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Research Article

Improved Bioavailability of a Water-Insoluble Drug by Inhalation of Drug-Containing Maltosyl-β-Cyclodextrin Microspheres Using a Four-Fluid Nozzle Spray Drier

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Abstract. We previously developed a unique four-fluid nozzle spray drier that can produce water-soluble microspheres containing water-insoluble drug nanoparticles in one step without any common solvent between the water-insoluble drug and water-soluble carrier. In the present study, we focused on maltosyl-β-cyclodextrin (malt-β-CD) as a new water-soluble carrier and it was investigated whether drug/malt-β-CD microspheres could improve the bioavailability compared with our previously reported drug/mannitol (MAN) microspheres. The physicochemical properties of bare drug microparticles (ONO-2921, a model water-insoluble drug), drug/MAN microspheres, and drug/malt-β-CD microspheres were evaluated. *In vitro* aerosol performance, *in vitro* dissolution rate, and the blood concentration profiles after intratracheal administration were compared between these formulations. The mean diameter of both drug/MAN and drug/malt-β-CD microspheres was approximately 3–5 μm and both exhibited high aerosol performance (>20% in stages 2–7), but drug/malt-β-CD microspheres had superior release properties. Drug/malt-β-CD microspheres dissolved in an aqueous phase within 2 min, while drug/MAN microspheres failed to dissolve in 30 min. Inhalation of drug/malt-β-CD microspheres enhanced the area under the curve of the blood concentration curve by 15.9-fold than that of bare drug microparticles and by 6.1-fold than that of drug/MAN microspheres. Absolute bioavailability (pulmonary/intravenous route) of drug/malt-β-CD microspheres was also much higher (42%) than that of drug/MAN microspheres (6.9%). These results indicate that drug/malt-β-CD microspheres prepared by our four-fluid nozzle spray drier can improve drug solubility and pulmonary delivery.

KEY WORDS: 4-fluid nozzle spray drier; inhalation therapy; maltosyl-β-cyclodextrin; microparticles; water-insoluble drug.

INTRODUCTION

Many promising drug candidates are poorly water soluble with inherently low mucosal permeability, greatly complicating the development of pharmaceutical formulations. There are many techniques for improving the water solubility, including the production of nano-scale drug particles. The use of nano-sized drug particles is one of the effective methods to improve the drug solubility: The increase of surface area of nanoparticles leads to the increase in dissolution velocity as described in Noyes–Whitney equation. The production of drug nanoparticles categorized into two methods: top-down method (milling method (1–6), high-pressure homogenizer (6,7), pulse laser (8) *etc.*) and bottom-up methods (simple precipitation, supercritical anti-solvent method (9) *etc.*). In any case, the handling of drug nanoparticles is sometimes difficult: nanoparticles easily aggregate each other and accordingly, the good property of nanoparticles such as enhancement of oral absorption is lost due to aggregation. While surfactant is required to stabilize drug nanoparticles and the combination of methods as described above is then attempted, one of the effective methods to preserve drug nanoparticles is to prepare microparticles containing drug nanoparticles, which is called as "drug nanocomposite". Although a few articles mention the nanocomposite (10,11), the process is complex and the scale-up problem is still remaining.

The four-fluid nozzle spray drier has a unique nozzle with two liquid and two gas passages, which allow drug and carrier to be dissolved in separate solvents, eliminating the need for a common solvent. This technique has been used for the production of nanocomposite by a one-step process. Although the detailed mechanism of the production of nanocomposite by using the four-fluid nozzle is still unclear, we proposed a tentative mechanism: After the drug solution (in organic

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solvent) and the aqueous solution are sprayed with high speed compressed together, the drug solution and aqueous solution collided at the end of the nozzle, and then, a powerful shock-wave is generated at that spot. The resulting droplets are mixed and the drug starts to crystallize at nano-scale through the "anti-solvent effect," which occurs by decreasing the solubility after the mixing of drug solvent and drug insoluble solvent (12,13). We previously described mannitol (MAN) microspheres containing nanosized polymeric or pharmaceutical drugs for oral administration or for inhalation as a dry powder (14–18).

In this study, we used maltosyl-β-cyclodextrin (malt-β-CD) instead of MAN as a water-soluble carrier for microparticles. These β-CDs are frequently used in pharmaceutical formulations to increase the solubility, stability, and bioavailability of various medical agents by forming non-covalent inclusion complexes (19). Although unmodified β-CDs are toxic at high doses and have limited aqueous solubility (20,21), modified CDs such as sulfobutyl ether derivatives of β-CD and malt-β-CD have lower cytotoxicity and neurotoxicity compared with β-CD (22–24).

The N-type Ca^{2+} channel blocker ONO-2921, originally developed for the treatment of neuropathic pain (25,26), was used as the model water-insoluble drug and encapsulated in microspheres suitable for inhalation. The native compound is practically insoluble in water and oral bioavailability is approximately 1% because of the first-past effects of cytochrome P450 3A4. Therefore, an alternative drug delivery system is necessary to improve ONO-2921 solubility and to maintain the activity by suppressing liver metabolism.

The lung is an attractive route for drug delivery because it has a large surface area (75–140 m^2) and a relative thinner air–blood barrier in the alveolar epithelium (<1 μm) (27,28). In addition, drugs delivered by inhalation can escape first-pass effects because the drug can enter the blood stream directly; therefore, pulmonary delivery is expected to improve drug absorption. In the present study, we investigated whether ONO-2921/malt-β-CD microspheres produced using a four-fluid nozzle spray drier could enhance the solubility and *in vivo* absorption of the drug following inhalation.

MATERIALS AND METHODS

Materials

The model drug ONO-2921 was kindly donated by Ono Pharmaceuticals Co. (Tokyo, Japan). malt-β-CD was purchased from Ensuiko Sugar Refining Co. (Tokyo, Japan), and MAN was purchased from Wako Pure Chemical Industries (Osaka, Japan). All reagents were of analytical grade.

Preparation of Drug-Containing Microspheres Using a Four-Fluid Nozzle Spray Drier

Spray-dried particles were prepared using a four-fluid nozzle spray drier (MDL-050; Fujisaki Electric, Tokushima, Japan) by a previously described method with minor modifications (17). In brief, a four-fluid nozzle has two chisel-shaped nozzles through which compressed air passes and two additional nozzles through which sample solutions pass (Fig. 1). Different liquids and gases can pass through individual nozzles. During operation, the pointed end of the nozzle functions as the flow site for the two liquid ports on either side. At the liquid flow site, the sample solutions are propelled by high-speed compressed air to the acceleration zones. Air streaming from the outer ports collides at the tip, and a powerful shock wave is generated at the collision focal spot. As a result, the solutions are atomized into droplets. The droplets are then dried by heated air and collected.

In order to produce ONO-2921/MAN or ONO-2921/malt-β-CD microspheres, the drug and polymer were first dissolved in separate solvents. ONO-2921 was dissolved in 2/1 acetone/methanol (*v*/*v*) at a final concentration of 1.67% *w*/*v*, while MAN (16.7% *w*/*v*) or malt-β-CD (5.01, 8.35, or 16.7% *w*/*v*) were dissolved in water. The final ratios were 1/10 (*w*/*w*) for ONO-2921/MAN and 1/3, 1/5, and 1/10 (*w*/*w*) for ONO-2921/malt-β-CD microspheres. The organic drug solution and the aqueous solutions of MAN or malt-β-CD were passed through different liquid passages of the four-fluid nozzle. Spray drying of the ONO-2921/MAN and ONO-2921/malt-β-CD microspheres was performed under the following conditions: inlet temperature, 60°C; outlet temperature, 35–44°C; supply rate for ONO-2921 and polymer solutions, 5 ml/min; spray air rate, 30 l/min; and spray air pressure, 8 kgf/cm^2. The yield percentage of each particle from the spray dryer was more than 90%.

Preparation of Bare Drug Microparticles Using a Two-Fluid Nozzle Spray Drier

Spray-dried drug particles were prepared using a model Pulvis mini-spray GB22 (Yamato Scientific Co., Tokyo, Japan) as described above (29). ONO-2921 (4% *w*/*v*) was first dissolved in methanol and then mixed with water for a final water/methanol ratio of 1/10 (*v*/*v*). Spray drying of ONO-2921 was performed under the following conditions: inlet temperature, 60°C; supply rate, 5 ml/min; and spray pressure, 3 kgf/cm^2. The bare ONO-2921 microparticles produced using a two-fluid nozzle spray drier are referred to as ONO-2921 spray-dried microparticles (ONO-2921 SD2). The yield percentage of each particle from the spray dryer was more than 90%.

Scanning Electron Microscopy

Particle structure was observed under an S-2250N scanning electron microscope (Hitachi, Tokyo, Japan) as previously described (14). In brief, the microparticles were coated with 25-nm-thick gold using a quick carbon coater (SC-701; Sanyu Electronics, Tokyo, Japan). The mean particle diameters (the horizontal Feret's diameter) were determined by image analysis of approximately 500–800 particles using an image analysis software (WinROOF; Mitani, Fukui, Japan). The mean particle diameter was defined as the median diameter of the cumulative curve of the number-basis particle size distribution.

High-Performance Liquid Chromatography

The ONO-2921 concentration in the collection solutions was determined by high-performance liquid

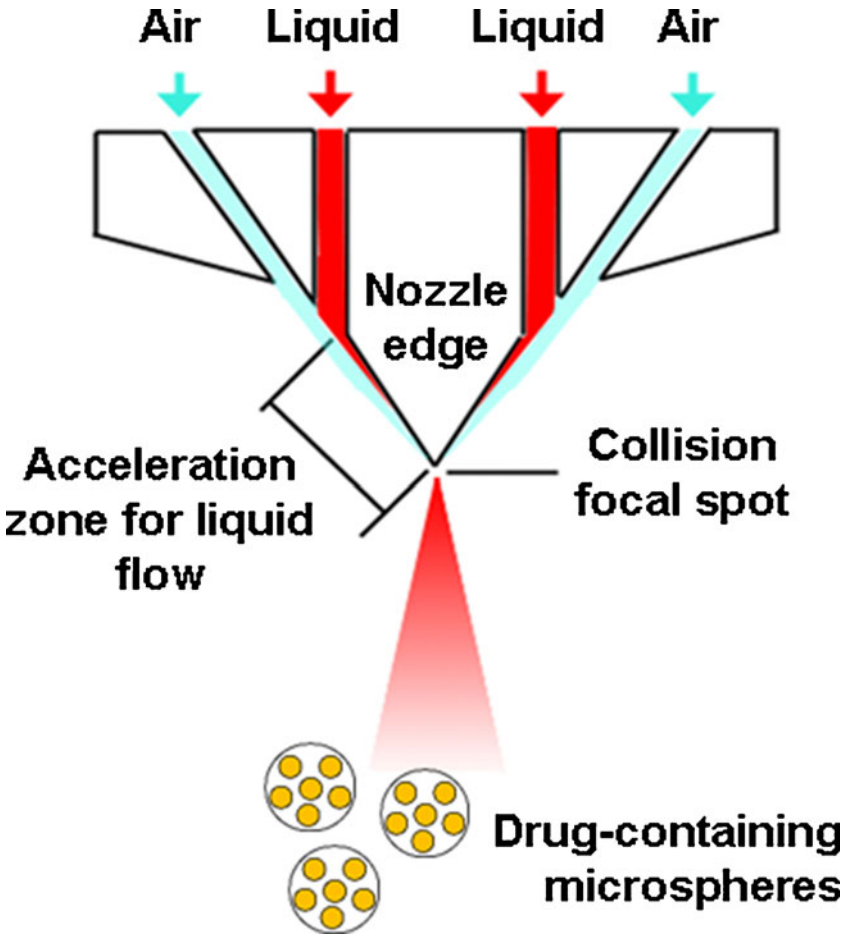

Fig. 1. Schematic illustration of a four-fluid nozzle used for one-step preparation of drug-containing microspheres

chromatography (HPLC) as previously described (16). In brief, the system consisted of a pump (Jasco-880-PU), a detector (Jasco-875), an integrator (Jasco-807-IT), and a YMC-Pack Pro C18 AS-302 column (4.6 mm×150 mm, S-5 μm; YMC Co., Ltd., Kyoto, Japan). The flow rate was 1.1 ml/min and the column temperature was maintained at 30°C. A 3:6:1 5 mM KH_2PO_4–5 mM K_2HPO_4/acetonitrile/methanol solution (*v*/*v*/*v*) was used as the mobile phase. The analysis was performed by UV detection at 210 nm.

In Vitro Aerosol Performance

In vitro aerosol performance of microspheres was evaluated using an AN-200 Andersen nonviable sampler (Tokyo Dyrec, Tokyo, Japan). In brief, cascade impactor is a standard equipment for evaluating inhalers as per the European Pharmacopoeia and United States Pharmacopoeia (30–32). A cascade impactor consists of a throat, a pre-separator, and eight stages (0–7). Each stage consists of a metal plate with small holes (nozzles) and a glass plate for collecting particles. A methanol/glycerin/water solution (8:1:1 *v*/*v*/*v*) is soaked into gauze and plated on the glass plates to retain the particles. A dual chamber-type inhalation device (Jethaler®; Hitachi, Tokyo, Japan) was used for inhalation. A hydroxypropyl methylcellulose capsule (size no. 2; Shionogi Qualicaps, Nara, Japan) was filled with 30 mg ONO-2921 SD2, ONO-2921/MAN, or ONO-2921/malt-β-CD microspheres. The test was performed at an inhalation rate of 28.3 l/min for 10 s. Prior to the inhalation study, the flow rate was calibrated several times with the inhalator containing an empty capsule. After aspiration, the particles that were deposited on the capsule, device, throat, pre-separator, and at each stage of the cascade impactor were rinsed off with 100-ml distilled water. The quantity of ONO-2921 in the rinse solution was determined by HPLC as described in the "Scanning Electron Microscopy" section.

In Vitro Dissolution Study

The dissolution profiles of ONO-2921 were examined using a dissolution tester (NTR-6100A; Toyama Sangyo, Osaka, Japan) and a paddle method as previously described (14). In brief, 10 mg sample was gently added to 900 ml of distilled water at 37±0.5°C using a rotating paddle (100 rpm) as the flow system. Aliquots of this solution were sampled at 0, 0.5, 1, 2, 5, 10, 30, and 60 min and the concentration of ONO-2921 in the test solutions were analyzed by HPLC as described in the "Scanning Electron Microscopy" section. The dissolution test was performed under sink conditions.

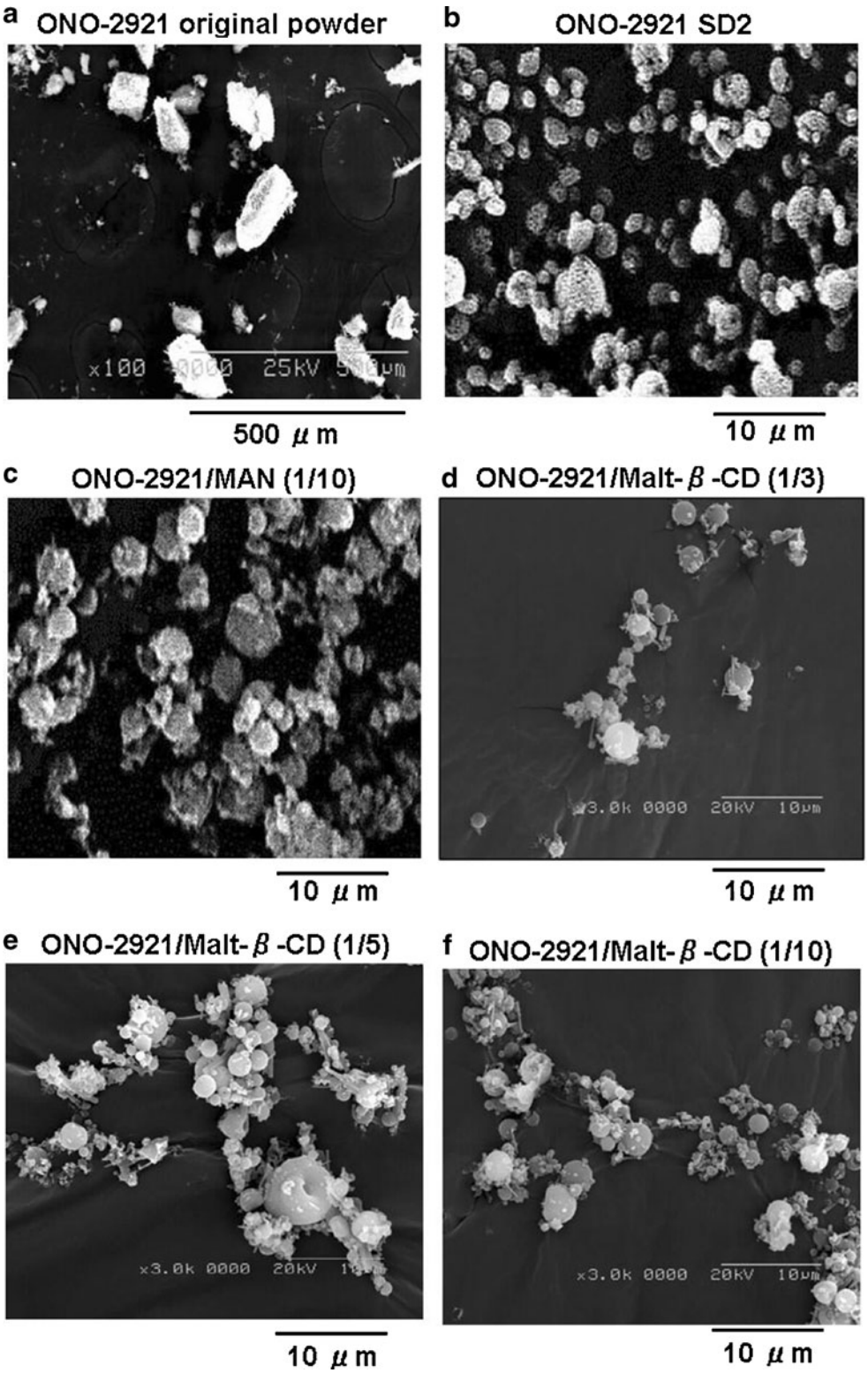

Fig. 2. SEM photographs of (**a**) ONO-2921 (original powder), (**b**) ONO-2921 (spray dried), (**c**) ONO-2921/MAN microspheres, and (**d–f**) ONO-2921/malt-β-CD microspheres at drug/polymer ratios of (**d**) 1/3, (**e**) 1/5, and (**f**) 1/10

Animals

Male Sprague–Dawley rats (age, 6 weeks; weight, 180–220 g) were purchased from Japan SLC (Shizuoka, Japan). The animals were provided food and water *ad libitum*, according to the Guidelines of Experimental Animal Care issued by the Prime Minister's Office of Japan. The experimental protocol was approved by the Committee of Animal Care and Use of Tokyo University of Pharmacy and Life Sciences.

Surgery

In order to administer microspheres through the intratracheal route, a series of surgery was performed in the rats referring to a previous study (17). In brief, a rat was anesthetized and placed in a hold with upper incisors hooked onto the frame and the body positioned with the face up. Carotid arterial cannulation was also performed using polyethylene tube (2.42 mm outside diameter×3 cm) inserted 1.5 cm into the trachea for cannulation.

Pharmacokinetics of ONO-2921

For the *in vivo* pulmonary absorption study, microspheres (ONO-2921, 2 mg/kg) were introduced by intratracheal administration using a veterinary dry powder insufflator (DP-4; Penn Century, Philadelphia, PA, USA) equipped with a three-way stopcock. Compressed air for releasing the particles was generated by depressing the plunger from 2 to 0.5 ml on the syringe scale. The delivery tube of the insufflator was inserted into the tracheal cannula and the rat's breathing was stopped for 2 s. Next, an air pulse was released through the three-way cock to deliver the particles, and the position was held for 5 s. The delivery tube was then removed from the cannula.

Blood samples (400 μl) were collected from the carotid arterial cannula at 1, 3, 5, 10, 15, 30, and 60 min after administration of the formulations. Plasma was obtained from the blood samples by centrifugation (10,000 rpm, 15 min, 4°C). Ethanol (300 μl) was added to 100 μl of plasma, the mixture was vortexed, and then centrifuged (10,000 rpm, 15 min, 4°C) to precipitate the plasma proteins. The supernatant was evaporated to dryness using a HVC-500 mini-centrifugal concentrator (Asahi Techno-glass, Chiba, Japan). The dried residue was dissolved in 200 μl mobile phase and the samples were then analyzed by HPLC.

The area under the blood concentration-time curve (AUC) was calculated by trapezoidal rule. Absolute bioavailability was calculated by the formula: $\text{Bioavailability} = (\text{AUC}_{\text{Inhalation}}/\text{Dose}_{\text{Inhalation}})/(\text{AUC}_{\text{i.v.}}/\text{Dose}_{\text{i.v.}})$. To obtain pharmacokinetic data following intravenous administration, the ONO-2921 SD2 suspension (2 or 15 mg/kg) was administered through the tail vein and blood was collected at the times indicated above. Blood samples were then treated as above to obtain samples for HPLC.

Phase Solubility Studies

The solubility of ONO-2921 was examined as previously described (33). Excess amount of ONO-2921 was added to 0–20 mM malt-β-CD solutions. The suspension was equilibrated at room temperature overnight, centrifuged for 10 min at 2,100 rpm, and then the supernatant was analyzed by HPLC as described in the "Scanning Electron Microscopy" section.

RESULTS AND DISCUSSION

Physicochemical Characteristics of Powder and Different Microsphere Formulations

Scanning electron microscopy (SEM) of the different microparticle formulations of ONO-2921 revealed that ONO-2921 powder formed rod-like structures (Fig. 2a), while ONO-2921 SD2 microparticles were nearly spherical (Fig. 2b). The ONO-2921/MAN (Fig. 2c) and ONO-2921/malt-β-CD microspheres prepared with different malt-β-CD concentrations (Fig. 2d–f) were also nearly spherical. The mean diameters of microspheres were approximately 3–5 μm.

In Vitro Aerosol Performance of Different Microsphere Formulations

In vitro aerosol performance of microspheres was evaluated using a cascade impactor and then HPLC (Fig. 3). Microspheres accumulated in the device, throat, and pre-separator, indicating that drugs were not released because of cohesion caused by hydrophobicity. On the other hand, a fraction of the microparticles was effectively delivered to stage 2–7. Indeed, more than 20% of ONO-2921 SD2, ONO-2921/MAN, and ONO-2921/malt-β-CD microspheres accumulated in stages 2–7. These regions are collectively termed the fine particle fraction. Particles in the range of approximately 0.4–5.8 μm are usually most effective for inhalant treatment because they can be delivered to the alveoli in the deepest regions of the lung (34,35). The diameters of prepared microparticles were 3–5 μm (Fig. 2), which was consistent with the results of *in vitro* aerosol performance.

Dissolution Profiles of ONO-2921 from Different Formulations

Dissolution testing was performed using the paddle method. ONO-2921 original powder and ONO-2921 SD2 microspheres did not dissolve in the aqueous phase, even after 60 min, while the ONO-2921/MAN microsphere formulation showed only slight release of ONO-2921 within 60 min. In contrast, ONO-2921/malt-β-CD microspheres rapidly released drug into the aqueous phase within 2 min (data not shown).

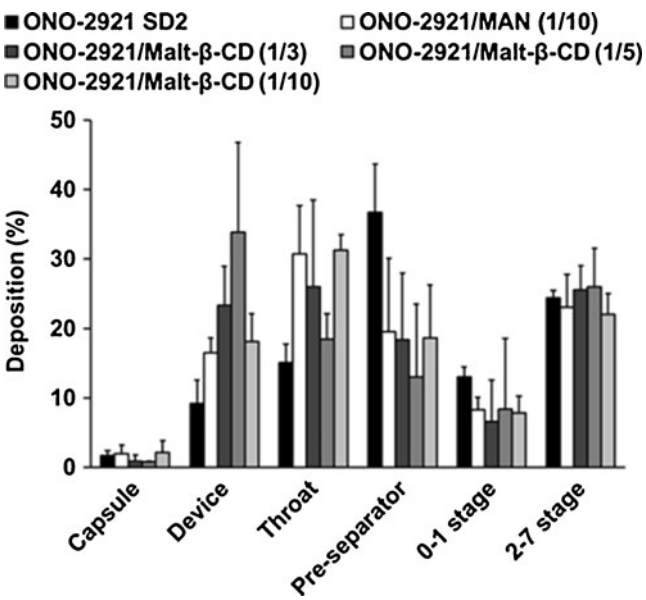

Fig. 3. Aerosol performances of ONO-2921 SD2, ONO-2921/MAN, and ONO-2921/malt-β-CD microspheres prepared at different drug/polymer ratios (1/3, 1/5, and 1/10). The concentration of ONO-2921 was determined by HPLC as described in the "MATERIALS AND METHODS" section. Each value represents the mean±SD (n=3)

Effect of Microsphere Formulation on Pulmonary Absorption

In order to make an *in vivo* rat model for pulmonary inhalation, a surgery of trachea cannulation was demonstrated, and then, the formulation was delivered into lung directly. The blood concentration profiles of ONO-2921 and AUCs of ONO-2921 after intratracheal administration of the different formulations again revealed enhanced solubility and bioavailability of the ONO-2921/malt-β-CD microsphere formulation (Fig. 4 and Table I). The blood concentration of ONO-2921 was below the detection limit following intratracheal administration of ONO-2921 SD2 (2 mg/kg dose) (data not shown), while a higher dose (15 mg/kg) caused a transient increase. The AUC value/dose of the ONO-2921/MAN microsphere formulation was 2.6-fold higher than that of ONO-2921 SD2 at 15 mg/kg. The ONO-2921/malt-β-CD microsphere formulation dramatically increased blood concentration as the ratio of ONO-2921 to malt-β-CD increased from 1/3 to 1/10. The AUC value/dose of ONO-2921/malt-β-CD (1/10) was 15.9- and 6.1-fold greater than those of ONO-2921 SD2 and ONO-2921/MAN microsphere formulations, respectively.

From the AUCs after the intravenous administration of ONO-2921 solution ("ELECTRONIC SUPPLEMENTARY MATERIAL" (ESM) Fig. 1), absolute bioavailability (pulmonary route against systemic route) was calculated (Table II). According to Ono Pharmaceutical (personal communications), oral bioavailability of ONO-2921 is only 1%. In contrast, the bioavailability following pulmonary administration was slightly enhanced (2.7%). The ONO-2921/MAN microsphere formulation improved the bioavailability to 6.9%. The bioavailability of the ONO-2921/malt-β-CD formulation dramatically increased as the amount of malt-β-CD increased from a drug/polymer ratio of 1/3 to 1/10. The bioavailability of ONO-2921 was almost 40% when the ratio of ONO-2921/malt-β-CD was 1/10.

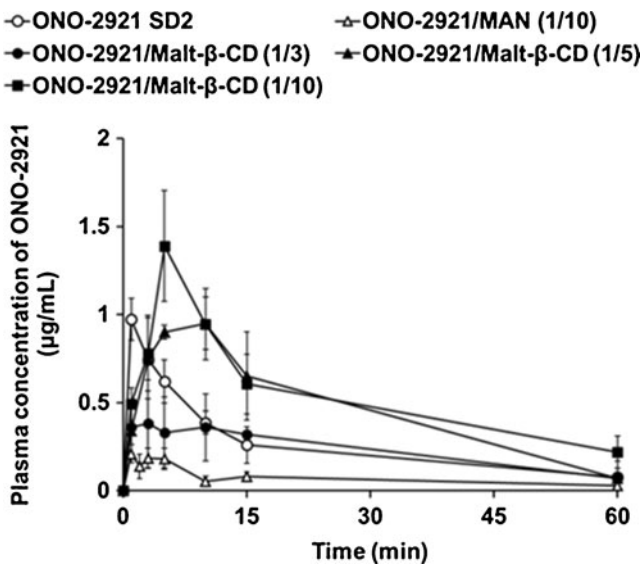

Fig. 4. Plasma concentration of ONO-2921 in rat blood after intratracheal administration of (**a**) ONO-2921 SD2 (ONO-2921, 15 mg/kg; *open circles*), (**b**) ONO-2921/MAN microsphere (ONO-2921, 2 mg/kg; *open triangles*), and ONO-2921/malt-β-CD microspheres at 1/3, 1/5, and 1/10 (2 mg/kg; *closed symbols*). The concentration of ONO-2921 was determined by HPLC as described in the "MATERIALS AND METHODS" section. Each value represents the mean±SE (n=3)

Table I. AUC_{0-60} of ONO-2921 in rats after intratracheal administration of different formulations (mean±SE, n=3)

Formulations	AUC_{0-60}/dose (min μg kg/ml mg)
ONO-2921 SD2	1.0±0.1
ONO-2921/MAN	2.6±0.8
ONO-2921/malt-β-CD (1/3)	3.7±0.3
ONO-2921/malt-β-CD (1/5)	10.3±1.8
ONO-2921/malt-β-CD (1/10)	15.9±4.2

From the results of enhanced bioavailability, we assume that the drug solubility may be enhanced by following two mechanisms: (1) Nano-sized drug particles prepared by the four-fluid spray nozzle may enhance the drug solubility. We found that ONO-2921/MAN microparticles also improved the bioavailability (Fig. 4 and Table II). This is consistent with our previous study using drug/MAN microparticles (15–17). (2) The dissolved drugs can form inclusion complex with malt-β-CD, and then, the inclusion complex may contribute the enhancement of drug solubility. We determined the *in vitro* phase solubility of ONO-2921 in malt-β-CD solutions (ESM Fig. 2). The bare ONO-2921 microparticles were dissolved in equimolar concentrations of aqueous malt-β-CD between 1 and 20 mM. The results suggested that ONO-2921 produced inclusion complexes with malt-β-CD *in vitro*. Although it is difficult to know the interaction between ONO-2921 and malt-β-CD *in vivo*, the results might help the understanding of *in vivo* behavior. On the other hand, an earlier study reported that even a physical mixture of modified CDs with a drug can modify drug dissolution behavior (36,37). Therefore, we cannot exclude the contribution of enhanced drug solubility. These inclusion complexes or *in situ* complexes between malt-β-CD and drug can enhance the dissolution of a drug.

We assume that it is difficult to form ONO-2921/malt-β-CD complexes after the spray drying. In the process of spray drying, the organic solution (ONO-2921) and the aqueous solution (malt-β-CD) are mixed in the nozzle and the resulting mixture immediately generated the mist of ONO-2921/malt-β-CD after spray drying. Therefore the time to form inclusion complexes is absolutely lacking in spray drying. The ONO-2921/malt-β-CD microparticles may form composite after the spray dryer as shown in Fig. 1 and the inclusion complex may be partially formed after the preparation of drug suspension for dissolution and pulmonary absorption study, although it is difficult to estimate the proportion of inclusion complexes *in vivo*.

Table II. Absolute bioavailability of ONO-2921 in rats after intratracheal administration of different formulations

Formulations	Bioavailability (%)
ONO-2921 SD2	2.7
ONO-2921/MAN	6.9
ONO-2921/malt-β-CD (1/3)	9.7
ONO-2921/malt-β-CD (1/5)	27.2
ONO-2921/malt-β-CD (1/10)	42

CONCLUSION

Our newly developed drug/malt-β-CD microspheres improved the *in vivo* absorption and bioavailability of the water-insoluble drug ONO-2921 when administered *via* the pulmonary route. These microspheres were prepared in one step using a four-fluid nozzle spray drier. Microparticle diameters distributed in the fine particle size range as revealed by SEM and *in vitro* aerosol performance assay suggests that our prepared microparticles are efficiently delivered to the lung. In addition, the results of *in vivo* pulmonary absorption study suggest that drug/malt-β-CD microspheres may rapidly dissolve in aqueous solution and be delivered efficiently into the blood stream. Since the mechanisms for the dramatic improvement in the absorption of drug/malt-β-CD microspheres compared to MAN microspheres remain unclear, further study about drug solubility, absorption, and the interaction of drug/malt-β-CD is required. However, the improved bioavailability achieved by this formulation will contribute to future studies examining the therapeutic effects of water-insoluble drugs following inhalation of microspheres.

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