasone propionate was measured. The peak concentration was achieved at 30 min after dosing. The plasma level showed gradual decrease, and it was below the detection limit by 8 h post dosing. In this study, kappa-carrageenan could maintain the high serum concentration of fluticasone propionate until 6 h after dosing in rats. Therefore, the application of kappa-carrageenan for inhalation formulation of antiasthmatic drugs might lead to decreasing the incidence of nocturnal and early morning wheezing in asthmatics.

On the other hand, the safety of carrageenans to pulmonary organ is an important matter of interest. Thus, we examined whether carrageenans have pro-inflammatory activity to the pulmonary tissues because lambda-carrageenan has been used as a phlogogenic material for evaluation of pharmacological activity of anti-inflammatory drugs (Winter et al., 1962). Neutrophils, total protein and LDH as inflammatory indicators in BALF at 24 h after dosing of 1% lambda-carrageenan were significantly augmented as compared to those of PBS. Lambda-carrageenan is highly sulfated, therefore, it would appear that lambda-carrageenan has certain pro-inflammatory activity to the pulmonary mucosa. Iota-carrageenan has lower content of sulfate group than lambda-carrageenan, and kappa-carrageenan has the lowest sulfate among these three. In proportion to content of sulfate group in carrageenans, magnitudes of these inflammation indicators in iota-carrageenan-

treated animals were less prominent than those in the lambda-carrageenan-treated. Furtheremore, kappa-carrageenan did not change the indicators. LDH, a cytoplasmic enzyme, should be released to extracellular spaces and collected by BAL only when cell lysis or cell membrane damage has occurred (Henderson, 1984). Todo et al. (2001) reported that the LDH activity in BALF at 24 h after intratracheal administration was about 10-fold higher with 0.25% Triton-X 100 than PBS. The LDH activities in BALF at 24 h after dosing of iota- and kappa-carrageenans were as low as that for PBS administration, therefore, it was suggested that iota- and kappa-carrageenans induced little or no pulmonary cell membrane damage.

In conclusion, the present findings suggested that iota- and kappa-carrageenans were effective to regulate the absorption rate of antiasthmatic drugs such as theophylline and fluticasone propionate. In addition, they did not cause local damage and inflammation to the pulmonary tissues. This approach using iota- and kappa-carrageenans may be useful to retain the local acting drugs to the lung tissues and to control the absorption the absorption rate of drugs from the lung to the systemic circulation.

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