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Applicability of DPI formulations for novel neurokinin receptor antagonist

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Abstract

A novel triple neurokinin receptor antagonist (TNRA) could have pharmaceutical efficacy for asthma and/or chronic obstructive pulmonary disease. TNRA is potentially developed as inhalation medicine. The aim of this investigation was to evaluate the applicability of dry powder inhaler (DPI) formulation for TNRA. DPI formulation containing lactose was used for this feasibility study. Mechanofusion process for surface modification was applied on lactose particles to prepare four different DPI formulations. The mixture of TNRA and lactose was administered to rats intratracheally using an insufflator. The deposition pattern and blood concentration profile of TNRA were evaluated. Although there was no significant difference in deposition on deep lungs between the four formulations, DPI formulations containing mechanofusion-processed lactose showed longer T_{max} and $t_{1/2}$ and higher $AUC_{0-\infty}$ and MRT compared to that containing intact lactose. On the other hand, the contact angle measurement showed that the mechanofusion process decreased the polar part of the surface energy of the lactose. Therefore, the prolongation of the wetting of the formulated powder mixture seemed to delay the dissolution of TNRA deposited in respiratory tract. It was concluded that DPI formulation containing mechanofusion-processed lactose could be suitable for inhalation of TNRA.

Keywords: Triple neurokinin receptor antagonist; Dry powder inhaler; Mechanofusion; Intratracheal administration; Wettability

1. Introduction

In human airways, the tachykinins substance P and neurokinin A (NKA) are the predominant neuropeptides that are released from sensory nerve endings by mechanical, thermal, chemical or inflammatory stimuli (Nieber et al., 1992; Heaney et al., 1998). They interact with neurokinin receptors and increase mucus secretion and increase permeability of the blood vessels resulting in plasma leakage and vasodilatation. Binding of the receptors in the smooth muscles causes bronchoconstriction (Chapman et al., 1998). Therefore, neurokinin receptor antagonists could be used for the treatment of asthma and chronic obstructive pulmonary disease (COPD). The target sites of neurokinin receptor antagonists are trachea and bronchi for asthma and bronchiole and deep lungs for COPD. Three types of neurokinin receptors are known and blocking all three receptors is supposed to be useful for the treatment of asthma (Advenier et al., 1992; Myers and Undem, 1993; Naline et al., 1996; Daoui et al., 1998; Amadesi et al., 2001; Canning et al., 2002; Myers et al., 2005). Triple neurokinin receptor antagonist (TNRA) is a potent triple neurokinin receptor antagonist synthesized in Daiichi Sankyo Co., Ltd. (Nishi et al., 2000) and significant protection against NKA-induced bronchoconstriction was observed in mild asthmatics when TNRA solution was orally administered (Schelfhout et al., 2006). On the other hand, because the target site of TNRA is located in the lungs, there is a potential need for the development of inhalation formulation to realize an alternative route of administration. Based on such background, we decided to evaluate the applicability of dry powder inhaler (DPI) formulation for TNRA using lactose particles with or without surface modification by mechanofusion process.

There are three types of inhalation formulation, i.e., dry powder inhaler (DPI), metered dose inhaler (MDI) and nebulizer. Among them, DPI is becoming the major one because it is free from anti-environmental propellants and easy to use. In addi-

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tion, the inhalation of DPI formulations has been recognized as valuable for both the local administration to respiratory tract (Kawashima et al., 1998; O'Hara and Hickey, 2000; Sharma et al., 2001; Smith et al., 2001) and systemic administration (Tronde et al., 2003; Bosquillon et al., 2004; Yamamoto et al., 2004). Various studies on the absorption through the lungs of both small and macro molecules have been conducted (Folkesson et al., 1990; Hastings et al., 1992; Dershwitz et al., 2000; Lombry et al., 2002). One of the most conventional DPI formulations is the mixture of micronized drug particles and larger lactose particles. Therefore, we chose the DPI formulation containing lactose to evaluate the applicability of the DPI formulations for TNRA.

Mechanofusion is a powder-processing technology through which lactose particles receive shearing stress. Mechanofusion process on lactose surface was reported to change the surface properties of powder particles (Nagai et al., 2006) and alter the inhalation profile of DPI formulations containing lactose and fine drug particles (Kumon et al., 2006).

2. Materials and methods

2.1. Materials

α-Lactose monohydrate, Pharmatose 325M (particle diameter: 60 μm ca., DMV, The Netherlands) as an excipient for DPI was used as received or after mechanofusion-processed. Magnesium stearate (Mg-St) was purchased from Taihei Chemical Industrial Co., Ltd. (Japan) and sucrose stearate S370F was purchased from Mitsubishi-Kagaku Foods Corporation (Japan).

1-{2-[(2*R*)2-(3,4-Dichlorophenyl)4-(3,4,5-trimethoxybenzoyl)morpholin2-yl]ethyl}spiro [benzo[*c*]thiophene-1(3H),4′-piperidine]-(2*S*)-oxide hydrochloride (triple neurokinin receptor antagonist: TNRA) was used as a drug substance. The chemical structure of TNRA is shown in Fig. 1. TNRA is the monohydrochloride of R-112075. R-112075-D_9 was R-112075 labeled with deuterium and used as the internal standard for LC/MS/MS measurement. Both TNRA and R-112075-D_9 were synthesized by Daiichi Sankyo Co., Ltd. (Japan). 1-Octanol, methanol, ethanol, dichloromethane and hexane were of extra pure grade and purchased from Wako Pure Chemical Industries, Ltd. (Japan).

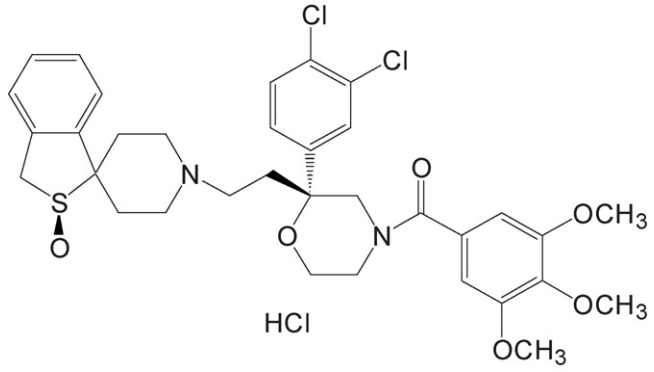

Fig. 1. Chemical structure of TNRA.

2.2. Mechanofusion-processed lactose

Surface modification was applied on Pharmatose 325M by mechanofusion using a rotor-type powder mixer, a Mechanofusion® AMS (Hosokawa Micron Corporation, Japan). The details are described elsewhere (Kumon et al., 2006). The particle size was unchanged by the process. Sucrose stearate and Mg-St were optionally used as coating materials. The mixing ratios of the additives were as follows: sucrose stearate/lactose = 1/99 (w/w) and Mg-St/lactose = 3/97 (w/w).

2.3. Measurement of particle size of lactose

The particle size of lactose was measured with a laser diffraction particle size distribution analyzer (Helos & Rodos, Sympatec GmbH, Germany).

2.4. Preparation of powder formulation

A TNRA powder was milled to a diameter of 2–3 μm with a Jet Mill Co-Jet system (Seishin Enterprise Co., Ltd., Japan). Milled TNRA and lactose were gently blended with a mortar and pestle at a ratio of 2:98 (w/w). For the Andersen Cascade Impactor test, 25 mg of each blend was loaded into size 2 HPMC capsules (Qualicaps Co., Ltd., Japan).

2.5. Andersen cascade impactor test

2.5.1. Cascade impactor test

The inhalation properties of the DPI formulations were evaluated using an Andersen Cascade Impactor (ACI, Copley, UK) with an inhalation device, a Jethaler (dual chamber type, Hitachi Unisia Automotive, Ltd., Japan). The method was the same as previously applied to another pharmaceutical compound (Kumon et al., 2006). In brief, the formulated powder was filled in a capsule and inhaled with the Jethaler at the flow rate of 30 L/min. The flow rate was determined as described in USP. The amount of TNRA deposited on each part of the ACI was quantified by HPLC analysis.

The fine particle fraction (FPF) is the percentage of powder collected from Stage 2 to Stage 7 and a filter at 30 L/min. FPF is given by Eq. (1):

$$\mathrm{FPF}\,(\%) = \frac{\text{TNRA collected from Stage 2 to Stage 7 and filter}}{\text{Entire dose}} \times 100 \quad (1)$$

2.5.2. Drug analysis

TNRA was analyzed by HPLC employing a mixture of acetonitrile and 0.01 mol/L sodium phosphate buffer (pH 7.0) (43:57%, v/v) as the mobile phase running at a flow rate of 1 mL/min ca. and UV detection at 254 nm. The HPLC system consisted of a pump (LC-10AD, Shimadzu Corporation, Japan), a UV detector (SPD-10A, Shimadzu Corporation, Japan) and an L-column ODS (15 cm × 4.6 mm i.d., particle size 5 μm, Chemicals Evaluation and Research Institute, Japan), which was maintained at 40 °C.

2.6. In vivo intratracheal administration study

The *in vivo* studies were conducted in compliance with the internal company policies and guidelines of Sankyo Co., Ltd.

2.6.1. Apparatus for intratracheal administration

A PennCentury Insufflator (Model DP-4; PennCentury Inc., USA) was used for the intratracheal (i.t.) administration.

2.6.2. Animals

Seven-week-old male CD (SD)/IGS rats were obtained from Charles River Laboratories Japan, Inc. (Japan). The rats were initially anesthetized with 50 mg/kg of Nembutal (Dainippon Sumitomo Pharma Co., Ltd., Japan) and remained anesthetized throughout the experiments.

2.6.3. Intratracheal administration of TNRA dry powder inhaler in rats

Approximately 2 mg of each powder mixture of TNRA and lactose (intact or mechanofusion-processed) was loaded into the insufflator. The trachea was incised between the fifth and sixth tracheal rings and the needle of the insufflator device was inserted to a depth of ca. 0.3 cm through a trachea incision in the rats (Fig. 2). The estimated amount of inhaled TNRA was 0.2 mg/kg of the body weight of the rats. Administration of the powder was performed by insufflation of 1 ml of the air contained in the syringe connected to the device.

2.6.4. Blood sampling and sample preparation

Blood samples were withdrawn from the jugular veins with a heparin rinsed syringe at 2, 5, 10, 15, 30, 60 and 120 min after the i.t. administration. The plasma was separated from the blood samples by centrifugation and stored at −80 °C until analysis.

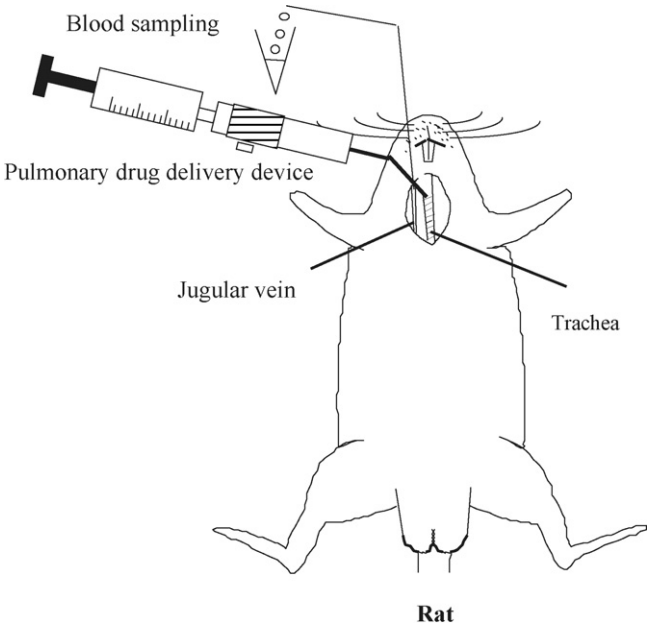

Fig. 2. Schematic diagram of intratracheal administration using pulmonary drug delivery device, dry powder insufflator DP-4.

The frozen plasma sample was thawed and diluted with 4% (w/v) BSA to inhibit the sample adsorption to the vessel wall. A 4% (w/v) BSA solution was also used as a control plasma sample. The diluted plasma was vortex-mixed with 0.03% of HCl and internal standard solution (R-112075-D_9 100 ng/ml in plasma). The mixture was loaded into an Oasis HLB cartridge (Waters Corporation) and eluted with methanol. The eluted solution was dried, redissolved with methanol, vortex-mixed with water and filtered with a 0.22 μm filter. An aliquot of the filtered sample was directly injected onto the LC/MS/MS system for analysis as described below (2.6.6). The concentration of TNRA was calculated with the concentration of R-112075, a free base form of TNRA, measured by LC/MS/MS. The data from these samples were used to construct the pharmacokinetic curves of the TNRA concentration in the blood versus the time. The same sample handling process was applied for the determination of precision and accuracy. The back-calculated calibration standard concentrations of the standard were within ±15% of their theoretical concentrations. The precision and accuracy of the quality control samples were within ±15%.

2.6.5. Sample preparation for the evaluation of pulmonary deposition pattern

The trachea, main bronchi, and lungs were excised immediately (5 min) or 2 h after the i.t. administration of the mixture of TNRA and lactose. The excised organs were divided into three samples: (1) trachea and main bronchi, (2) peripheral part of the lungs and (3) central part of the lungs. Each sample was stored at −80 °C until analysis. For the preparation of the analytical samples, the frozen organ samples above were weighed and homogenized with water with an ultrasonic homogenizer. The homogenate was vortex-mixed with water, internal standard solution (R-112075-D_9 100 ng/mL in plasma) and diethyl ether. The mixture was centrifuged at 4 °C and the supernatant was dried at 40 °C under a nitrogen stream. The residue was redissolved with methanol and mixed with water. Then the mixture was filtered with a 0.22 μm filter and directly injected onto the LC/MS/MS system for analysis.

2.6.6. LC/MS/MS analysis for blood samples

An Agilent 1100 system (Agilent Technologies, Inc., USA) consisting of a vacuum degasser, single pump and an auto sampler was used for the solvent and sample delivery. An Applied BioSystems MDS Sciex (Canada) API 4000 system with a TurboIonSpray ionization (ESI) source was used for mass analysis and detection. Chromatographic separation was achieved under isocratic conditions on an XTerra MS C18 column (15 cm × 2.1 mm i.d., particle size 5 μm, Waters, USA) with a guard column at 40 °C.

The mobile phase consisted of methanol/0.02 mol/L ammonium acetate/formic acid (50:50:0.1, v/v/v) at a flow rate of 0.20 mL/min. The mass spectrometer was operated in the positive ion mode with the probe at 500 °C. Multi Reaction Monitoring (MRM) mode was chosen to perform the MS/MS detection. Quantitation was conducted by monitoring of the transitions m/z 673.2 → m/z 195.0 for R-112075 (TNRA) and

m/z 682.4 → m/z 204.1 for the internal standard (R-112075-D_9), respectively.

2.6.7. LC/MS/MS analysis for lung homogenate samples

The same LC/MS/MS equipment was used as that used for the blood sample analysis described in 2.5.6. Chromatographic separation was achieved under gradient conditions on an XTerra RP18 (5 cm × 2.1 mm i.d., particle size 3.5 μm, Waters, USA) at 40 °C. The time program of chromatograph was a linear gradient from 0–25% solvent B in 0–5 min and 0% solvent B in 5–8 min. The mobile phase consisted of solvents A and B, as follows: solvent A: water–methanol–formic acid–ammonium acetate (550:450:1:0.77, v/v/v/w) and solvent B: methanol–formic acid (1000:1, v/v). The total flow rate was 0.20 mL/min. The same MS/MS detection was conducted as described in 2.5.6.

2.6.8. Pharmacokinetic studies

The pharmacokinetic parameters were calculated by non-compartmental analysis using WinNonlin™, version 4.0.1 (Pharsight Corporation, Palo Alto, USA). The observed peak plasma concentrations (C_{max}) and the times to reach them (T_{max}) were derived directly from the plasma concentration data for each animal. The area under the plasma concentration–time curve (AUC) was calculated using the linear/logarithmic trapezoidal rule. The area from the last observed data point to infinite time was obtained by extrapolation. The elimination rate constant (k) was estimated by linear regression of the last three to six time points of the log concentration versus the time curve. The plasma elimination half-life ($t_{1/2}$) was calculated as $\ln 2/k$. The mean residence times (MRT) were calculated as the ratio between the areas under the first-moment versus the time curve (AUMC) and AUC. All results shown in the tables are expressed as the mean ± standard deviation. A Student's t-test or Welch's t-test was performed to demonstrate statistical differences.

2.7. Measurements of dispersive and polar part of surface tension

Sorption measurements using the Washburn method were applied to determine the surface energy of the lactose samples (Kiesvaara and Yliruusi, 1993). The contact angles of the lactose were measured with a Krüss Tensiometer K100 (Krüss GmbH, Germany).

Compacts of the powder (300 mg) were prepared in a highly polished aluminum vessel (2.5 mm × 10 mm) with a filter base using centrifugation by a Himac CR 21G (Hitachi High-Technologies Corp., Japan). The vessel was suspended from a balance and one of the test liquid (1-octanol, methanol, ethanol, dichloromethane and hexane) was placed in a clean glass dish. The temperature of the liquids was controlled at 20 ± 0.5 °C, by flowing water from a circulator. The glass dish was raised at the speed of 6 mm/min by means of a motorized platform to contact the powder plate. After the vessel had contacted the liquid, the speed at which the liquid rose through the bulk powder was measured by recording the increase in weight as a function of time. Three compacts of the same powder were subjected to measurements with every liquid.

Bulk powder through which a liquid flows can be regarded as being similar to a bundle of capillaries. With the assumption that the bulk densities of the lactose samples were uniform, the capillary radius for the bulk powder was replaced by a constant determined with hexane. For measurements with other liquids, this constant can be inserted into the Washburn equation, so that the advancing contact angle could be determined for other liquids. From the contact angle data, the surface energy of a solid can be calculated using Krüss tensiometer software (Laboratory Desktop, Version 2.5, Krüss GmbH, Germany).

Table 1
The particle size of lactose before and after mechanofusion-processing (μm)

	Intact	Mechanofusion processed lactose		
		Without additive	With sucrose stearate	With Mg-St
$D_{10\%}$	32 ± 3	34 ± 0	36 ± 1	29 ± 0
$D_{50\%}$[a]	63 ± 2	48 ± 1	61 ± 2	58 ± 0
$D_{90\%}$	95 ± 5	85 ± 0	90 ± 0	85 ± 2

Data represented as the mean ± S.D. (n = 3).
[a] Values of $D_{50\%}$ were cited from our previous report (Kumon et al., 2006).

3. Results

3.1. In vitro inhalation profile of TNRA DPI formulations

The particle size of four types of lactose used for the DPI formulations is shown in Table 1 and no significant change of particle size was observed through mechanofusion process. On the other hand, ACI study showed that all three types of mechanofusion-processed lactose (Formulation 2–4) resulted in different inhalation profiles compared with intact lactose (Formulation 1) (Fig. 3). The deposition profile change by the mechanofusion process was quite similar to that observed in the same ACI study for the other pharmaceutical compound (Kumon

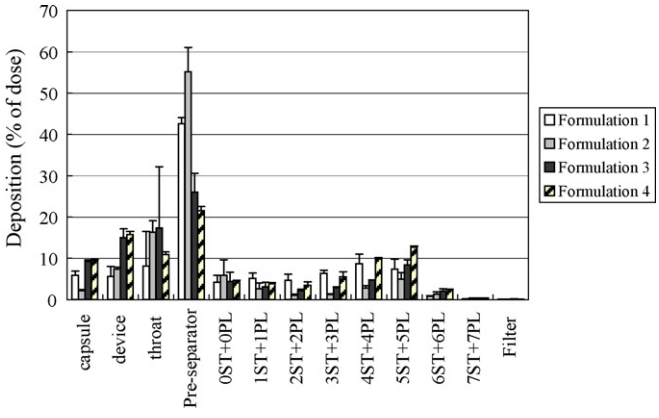

Fig. 3. *In vitro* inhalation profile of TNRA mixed with intact or mechanofusion-processed lactose. The surface modification applied on lactose for each formulation was as follows: intact (Formulation 1), mechanofusion-processed without additive (Formulation 2), mechanofusion-processed with sucrose stearate (Formulation 3), and mechanofusion-processed with Mg-St (Formulation 4).

et al., 2006). Namely, the DPI formulation containing lactose mechanofusion-processed without additive (Formulation 2) showed higher deposition on the pre-separator and lower deposition on the lower stages of the ACI. As well, the DPI formulation showed less deposition on the pre-separator when it contained lactose mechanofusion-processed with sucrose stearate or Mg-St (Formulation 3 or 4). The deposition on the lower stages of the ACI increased when the lactose was mechanofusion-processed with Mg-St. In both cases of TNRA and Compound A in previous study (Kumon et al., 2006), lactose processed with Mg-St resulted in higher deposition on the lower stages of the ACI than the other lactose. Correlated with these tendencies, FPF, a measure of the extent of deposition into the deep lungs, were calculated as follows. Intact lactose, 28.1%; lactose mechanofusion-processed without additive, 11.7%; lactose mechanofusion-processed with sucrose stearate, 20.9%; lactose mechanofusion-processed with Mg-St, 34.6%.

3.2. In vivo intratracheal administration study with rats

3.2.1. Time-courses of plasma levels of TNRA after administration of TNRA DPI

Fig. 4 illustrates the concentration versus time profiles of TNRA with a single i.t. dose administered to individual rats for each group. An hour after the powder insufflations, formulations composed of mechanofusion-processed lactose showed higher concentrations than the formulations composed of intact lactose (significance, ANOVA $P<0.05$). The pharmacokinetic parameters were calculated and are summarized in Table 2. T_{max}, $t_{1/2}$, $AUC_{0-\infty}$ and MRT increased when the lactose were mechanofusion-processed (significance, ANOVA $P<0.05$). Fig. 4 also shows that the drug concentration peak was the steepest with the formulation containing intact lactose.

3.2.2. Pulmonary deposition patterns of TNRA after intratracheal dosing

The pulmonary deposition patterns of TNRA are shown in Fig. 5. After 5 min, TNRA was deposited mainly in the trachea and main bronchi for all four formulations (Fig. 5a). There were no significant differences in the lung deposition pattern between all the formulations. The total deposition amount of TNRA in the trachea and lungs was approximately 50% of dose (Fig. 5b). After 2 h, amount of TNRA remained on the tracheal and pulmonary mucosa was only less than 1% of dose and there was no difference among all the formulations (Fig. 5c).

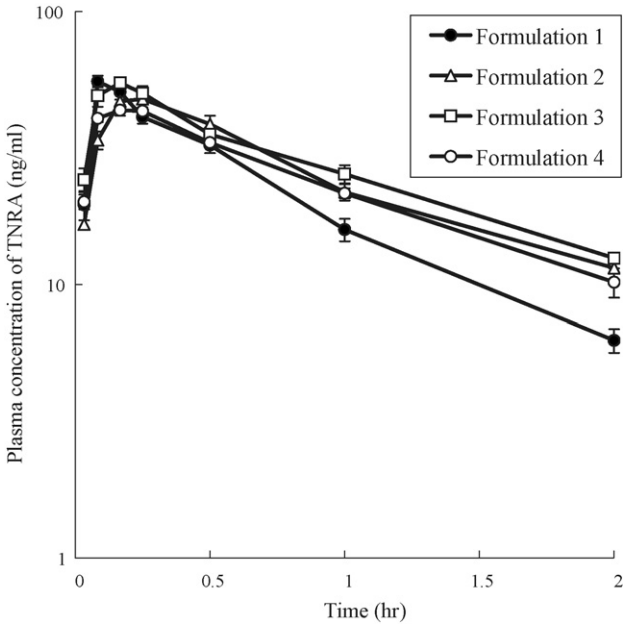

Fig. 4. Plasma concentration profile of TNRA. The surface modification applied on lactose for each formulation was as follows: intact (Formulation 1, ●), mechanofusion-processed without additive (Formulation 2, △), mechanofusion-processed with sucrose stearate (Formulation 3, □) and mechanofusion-processed with Mg-St (Formulation 4, ◯).

3.3. Surface energy of mechanofusion-processed lactose

The surface energy of the lactose was measured by the contact angle method and the results are shown in Fig. 6. A decrease of the polar part of the surface tension was observed when mechanofusion was applied. This suggested that the mechanofusion process decreased the wettability of the lactose. The correlation coefficients for the fitting of each experiment were over 0.98 in the linear plot of the Fowkes and Owens, Wendt, Rabel and Kaelble method.

Table 2
Nonparametric pharmacokinetic parameters of TNRA after intratracheal administration of DPI formulation to rats

Formulation number	Formulation 1	Mechanofusion processed lactose		
		Formulation 2 (without additive)	Formulation 3 (with sucrose stearate)	Formulation 4 (with Mg-St)
Dose (μg/kg)	187 ± 12	196 ± 6	194 ± 3	192 ± 4
$t_{1/2}$ (h)	0.629 ± 0.036	0.899 ± 0.181*	0.988 ± 0.121**	0.875 ± 0.096**
T_{max} (h)	0.083 ± 0.000	0.229 ± 0.042**	0.167 ± 0.068**	0.167 ± 0.083**
C_{max} (ng/mL)	55.5 ± 5.4	51.0 ± 6.7	56.4 ± 3.1	45.0 ± 4.0*
AUC_{0-2h} (ng h/mL)	42.8 ± 5.7	51.5 ± 5.7	55.8 ± 5.9*	48.4 ± 3.5
AUC_{0-h} (ng h/mL)	48.5 ± 7.2	66.8 ± 9.7*	73.6 ± 5.0**	69.9 ± 8.6*
MRT (h)	0.90 ± 0.05	1.33 ± 0.26*	1.40 ± 0.15**	1.34 ± 0.05**

Data represented as the mean ± S.D. (Formulation 1, 4: $n=3$, Formulation 2, 3: $n=4$).
* $P<0.05$.
** $P<0.01$.

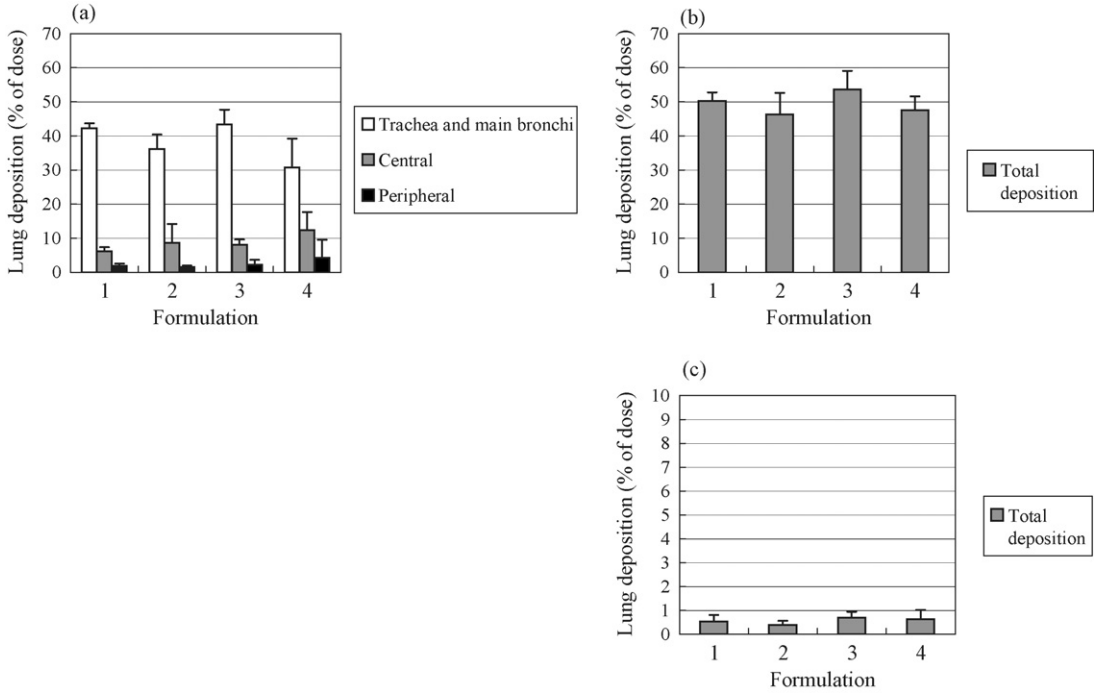

Fig. 5. Distribution of TNRA intratracheally administered to rats: (a) deposition in each part of the lungs after 5 min; (b) total lung deposition after 5 min; (c) total lung deposition after 2 h. The surface modification applied on lactose for each formulation was as follows: intact (Formulation 1), mechanofusion-processed without additive (Formulation 2), mechanofusion-processed with sucrose stearate (Formulation 3), and mechanofusion-processed with Mg-St (Formulation 4).

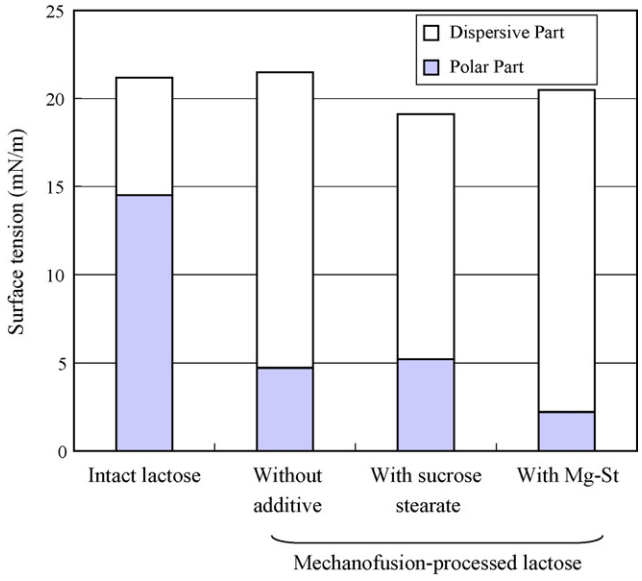

Fig. 6. Surface tension of lactose obtained by contact angle measurement.

4. Discussion

By using the mechanofusion-process with or without additive, TNRA DPI formulations with different inhalation properties were prepared (Fig. 3). The higher deposition on pre-separator suggested the less extent of separation of TNRA and lactose particles of Formulation 2. In contrast, the highest FPF was obtained by DPI formulation containing lactose mechanofusion-processed with Mg-St. The physical mixture of lactose and sucrose stearate or Mg-St prepared with a mortar and pestle did not show high FPF value. Therefore, it was suggested that the mechanofusion process was essential to improve FPF.

The pharmacokinetics of TNRA was altered when the lactose was mechanofusion-processed (Fig. 4 and Table 2). Namely, T_{max}, $t_{1/2}$, $AUC_{0-\infty}$ and MRT were increased by the mechanofusion process on the lactose. The TNRA elimination profile should be the same regardless of the formulations once the drug molecules were absorbed and reached the blood stream. Therefore, the increased $t_{1/2}$ and MRT may be related to the so-called flip-flop phenomenon. The absorption rate of dissolved TNRA is expected to be very fast based on the following information (data not shown). When TNRA solution was administered to Wister-Imamichi and F334/DuCrj rats via i.t. and i.v. routes, the similar time course profiles of blood concentration were observed (Yabe and Tanaka, 2003). Furthermore, when TNRA was administered as powder, the sustained absorption was observed suggesting that dissolution of TNRA limited the rate of absorption. Therefore, it seemed that dissolution rates of TNRA were different between the DPI formulations with intact lactose and mechanofusion-processed lactose.

Neurokinin receptor antagonists are expected to express its pharmaceutical efficacy for asthma at the area from trachea to bronchi and for COPD at bronchiole and deep lungs. The absorption of TNRA was considered to occur through such target area after the i.t. administration. Therefore, we consider that $AUC_{0-\infty}$ reflects the amount of TNRA reached to the target area, although some drug molecules are possibly exert pharmaceutical action at the target area and decomposed before reaching the blood stream. Based on this consideration, DPI formulations containing mechanofusion-processed lactose can be regarded as preferable for TNRA due to their higher $AUC_{0-\infty}$.

As we did not evaluate the pharmacokinetics of TNRA solution in this study, exact absolute bioavailability could not be determined. However, it is expected to be higher than 36% for Formulation 1, and higher than 50% for Formulation 2–4 by reference to pharmacokinetic parameters of TNRA solution in another strain of rats namely, $AUC_{0-\infty}$ upon i.v. administration of TNRA was 136 ng h/mL at the dose of 0.2 mg/kg in F334/DuCrj rats. By using this value, bioavailability of DPI formulation was calculated to be 36% for Formulation 1 and 50% ca. for Formulation 2–4. In addition, $t_{1/2}$ was suggested to be shorter in CD(SD)/IGS than in F334/DuCrj because $t_{1/2}$ of i.t. administration of powder formulation (Formulation 1) to CD(SD)/IGS rats was 0.63 ± 0.04 h and that of i.v. administration to F334/Crj rats was 0.94 ± 0.44 h. Therefore, it is reasonable to expect that real bioavailability is more than these values. To perform exact pharmacokinetic analysis, TNRA solution (i.v. and i.t.), DPI formulation of TNRA (i.t.) should be evaluated in the same system.

Although the pharmacokinetic parameters were different between the DPI formulations containing intact lactose and mechanofusion-processed lactose, the deposition patterns of TNRA *in vivo* were similar to each other at the both excision time points of 5 min and 2 h after the administration (Fig. 5). The total deposition amount of TNRA in the trachea and lungs was about 50% of dose at 5 min (Fig. 5b) and less than 1% of dose at 2 h (Fig. 5c), suggesting rapid absorption of TNRA via mucosal membrane once dissolved as mentioned above. Because TNRA is metabolized at liver and excreted via bile duct into intestine (in-house data), it is difficult to consider that TNRA was decomposed so rapidly to be responsible for the clearance in the lung. As T_{max} values were 0.083–0.229 h (5–14 min), certain amount of drugs deposited in deep lungs would be dissolved and absorbed before detection at 5 min. Therefore, TNRA deposition on trachea and main bronchi (Fig. 5a) suggested that the TNRA in deep lungs was absorbed quickly and only the TNRA deposited on upper part of respiratory tract was observed. Then, the difference of the deposition properties in the ACI studies was not reflected in the *in vivo* deposition in the lungs. For the advance study, it would be better to evaluate the administration method by using drugs of slow absorption and/or *in vivo* deposition at shorter time point. Considering the range of FPF values of 11.7–34.6%, the rest of 65.4–88.3% of TNRA deposited trachea and bronchi and the difference of FPF could be unclear in lung deposition. As the majority of the TNRA was deposited on trachea and bronchi with lactose, the PK profile seemed to be strongly affected by the absorption from those parts.

In this study, it cannot be excluded that *in vivo* administration with an insufflator puffed all the powder in a burst and the mixture of TNRA and lactose was trapped at the forks of trachea. Even the various insufflators are widely used for the *in vivo* DPI evaluations (Todo et al., 2001; Bosquillon et al., 2004; Codrons et al., 2004; Yamamoto et al., 2004), comparison of particle size distribution between two devices is necessary.

Contact angle measurement suggested that the mechanofusion process decreased the polarity of lactose particle surface and made them less wettable (Fig. 6). As well, the surface acidity parameter KD/KA measured by IGC decreased when lactose was mechanofusion-processed (Kumon et al., 2006). The acidity change of the IGC parameter implied that the surface property as an electron-donor or electron-acceptor was changed by the mechanofusion process. Accordingly, the electron locating condition of the surface was considered to be changed by mechanofusion process.

Based on the observations above, we consider that the decrease of wettability of lactose caused by the mechanofusion process altered the TNRA dissolution profile. As the PK profile seemed to be strongly affected by TNRA deposited on trachea and bronchi with lactose, the wettability of the powder mixture would change the blood concentration profile. TNRA particles in a powder mixture was considered to be absorbed in two ways, i.e., as TNRA on the lactose particles, or as TNRA particles being directly deposited on or close to the lung mucosa. TNRA particles on lactose started to dissolve after the lactose particles had dissolved or after the lung mucus had intruded into the powder mixture and reached the TNRA particles. In this mechanism, the lactose wettability should largely affect the rate of TNRA absorption. The prolongation of wetting of the powder mixture leads to a delay of the dissolution of TNRA deposited on the lactose. That is, it caused long continuous absorption followed by $t_{1/2}$ elongation and increase of MRT. On the other hand, TNRA deposited directly on the mucosa dissolved quickly and absorption occurred in the same way between the four formulations, because the lactose should have no effect on the free TNRA absorption mechanism.

In addition, in this study, even mechanofusion without any additive demonstrated a potential to enhance the duration of the action of TNRA DPI formulations. This result suggested the possibility that the mechanofusion process could control the release and absorption of drugs administered intratracheally without a change of excipients in the DPI formulation in the case that the drug is quickly absorbed from wide area of respiratory tract mucosa. This information could be valuable, since the use of excipients for inhalation medicine is limited because of the potential stimulation to the trachea and lungs (American Academy of Pediatrics, 1997; Hussain et al., 2004).

5. Conclusion

It was suggested that TNRA showed high bioavailability when it was administered intratracheally as DPI formulation with mechanofusion-processed lactose. All the DPI formulations studied delivered high percentage of TNRA to trachea and main bronchi which were its target area for asthma. Therefore, DPI containing lactose was shown to be preferable for inhalation formulation of TNRA. The observation in this study suggested that the mechanofusion process would be an effective method to extend the duration of the action of dry powder inhalation drugs which are quickly absorbed from wide area of respiratory tract mucosa.

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