Surface-modified Antiasthmatic Dry Powder Aerosols Inhaled Intratracheally Reduce the Pharmacologically Effective Dose

Yoshiaki Kawashima,[1,3] Takanori Serigano,[1] Tomoaki Hino,[1,2] Hiromitsu Yamamoto,[1] and Hirofumi Takeuchi[1]

Received Received May 3, 1998; accepted August 11, 1998

Purpose. The aim of this study was to construct a reliable dry powder inhalation (DPI) testing system for use in guinea pigs. Using this system, we were able to demonstrate the superiority of pulmonary administration of hydrophilically surface-modified pranlukast hydrate powder (SM-DP) over IV and PO administration as reflected in improved pharmacological action. Our ultimate aim is the development of an ideal treatment system for bronchial asthma involving topical administration to the lung.

Methods. The reliability of the present DPI system was validated by continuously monitoring the concentration and particle size distribution of aerosols generated with an ambient particulate monitor and an Andersen air sampler, respectively. The pharmacological effect of SM-DP intratracheally administered to guinea pig was investigated by measuring the degree of bronchoconstriction and microvascular leakage induced by leukotriene D_4.

Results. The mass concentration of aerosols generated by the DPI system was stable and the mass median aerodynamic diameter of aerosols insufflated from the respirator of the DPI system ranged from 1.4 to 1.7 μm, within respirable limits. Inhibition of bronchoconstriction and airway microvascular leakage induced by leukotriene D_4 was achieved successfully with a dramatically lower dose of DP, or a further lower dose of SM-DP, comparable with that of the drug solution injected intravenously. The plasma pranlukast hydrate level with SM-DP at 50% inhibition of bronchoconstriction and airway microvascular leakage was reduced to 1/10 or less that following IV and PO administration.

Conclusions. The hydrophilically surface-modified pranlukast hydrate powders were ideally aerosolized by the present DPI system, and were uniformly deposited in the lung lobes after inhalation. The pulmonary administration system with SM-DP is strongly recommended as an ideal system for the treatment of bronchial asthma in order to avoid systemic side-effects due to a dramatically reduced ED_{50}, comparable with or lower than IV, and the low plasma concentration of drug, 1/12 or less than that following IV and PO administration.

KEY WORDS: dry powder inhalation; aerosol therapy; intratracheal administration; leukotriene inhibitor; pranlukast hydrate; leukotriene D_4.

INTRODUCTION

Many approaches involving the use of drug inhalation aerosols have been tried for the treatment of asthma, chronic bronchitis, emphysema, cystic fibrosis, pulmonary infections, and other diseases (1). This is because of the possibility of significantly reducing the clinically effective dose and, thus, improving drug safety via topical administration to the lung (2). The orally active peptide leukotriene antagonist for bronchial asthma, pranlukast hydrate (PH), is a promising new candidate for pulmonary delivery.

We have been developing a dry powder inhalation system of PH to improve the efficiency of its delivery by inhalation to the deep lung by hydrophilic surface modification using hydroxypropyl-methylcellulose phthalate nanospheres (HPMCP-NS) (3). This surface modification was found to improve dramatically the dissolution rate of a hydrophobic drug like PH in pH 6.5 phosphate buffer as well as its inhalation properties *in vitro*. These findings suggested strongly that the drug particles deposited on the lung mucosa at pH = 5.7 to 7.5 would dissolve rapidly, resulting in improved therapeutic effects.

The aim of present study was to construct a reliable dry powder inhalation testing system to investigate that pharmacological effect of drug delivered to the lung *in vivo* using a small animal, e.g., guinea pig, and to evaluate the pharmacologically effective dose and systemic side-effects at the same time. Using this system, the advantages of hydrophilic surface-modified PH powder for inhalation were demonstrated as far as developing an ideal PH delivery system via topical administration to the lung was concerned.

MATERIALS AND METHODS

Chemicals and Surface Modification of Drug Powder for Inhalation

The following chemicals and drug were used: leukotriene D_4 (LTD_4; Cayman Chemical Company, USA) dissolved in ethanol; Evans blue (Nacalai Tesque, Japan) dissolved in 1/15 M phosphate-buffered saline (pH 7.4, Nissui Pharmaceutical, Japan); sodium pentobarbital (Nembutal injection, Abbott, USA); formamide (Nacalai Tesque, Japan); sodium carboxymethylcellulose (Kishida Chemical, Japan); polyoxyethylene sorbitan monooleate (Tween 80, Kishida Chemical, Japan); pranlukast hydrate (4-oxo-8-[4-(4-phenylbutoxy) benzoylamino]-2-(tetrazol-5-yl)-4*H*-1-benzopyran hemihydrate, solubility in water 1.2 μg/ml, mean particle diameter 2.13 μm, determined by laser diffraction with a dry disperser (LDSA-2400A and PD-10S, Tohnichi Computer, Japan), supplied by Ono Pharmaceutical, Japan). PH has a potent antiasthmatic effect due to leukotriene inhibition (4,5).

PH with its surface modified by HPMCP-NS was prepared as described in the previous paper (3). The PH particles were introduced to aqueous colloidal HPMCP dispersions (NS) prepared by emulsion-solvent diffusion techniques developed by us (6,7), followed by freeze-drying the resultant aqueous dispersions. *The amount of HPMCP-NS added to the drug was 10%.*

PH for oral administration was suspended in 0.5% sodium carboxymethylcellulose solution while PH for intravenous administration was dissolved in tris-buffer (pH 9.0) containing 0.1% of Tween 80.

[1] Gifu Pharmaceutical University, 5-6-1, Mitahora-higashi, Gifu 502-8585, Japan.

[2] Present address: Faculty of Pharmaceutical Science, University of Tokushima, 1-78-1, Sho-machi, Tokushima 770-0044, Japan.

[3] To whom correspondence should be addressed. (e-mail: yoshiaki@gifu-pu.ac.jp)

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Dry Powder Inhalation Testing System

The dry powder inhalation testing system was a novel one as shown in Fig. 1. PH powder loaded into the reservoir with a diameter of 7 or 14 mm, equipped with a powder dispersing generator (RBG-1000, Palas, Germany) was aerosolized into the aerosol reservoir (chamber) using pressurized air. During the generation of PH powder aerosols, the mass concentration of aerosol dispersed inside the chamber was continuously monitored with an ambient particulate monitor (TEOM series 1400, Rupprecht & Patashnick, USA). After the mass concentration of aerosols reached equilibrium, the constant volume of aerosols (5 ml) in the chamber was insufflated into the lung of the test animal with a respirator (Model 683, Harvard Apparatus, USA) operated at 70 strokes/min. The particle size distributions of the aerosols in the aerosol reservoir and the aerosols insufflated from the respirator were determined by an Andersen air sampler (AN-200, Andersen, USA). The amount of aerosol powders insufflated throughout the respirator was measured by passing them through a capturing solvent (50 mM sodium bicarbonate solution/ethanol = 1/1) for 5 min. The PH dissolved in the solvent was determined by reverse-phase HPLC using a UV detector (LC-6A and SPD-6AV, Shimadzu, Japan). The HPLC system consisted of an ODS column (Inertsil ODS-2, GL Science, Japan) and a mobile phase of 0.02 M potassium dihydrogenphosphate/acetonitrile/methanol (8:10:1, v/v) at a flow rate of 1.0 ml/min. The UV detection wavelength was 260 nm and 4-hydroxybenzoic acid isoamyl ester was used as internal standard.

Animals and Test Procedure for Measurement of LTD_4-Induced Bronchoconstriction and Airway Microvascular Leakage

The method for measuring bronchoconstriction was essentially a modification of the Konzett-R__ssler technique (8).

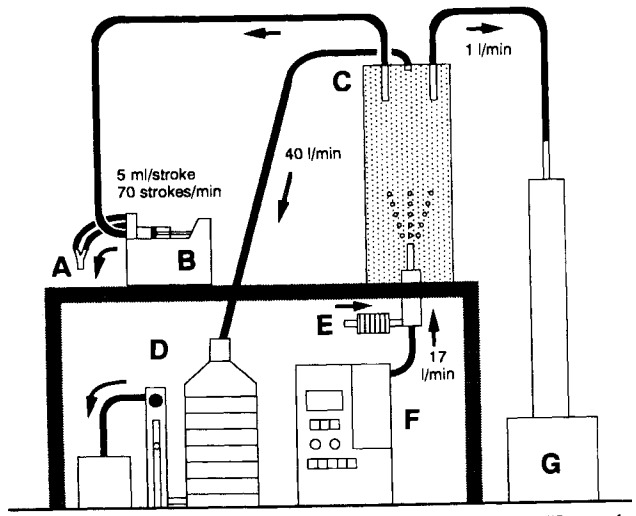

Fig. 1. Apparatus for insufflating guinea pig lung with PH powder aerosols. A: Y-shaped polyethylene tube for cannulation of guinea pig lung; B: constant volume respirator operated at 5 ml/stroke and 70 strokes/min (Model 683, Harvard, USA); C: aerosol reservoir (acrylic chamber; ϕ120 mm × 520 mm, Volume: 5880 ml); D: aerosol sampler (AN-200, Andersen, USA); E: atmospheric pressure regulator; F: powder-dispersing generator (RBG-1000, Palas, Germany); G: ambient particulate monitor (TEOM series 1400, Rupprecht & Patashnick, USA).

Six-week old male Hartley guinea pigs were used, they weighed 300 to 400g and were fasted for 24 hr before the experiments. The animals were anesthetized with sodium pentobarbital (75 mg/kg, i.p.). A small Y-shaped cannula connected to a constant volume respirator was inserted into the trachea, and the animals were artificially ventilated with a constant volume of 5 ml at a frequency of 70 strokes/min. Changes in insufflation pressure at a constant airflow were measured by a bronchospasm transducer (7020, Ugo Basil, Italy) connected to the side-arm of the tracheal cannula. The jugular vein was cannulated for the administration of Evans blue and LTD_4. One minute after the administration of Evans blue (20 mg/ kg), bronchoconstriction was induced by intravenous injection of LTD_4 (2 μg/kg) via the jugular cannula. LTD_4-induced bronchoconstriction was measured for 10 min and represented as a percentage of the maximal increase in insufflation pressure achieved by clamping the trachea. The % inhibition of bronchoconstriction *during this 10 min period* was calculated by Eq. (1).

$$\text{Inhibition (\%, Bronchoconstriction)} = \left(1 - \frac{AUC_{Treated}}{AUC_{Vehicle}}\right) \times 100 \quad (1)$$

where, $AUC_{Vehicle}$ and $AUC_{Treated}$ are the areas under the curve of the bronchoconstriction response of the vehicle-treated control and PH treated animal, respectively.

After measurement of bronchoconstriction, the animals were exsanguinated. The chest cavity was then opened and Evans blue was washed out by perfusing with 0.9% NaCl from the pulmonary artery into the left atrium. The airways and lungs were then removed and freed of extraneous connective tissues. The extrapulmonary airway was divided into trachea and main bronchi, and the intrapulmonary airway was stripped of parenchyma. The wet weight of each tissue was measured. The Evans blue contained in each tissue was completely extracted with 5 ml formamide after being allowed to stand for at least 24 hr. The amount of Evans blue in the extract was determined spectrophotometrically at 620 nm (UV-160A, Shimadzu, Japan), and the content of Evans blue was expressed as ng/mg tissue. The airway microvascular leakage of Evans blue was calculated by Eq. (2).

$$\text{Inhibition (\%, Airway microvascular leakage)} = \frac{AML_{Vehicle} - AML_{Treated}}{AML_{Vehicle} - AML_{Baseline}} \times 100 \quad (2)$$

where, $AML_{Vehicle}$, $AML_{Baseline}$ and $AML_{Treated}$ are the airway microvascular leaked Evans blue per tissue weight of vehicle-treated control, baseline and PH-treated animal, respectively. The baseline was determined by administration of 0.9% NaCl containing Evans blue.

Dry powder aerosols of original (DP) and surface-modified PH (SM-DP) using HPMCP-NS generated from the inhalation testing system were inhaled intratracheally by guinea pigs via the respirator, as described above. A dose of approximately 10, 30 or 100 μ g/kg for the original PH and one further dose of 3 μg/kg added to the dose series for SM-DP were administered 60 min before LTD_4 challenge, respectively. The pulmonary administered dose was calculated by Eq. (3).

$$\text{Administered dose } (\mu g/kg) = \frac{G \times T - E}{W} \quad (3)$$

where, G, T, E and W are the amount of PH aerosols generated

from the respirator (μg/min), inhalation time (min), amount of PH exhaled by the animal (μg) and body weight (kg), respectively. *The amount of PH exhaled by the animal (E) was determined in a pilot experiment.*

As a reference, PH was administered intravenously (IV) or orally (PO) to guinea pigs. For IV dosing, 10, 30 or 100 μg/kg PH dissolved in tris-buffer (pH 9.0) containing 0.1% Tween 80 (1 ml/kg) was administered 1 min before the LTD_4 challenge. For PO dosing 1, 3 or 10 mg/kg PH suspended in 0.5% sodium carboxymethylcellulose solution (10 ml/kg) was administered 60 min before the LTD_4 challenge.

Pulmonary Deposition Patterns of PH After DP Dosing

After PH dry powder aerosols were administered (approx. 30 μg/kg), the lungs were immediately excised. The right and left lungs were divided into 3 lobes (upper, middle and lower lobes) and 2 lobes (upper and lower lobes), equally at the middle point of the long axis, respectively. Each lobe was homogenized in ethanol and centrifuged at 10000 rpm for 15 min. Then the supernatant was centrifugally evaporated, and redissolved with 0.2 ml HPLC mobile phase containing internal standard (4-hydroxybenzoic acid isoamyl ester). A reverse-phase HPLC equipped with UV detector was used to determine PH.

Table I. Deposition Patterns of PH in the Guniea Pig Lung After IF Administration

Tissue	% distribution	μg/g tissue
Trachea	11.8 ± 5.1	7.10 ± 3.67
Main Bronchi	0.8 ± 0.3	0.66 ± 0.23
Right Upper	5.7 ± 1.3	1.79 ± 0.44
Middle	12.2 ± 2.6	1.47 ± 0.31
Lower	33.1 ± 6.1	2.12 ± 0.19
Left Upper	15.3 ± 2.5	1.64 ± 0.26
Lower	21.1 ± 4.3	1.70 ± 0.26
Total Right	51.0 ± 4.2	1.79 ± 0.25
Total Left	36.4 ± 2.7	1.67 ± 0.17

Each value represents the mean ±S.E. of 5 animals.

Determination of Plasma PH Concentration

Blood samples were collected from the other side of the jugular vein just before the LTD_4 challenge. Then 0.2 ml plasma (0.4 ml in the case of DP and SM-DP) was mixed with 3 ml ethanol containing internal standard. The resultant mixture was shaken by vortex mixer and centrifuged at 3000 rpm for 10 min. The PH content of the supernatant was determined by reverse-phase HPLC as described above.

RESULTS AND DISCUSSION

Validation of Dry Powder Inhalation Testing System

The mass median aerodynamic diameters of the original PH aerosols in the chamber and from the respirator were 2.02 μm (*0.9–5.7 μm; 82.6%*) and 1.67 μm (*0.65–7.0 μm; 88.9%*), respectively. The original particle size of PH was 2.13 μm. The mass median aerodynamic diameters of the surface-modified PH aerosols in the chamber and from the respirator were 1.55 μm (*0.9–5.7 μm; 83.8%*) and 1.40 μm (*0.65–7.0 μm; 88.8%*), respectively. The average particle size of the surface-modified PH was 2.56 μm. This suggests that the aerosols generated by the respirator were dispersed ideally into discrete particles, although there was a slight reduction in particle size

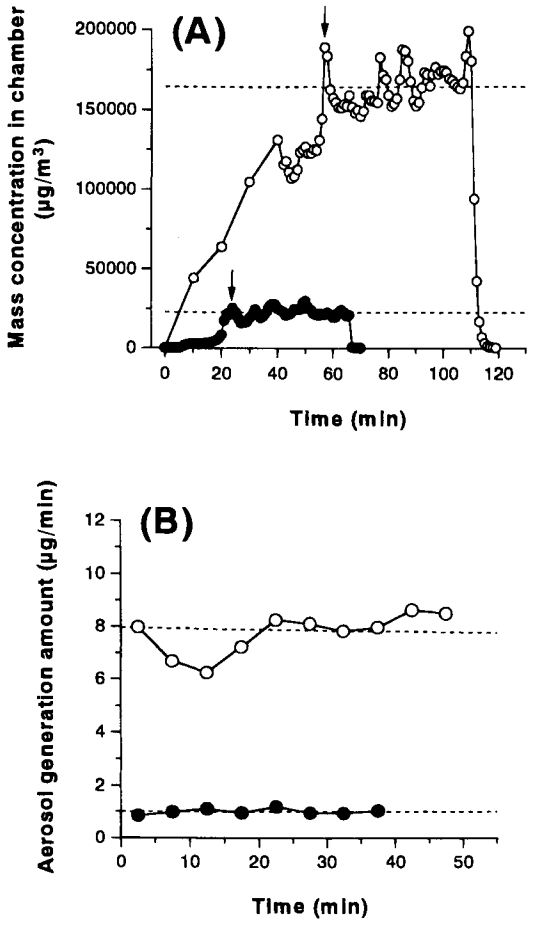

Fig. 2. Typical time-courses of aerosol concentration in chamber (A) and amount of aerosol generated from respirator (B). (●) 7 mm ϕ, (○) 14 mm ϕ powder-loading reservoir. The arrows in the figure (A) indicate the onset of capturing the aerosol generated from the respirator. The dotted line represents the mean value during the aerosol capturing.

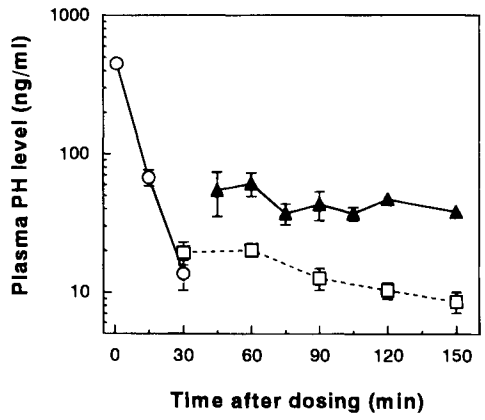

Fig. 3. Mean plasma concentration-time profiles following administration of PH to guinea pigs. (○) IV 30 μg/kg, (▲) PO 1 mg/kg, (□) DP 30 μg/kg. $AUC_{0\text{-}150}$ (μg* min/ml) are 3937 (IV), 5950 (PO) and 2017 (DP). C_{max} (ng/ml) are 61.2 (PO) and 20.3 (DP). Each value represents the mean ±S.E. of 3–8 animals.

due to the deposition of coarse particles inside the tube or sedimentation with gravity in the chamber.

In the previous paper (9), the deposition efficiency and site of inhaled aerosols in the respiratory tract were found to be critically influenced by their aerodynamic diameter, size distribution, shape and density. It has been reported that inhaled particles larger than 10.0 μm are generally deposited in the upper respiratory tract because of their inertial impaction, and those smaller than 0.5 μm are exhaled without deposition. Therefore, an aerodynamic diameter between 1.0 and 6.0 μm is thought to be most effective for pulmonary delivery (10). Accordingly, it was assumed that the particle size distribution of aerosols generated in the chamber or insufflated from the respirator were suitable for evaluating the pulmonary deposition patterns of inhaled powder aerosols and the pharmacological effect of PH.

Typical time-courses of aerosol mass concentration in the chamber (aerosol reservoir) and the amount of aerosol generated from the respirator after reaching equilibrium concentration in the aerosol reservoir are shown in Fig. 2 (A) and (B), respectively. For both aerosol-generating conditions, the aerosol concentration in the aerosol reservoir was reasonably stable after a period of induction to attain equilibrium. The amount of aerosol insufflated from the respirator was also stable. These findings are a validation that the dry powder inhalation testing system constructed for the present study operated reliably as far as the pulmonary administration of drug powder aerosols was concerned. Drug dosing could be altered quantitatively by varying the administration time and aerosol concentration.

Pulmonary Deposition Patterns of PH Dry Powder Aerosols

The pulmonary deposition distribution of PH after inhalation of PH aerosols is shown in Table I. About 90% of the inhaled PH was found in the right and left lobes, and the amount

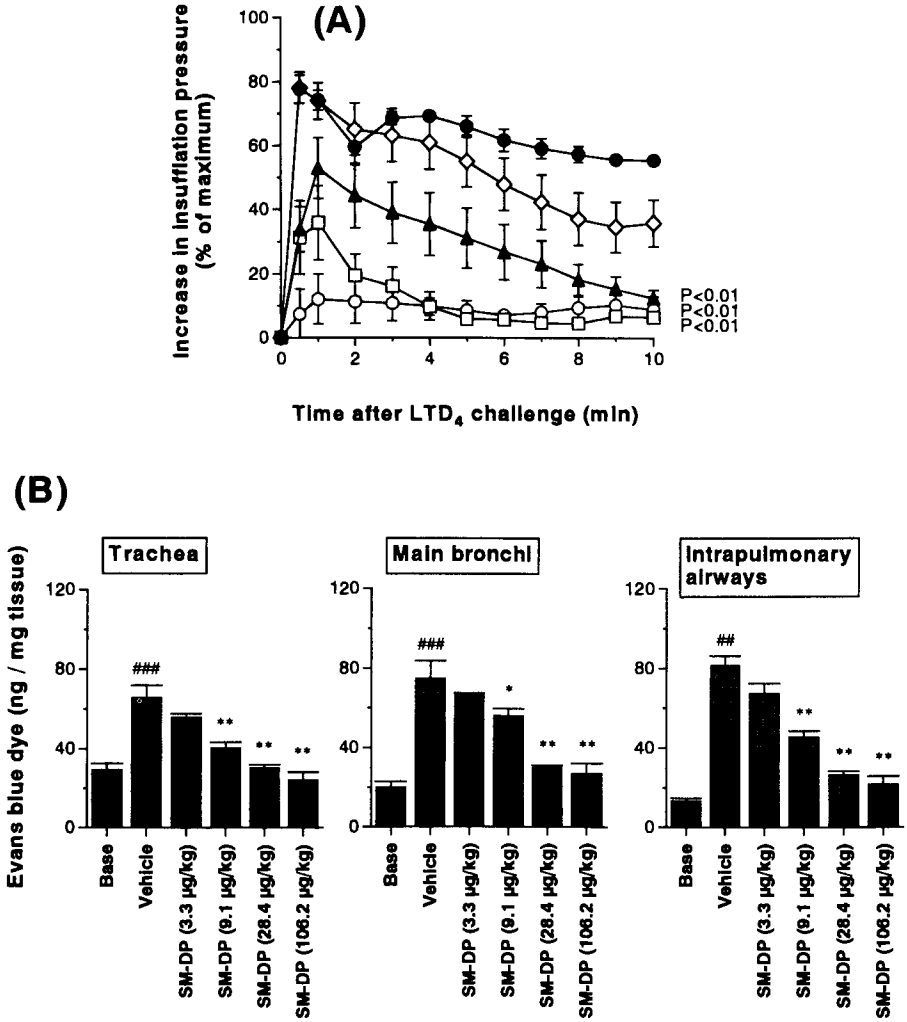

Fig. 4. Effects of PH of SM-DP on LTD_4-induced bronchoconstriction (A) and microvascular leakage (B) in guinea pigs. (●) Vehicle, (◇) 3.3 μg/kg, (▲) 9.1 μg/kg, (□) 28.4 μg/kg, (○) 106.2 μg/kg. Each value represents the mean ±S.E. of 5 animals. $P < 0.01$ significant difference compared with the vehicle using two-way analysis of variance followed by Dunnett's *t*-test. *$P < 0.05$, **$P < 0.01$: significant difference compared with the vehicle using Dunnett's *t*-test. ##$P < 0.01$, ###$P < 0.001$: significant difference compared with the baseline value using Student's unpaired *t*-test or Mann-Whitney's U-test.

deposited per unit tissue weight was fairly uniform. It has been frequently reported that the amount of inhaled aerosol deposited is different for the right and left lobes, due to an anatomical difference in the lungs (11,12). The uniform aerosol deposition shown in this table was obtained by the application of fine aerosols with a mean diameter of 1.67 μm, and resulted in the effective diffusion of aerosol particles in the air stream.

Time-Courses of Plasma Levels After Administration of PH

Mean plasma concentration-time profiles of PH administered via IV, PO and DP are shown in Fig. 3. It was found that the C_{max} of PO and DP was reached at 60 min after administration. Consequently, the pharmacological evaluation of PH was carried out at this time as discussed in the following paragraph. The AUC between 0 and 150 min, and C_{max} of DP were lower than those following IV and PO dosing, confirming the safety of DP and its usefulness in avoiding any systemic side-effects of PH.

Inhibition of LTD_4-Induced Bronchoconstriction and Microvascular Leakage by Administration of PH Dry Powder Aerosols

The intravenous administration of LTD_4 produced a biphasic bronchoconstriction with peaks at 30–60 sec and 4–5 min as reported previously (4). In the present study, dose-dependent inhibition of LTD_4-induced bronchoconstriction was observed for both SM-DP (Fig. 4 (A)) and DP (data are not shown). Intravenous and oral administration of PH inhibited LTD_4-induced bronchoconstriction in a dose-dependent manner as reported previously (4). The effects of the route of administration of PH on the % inhibition of LTD_4-induced bronchoconstriction are shown in Fig. 5 (A). Oral administration of PH proved that much higher dosing (about 190 times) was required to obtain 50% inhibition, compared with pulmonary (SM-DP) and IV administration. In fact, the dose of SM-DP required for 50% inhibition (ED_{50}) was comparable with or lower than that for IV administration.

LTD_4 produced significant airway microvascular leakage into the trachea, main bronchi and intrapulmonary airways. Intravenous or oral PH inhibited LTD_4-induced airway microvascular leakage in a dose-dependent manner. Dose-dependent inhibition of LTD_4-induced airway microvascular leakage was observed with SM-DP (Fig. 4 (B)) or DP (data are not shown). The effects of the route of administration of PH on the % inhibition of LTD_4-induced airway microvascular leakage are shown in Fig. 5 (B). The superiority of SM-DP in reducing the dose required for inhibition, compared with that of oral administration, was also clear.

The ED_{50} values obtained from Fig. 5 are summarized in Table II. These results indicate that dry powder inhalations (especially SM-DP) of PH are extraordinarily effective in reducing the ED_{50} for both bronchoconstriction and microvascular leakage, which was comparable with or greater than that of IV. It appears that the pulmonarily administered PH of DP was successfully absorbed into pulmonary tissues and inhibited the action of LTD_4. A further reduction in ED_{50} values was achieved by altering the PH surface with hydrophilic modifier (HPMCP-NS). This was due to the rapid dissolution and absorption of

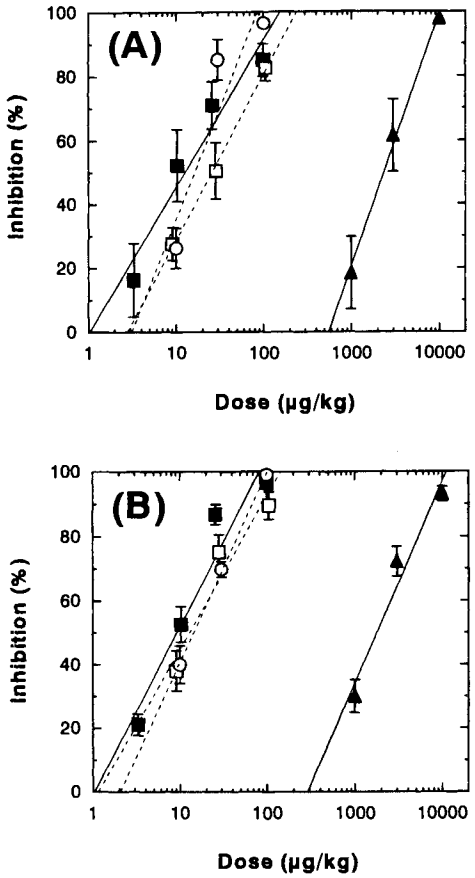

Fig. 5. Effects of route of administration of PH on the % inhibition of LTD_4-induced bronchoconstriction (A) and microvascular leakage (B) in guinea pigs. (○) IV, (▲) PO, (□) DP, (■) SM-DP. Each value represents the mean ±S.E. of 5 animals.

PH following hydrophilic surface modification with HPMCP-NS (3).

These results suggest that the intratracheal administration of surface modified PH aerosols effectively reduced the clinically required dose, leading to improvements in the safety of PH administration.

PH Level in Plasma before the Pharmacological Evaluation Test

There was no clear relationship between the PH plasma AUC and the % inhibition of LTD_4-induced bronchoconstriction and airway microvascular leakage. Therefore, the relationship between the plasma concentrations of PH just before LTD_4 challenge and the % inhibition was investigated as shown in Fig. 6 (A) and (B), respectively. IV and PO exhibited similar profiles, which shifted significantly to the right of DP or SM-DP. This finding indicates that PH administered by IV or PO inhibits LTD_4, only when delivered to the pulmonary LT receptors with the circulating blood. In contrast, PH administered by DP or SM-DP is directly absorbed into pulmonary tissues rather than delivered by the blood flow. Taburet and Schmit (13) also reported that the therapeutic effects depended on the local tissue concentration of drug, which might not be directly related to the plasma drug concentrations after topical administration (e.g. via aerosol delivery to the lung).

Table II. Effect of Administration Route of PH on LTD_4-induced Bronchoconstriction or Microvascular Leakage in Guinea Pigs

	ED_{50} (μg/kg)				
		Microvascular leakage			
Route	Bronchoconstriction	Trachea	Main bronchi	Intrapulmonary airways	Mean
IV	16.4	11.9	15.6	21.0	14.4
PO	2372	1823	1871	1677	1784
IF	25.2	10.6	18.8	11.8	12.9
SM-IF	12.6	5.8	14.0	10.1	9.2

The plasma PH levels after SM-DP were reduced to 1/12.4–1/14 compared with those following IV or PO administration as far as inhibiting bronchoconstriction or airway microvascular leakage was concerned. This finding also indicates the usefulness of SM-DP in reducing any systemic side-effects by reducing the concentration in the circulating blood.

CONCLUSIONS

The present dry powder inhalation testing system generated ideally dispersed aerosols of drug powders, which were intratracheally administered to the lung of guinea pigs by respirator. The inhaled powders were uniformly deposited into the right and left lobes. The safety and usefulness of powder inhalation was proved by the reduction in the AUC and Cmax of plasma PH levels compared with IV and PO administration. The pulmonary administration of surface-modified PH powders using HPMCP-NS effectively inhibited LTD_4-induced bronchoconstriction and airway microvascular leakage in a dose-dependent manner in the same way as intravenous administration of PH. This dry powder inhalation system reduced significantly the ED_{50} making it comparable with or greater than that after IV administration. The plasma concentration of surface-modified PH administered pulmonarily to give 50% inhibition of bronchoconstriction and airway microvascular leakage (ED_{50}) was reduced significantly, to 1/12 or less than that following IV and PO administration, reducing any systemic side-effects due to the lower blood concentrations of drug. Consequently, the present SM-DP system is strongly recommended as an ideal system for the treatment of bronchial asthma.

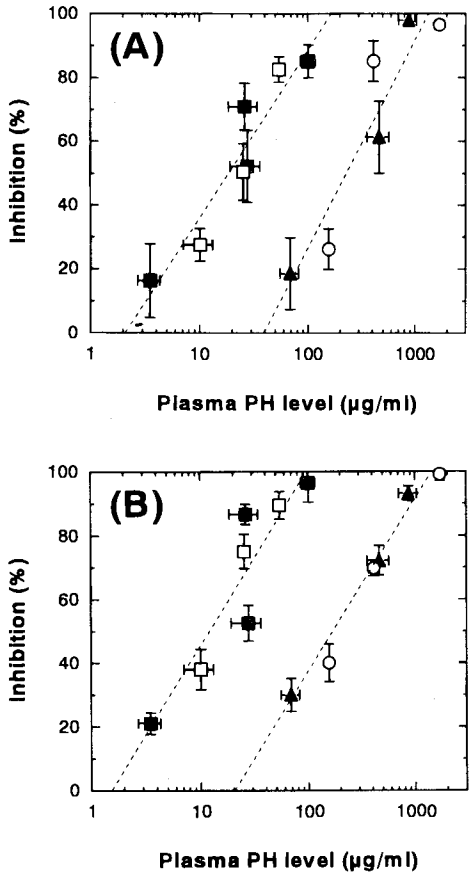

Fig. 6. Relationship between plasma PH level and the % inhibition of LTD_4-induced bronchoconstriction (A) and microvascular leakage (B) in guinea pigs. Symbols as in Fig. 4. Each value represents the mean ±S.E. of 5 animals. Plasma PH levels at 50% inhibition of bronchoconstriction were 238.9 (IV), 220.7 (PO), 21.5 (DP) and 16.7 (SM-DP). Plasma PH levels at 50% inhibition of airway microvascular leakage were 214.5 (IV), 162.3 (PO), 13.6 (DP) and 11.9 (SM-DP).

ACKNOWLEDGMENTS

The authors wish to thank Ono Pharmaceutical for providing technical support for the in vivo evaluation. The useful technical advice of Prof. H. Nagai and Dr. H. Tanaka, and the technical assistance of Mr. K. Mimura are gratefully acknowledged. Part of the present research was financially supported by a Grant in Aid for Scientific Research (B)(2) No. 08457595 from the Ministry of Education, Science, and Culture, Japan.

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