Development of Dried Emulsion/Mannitol Composite Microparticles through a Unique Spray Nozzle for Efficient Delivery of Hydrophilic Anti-tuberculosis Drug against Alveolar Macrophages

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As alveolar macrophages are attractive targets for the treatment of tuberculosis, effective methods for delivery to alveolar macrophages are under development. We investigated a pulmonary formulation for the efficient delivery of high water-soluble drugs at high concentration targeting alveolar macrophages. In this study, a surfactant-coated high water-soluble drug complex (SDC, a hydrophobic dried emulsion), which can preferably target alveolar macrophages and be expected to deliver drug at a high concentration, was prepared in the first process. OCT313, a high water-soluble sugar derivative with anti-tuberculosis activity was used. Then, a unique two-solution, mixing-type nozzle was used to prepare the SDC nanoparticles in mannitol (MAN) microparticles (SDC/MAN microparticles) because it was difficult to disperse the SDC nanoparticles in aqueous solution. The single micron size of OCT313–SDC/MAN microparticles contained OCT313–SDC nanoparticles (mean particle size of OCT313–SDC nanoparticles, 277.9 nm; drug contents, 1.31 ± 0.041 wt%). We found that the treatment of SDC/MAN microparticles exhibited significantly higher drug accumulation in macrophage cells (Raw264.7 cells, 7.5-fold, at 4h after treatment) in vitro and in alveolar macrophages in rats (9.1-fold, at 4h after treatment) in vivo than that of drug alone. These results suggest that the SDC/MAN microparticle formulation prepared by spray drying through a two-solution mixing-type nozzle provides efficient delivery of a water-soluble drug targeting alveolar macrophages and may be useful for tuberculosis treatment.

Key words tuberculosis; alveolar macrophage; nanocomposite particle; surfactant-coated drug complex (SDC); two-solution mixing-type spray nozzle

INTRODUCTION

Tuberculosis (TB) is an infectious disease, particularly rampant in Asia and Africa. According to a WHO report, approximately 10 million and 1.6 million people were infected with TB or died from TB worldwide, respectively, in 2017.1 The mechanism of TB, including the infection route and factors leading to the re-emergence of the disease and its variants, has been studied in detail.2–5 In the process of the development of TB, Mycobacterium TB (MTB), which is the causative organism of human TB, invades the trachea, bronchi, and alveoli of the lungs through the air, and some of the bacteria are ingested and sterilized by alveolar macrophages. Macrophages offer resistance against TB by inflammatory cytokine secretion through Toll-like receptors and by digestion in lysosomes and autophagosomes.6 The remaining bacteria are not sufficiently sterilized, not digested, and hide in the cytoplasm of alveolar macrophages, which results in the proliferation of MTB. Subsequently, MTB hides and persists in alveolar macrophages for a long time and starts to grow again if the host’s immunity is compromised. Eventually, a relapse of TB is observed. Drug administration in clinics is commonly performed by oral or intravenous routes, and drug delivery efficiency to alveolar macrophages, a possible target for TB, is currently limited. The lower efficiency for delivery to target sites can lead to the administration of high doses, which can cause systemic side effects in addition, and long-term drug administration can lead to drug noncompliance. New therapeutic agents against drug-resistant TB have been under development,7–8 while drug-resistant MTB due to drug noncompliance has been seen in several clinical cases. 

Pulmonary administration by inhalation is an attractive route for drug delivery, and is particularly useful for lungs diseases.9–11 Inhalation administration can deliver drugs to disease sites in the lung directly. In addition, the pulmonary route can avoid the first-pass metabolic effect in the liver, in contrast to the oral route, and can prevent nonspecific drug distribution that occurs in the body by systemic administration. To date, various drug formulations for inhalation administration have been studied. It is possible to reach the alveoli in the deep lung tissue by controlling the aerodynamic diameter of an inhaled formulation, and particle formulations with aerodynamic diameters in the range of 1–5 µm have high deposition efficiency in the respiratory tract including the alveoli. In addition, various drug carriers (e.g., liposomes,12–16 emulsions,17,18 poly(lactic-co-glycolic acid) particles,19–21 polymers22) have been studied extensively as carriers for pulmonary administration, although design of drug carriers depends on the target disease or the therapeutic drug. In the case of alveolar macrophages, the cells preferentially ingest...
lipophilic nanoparticles and microparticles. However, several issues, including encapsulation efficiency, drug concentration, and safety, require further study.

We developed a method that uses a spray dryer equipped with a special spray nozzle to handle and preserve drug nanoparticles within microparticles.23–26) This “two-solution mixing type” spray nozzle is separated into two passages: a water-solution containing a dissolved water-soluble carrier, including mannitol (MAN), and a drug/polymer solution dissolved in organic solvent forced through the passages. Subsequently, the solutions are mixed in the nozzle, and the mixture is immediately spray-dried. Drug/polymer nanoparticles are produced in the mixing process by the anti-solvent effect, which results from the change in the solubilities of the compounds in the mixed solution. We have reported that these nanoparticles are useful for not only the storage of nanoparticles but also the improvement of solubility and bioavailability in the intestinal tract and pulmonary areas.

We previously synthesized and studied a derivative of N-acetyl-D-glucosamine, named OCT313.27) OCT313, which is a monosaccharide derivative of glucose, is a water-soluble compound. OCT313 has potential as a novel anti-TB drug and is useful for the treatment of patients resistant to conventional anti-TB drugs.28) However, it is thought that the delivery of OCT313 into alveolar macrophages by oral or intravenous administration would be extremely difficult. Pulmonary delivery of a new type of TB-drug targeting alveolar macrophages at high drug concentrations is a possible strategy for efficient TB therapy.

In this study, to achieve efficient delivery of water-soluble drugs to alveolar macrophages, a two-solution mixing type nozzle was used to develop a novel dry powder inhalation formulation consisting of nanocomposite particles with high encapsulation efficiency (Fig. 1). A surfactant-coated drug complex (SDC) is a hydrophobic dried emulsion formed by the water-in-oil emulsion technique.29) SDC nanoparticles are expected to form nanoparticles of water-soluble drugs with high encapsulation efficiency and to be preferably taken up by macrophages because of its lipophilic surface property. A two-solution mixing nozzle was used for the spray drying of semi-solid SDC nanoparticles which are difficult to disperse and handle. SDC nanoparticles encapsulated in MAN microparticles (SDC/MAN microparticles) were produced through the spray-drying process. In the present study, the SDC nanoparticles and SDC/MAN microparticles were characterized, and the intracellular uptake of SDC/MAN microparticles in alveolar macrophages via pulmonary route was evaluated in vitro and in vivo in terms of drug delivery efficacy.

**MATERIALS AND METHODS**

**Reagents** Calcein was purchased from Dojindo Molecular Technologies Inc. (Osaka, Japan). NaOH, HCl, cyclohexane, and isopropanol were purchased from Wako Pure Chemical Industries Inc. (Osaka, Japan). The Ryoto sugar ester series, which are food emulsifiers as well as hydrophobic surfactants, were kindly provided by Mitsubishi-Kagaku Foods Co. (Tokyo, Japan). These structures and properties are shown in Table 1 (the same information can be obtained from the homepage of Mitsubishi-Kagaku Foods Co.). ER-290 and ER-190 consist of sucrose erucate, L-195 of sucrose laurate, O-170 of sucrose oleate, P-170 of sucrose palmitate, and S-170 of sucrose stearate. MAN was kindly provided by Mitsubishi Shoji Foodtech Co. (Tokyo, Japan). OCT313 (2-acetamido-2-deoxy-beta-D-glucopyranosyl N,N-dimethyldithiocarbamate) was synthesized as previously described.27)

**Preparation of SDC Nanoparticles** SDC nanoparticles were prepared as previously described,29) with some modifications. In brief, 5mL aqueous solution of OCT313 (62mM, equivalent to 100mg) or calcein (10mM, equivalent to 31mg) and 10mL surfactant solution (ER-290, ER-190, L-195, or O-170 were dissolved in cyclohexane, 50mg/mL) were mixed in a 50mL round-bottom flask. A Polytron homogenizer (PT3100; Kinematica AG, Luzern, Switzerland) was used to emulsify the mixture at 26000rpm for 2min to form stable water-in-oil emulsions. The resulting emulsions were frozen at −80°C for 1h and subsequently lyophilized by freeze drying.

![Fig. 1. Scheme for the Preparation of Spray-Dried SDC/MAN Microparticles Using a Two-Solution, Mixing-Type Spray Nozzle](image)

Table 1. The Properties of the Sucrose Fatty Acid Esters Used to Prepare SDC

<table>
<thead>
<tr>
<th>Name</th>
<th>HLB</th>
<th>Fatty acid (C number)</th>
<th>Purity of fatty acid (%)</th>
<th>Physical state</th>
<th>Solubility for cyclohexane</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-195</td>
<td>1</td>
<td>Lauric acid (12)</td>
<td>95</td>
<td>Solid</td>
<td>Dissolved</td>
</tr>
<tr>
<td>P-170</td>
<td>1</td>
<td>Palmitic acid (16)</td>
<td>70</td>
<td>Powder</td>
<td>Undissolved</td>
</tr>
<tr>
<td>O-170</td>
<td>1</td>
<td>Oleic acid (18)</td>
<td>70</td>
<td>Paste</td>
<td>Easily dissolved</td>
</tr>
<tr>
<td>S-170</td>
<td>1</td>
<td>Stearic acid (18)</td>
<td>70</td>
<td>Powder</td>
<td>Undissolved</td>
</tr>
<tr>
<td>ER-190</td>
<td>1</td>
<td>Erucic acid (22)</td>
<td>90</td>
<td>Paste</td>
<td>Easily dissolved</td>
</tr>
<tr>
<td>ER-290</td>
<td>2</td>
<td>Erucic acid (22)</td>
<td>90</td>
<td>Paste</td>
<td>Easily dissolved</td>
</tr>
</tbody>
</table>

The information was obtained from the manufacture’s homepage. One gram of sucrose fatty acid ester was mixed with 10mL cyclohexane. The solubility for cyclohexane was visually checked.
(FD-1; EYELA, Tokyo, Japan) overnight. The resulting viscous materials were used as OCT313–SDC (or calcein–SDC).

For measuring the encapsulation efficiency of SDC formulations, methanol was added to SDC formulation to destroy the structure of SDC. The samples were evaporated to remove methanol and water was added. The absorbance for wavelength 274 nm was measured using a UV-VIS spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) to calculate the OCT313 concentration. For the determination of calcein content in microparticles, calcein content in water was determined by measuring fluorescence (excitation, 355 nm; emission, 535 nm) using a microplate reader (Wallac 4000 ARVO multi-label counter; PerkinElmer, Inc., Waltham, MA, U.S.A.).

Preparation of SDC/MAN Microparticles by Spray Drying A Twin Jet Nozzle RJ10 TLM1 spray dryer equipped with a two-solution mixing type spray nozzle (Ohkawara Kakohki Co., Ltd., Kanagawa, Japan) was used to prepare the SDC/MAN microparticles as previously described, with minor modifications. In brief, OCT313–SDC or calcein–SDC was dispersed in isopropanol at a concentration of 0.30% (w/v). MAN was dissolved in water at a concentration of 3.0% (w/v). Subsequently, the SDC and MAN solutions were forced through different passages in the spray dryer, and the solutions were mixed in the mixing chamber of the spray nozzle. The mixture was immediately sprayed using the spray nozzle. The generated SDC/MAN mist was subsequently dried with heated air, and the produced SDC/MAN microparticles were collected as sample. The spray-drying conditions were as follows: mixing ratio of SDC/MAN, 1/15 (w/w); flow rate, 2.0 g/min (SDC solution), 3.0 g/min (MAN solution); inlet temperature, 145°C; and spray air pressure, 0.1 MPa. OCT313–MAN or calcein–MAN was prepared by spraying through a single passage of the same spray dryer as the control. Spray-dried samples (approximately 4–5 mg) were dissolved in pure water, and the drug contents in the mixture were determined as previously described.

Observation of Spray-Dried Microparticles and Size Determinations of SDC Nanoparticles in SDC/MAN Microparticles An S-4300 scanning electron microscope (SEM) (S-4300; Hitachi, Tokyo, Japan) was used to observe the particle appearances of the microparticle formulations. Before the observations, a quick carbon coater (E-102 ion sputtering device; Hitachi) was used to coat the microparticles with Pt–Pd, which was subsequently observed using an SEM.

To measure the size of the SDC nanoparticles encapsulated within the MAN microparticles, OCT313–SDC/MAN microparticles or calcein–SDC/MAN microparticles were added to water, and MAN was dissolved in water. A dynamic light scattering particle sizer (ZetaSizer Nano-ZS; Malvern Instruments Ltd., Malvern, U.K.) was used to measure the mean diameters of the SDC nanoparticles.

Cell Culture A macrophage-like murine cell line, Raw264.7 cells was obtained from ATCC (Manassas, VA, U.S.A.). The cells were maintained with Dulbecco’s modified Eagle’s medium (DMEM) (Wako Pure Chemical Industries, Ltd.) containing 10% fetal bovine serum (Invitrogen, Carlsbad, CA, U.S.A.) and penicillin/streptomycin and incubated at 37°C in a CO₂ incubator.

Observation of Intracellular Distribution of Calcein Derived from Calcein Microparticles by Confocal Microscopy The cells were seeded in 35-mm glass-bottom dish at a density of 1.0 × 10⁵ cells/well and incubated for 24 h. Then, the calcein–MAN or calcein–SDC/MAN formulation (20 µM calcein/well) was added to cells in serum-free medium. At 1 h or 4 h after the incubation, the cells were washed with PBS and the fluorescence of calcein in cells was observed by confocal microscopy (LSM 510 META; Carl Zeiss, Oberkochen, Germany).

Measurement of Intracellular Uptake of Calcein Derived from Calcein Microparticles by Flowcytometry The cells were seeded into a 35-mm dish at a density of 2.0 × 10⁵ dish and incubated for 24 h. Then calcein–MAN or calcein–SDC/MAN formulation (20 µM calcein/well) was added to the cells in a serum-free medium. At 1 or 4 h after the incubation, the cells were washed with PBS, trypsinized, and then collected. The cell suspension was immediately used for the measurement by flowcytometer (BD FACSVerse™, BD Biosciences, San Jose, CA, U.S.A.). A total 10000 cells were analyzed by FACSuite software (BD Biosciences). The mean fluorescence of calcein (fluorescein isothiocyanate (FITC)-A) was normalized by that of calcein–MAN (4 h).

Animals and Surgical Preparation for Intratracheal Administration All animal experiments were conducted in accordance with the guidelines of the ethics committee of the Graduate School of Pharmaceutical Sciences, Nagoya City University. Male Wistar rats were purchased from Japan SLC (Shizuoka, Japan) and the body weight between approximately 300–400 g was used for the study.

The surgical preparation for intratracheal administration and the administration itself were performed as previously described. In brief, the rats were anesthetized and placed on a holder. For cannulation, a polyethylene tube was inserted into the trachea at the middle of the fifth and sixth tracheal cartilages from the glabella thyroidea. A veterinary dry powder insufflator (Model DP-4; Penn Century, Wyndmoor, PA, U.S.A.) with compressed air combined with a three-way cock and syringe pressure were used to perform the intratracheal administration.

Measurement of in Vivo Uptake of OCT313 by Alveolar Macrophages after the Administration of OCT313 Microparticles After the surgery preparation for intratracheal administration as described in the previous section, OCT313–SDC/MAN microparticles or OCT313–MAN microparticles (200 µg/kg OCT313) were intratracheally administered. At 1 or 4 h after administration, 5 mL PBS was intratracheally administered, and bronchoalveolar lavage fluid (BALF) was collected. The process of BALF collection from the rats was repeated three times. The BALF was centrifuged at 1500 rpm for 5 min, and the supernatant was removed. Next, 5 mL PBS was added to the alveolar macrophages, which were resuspended using a pipette. A 500-µL aliquot of alveolar macrophage suspension in PBS was loaded onto 500-µL Centricoll™ (Percoll-like colloidal silica coated with polyvinylpyrrolidone, Sigma-Aldrich, St. Louis, MO, U.S.A.) and centrifuged at 4500 rpm for 15 min. The alveolar macrophages were isolated from the layer between the Centricoll™ and solution. The alveolar macrophages were collected and washed three times with PBS. The cells were preserved in a deep freezer as samples.

The content of OCT313 was measured by HPLC (UFLC-20A HPLC system; Shimadzu, Kyoto, Japan). The cell samples were treated with methanol, sonicated, and filtered.
with 0.45 \mu m pores to prepare samples for the analysis by HPLC. The typical HPLC condition was as follows: column, Inertsil ODS-80A column (2.1 \times 150-mm; GL Science, Tokyo, Japan); mobile phase, water/acetonitrile = 70/30; wavelength, 274 nm; flow rate, 0.2 mL/min; temperature, 37°C; and flow time 20 min. The final content (%) of OCT313 in macrophage fraction was calculated from the administered drug amount.

**Statistical Analysis**  One-way ANOVA with Bonferroni’s multiple comparison test and two-tailed \( t \)-test were conducted using GraphPad Software (La Jolla, CA, U.S.A.). \( p \) values < 0.05 were considered to indicate statistical significance.

**RESULTS**

**Characterization of SDC Formulations**  In the beginning of the present study, the SDC formulation, which was a dried emulsion, was prepared using the water-in-oil emulsion method to encapsulate the water-soluble drug. Six sugar fatty esters were used to investigate the optimal hydrophobic surfactant (Table 1). Dissolution in cyclohexane was necessary to prepare the SDC formulations; accordingly, S-170 and P-170 were removed for subsequent experiments at the point of solubility which was visually checked, and L-195, O-170, ER-190, and ER-290 were used for the preparation of the SDC formulations. Next, we determined the encapsulation efficiency of OCT313, which is a sugar derivative with anti-TB activity. The order of the encapsulation efficiency was ER-290 > ER190 = L-195 > O-170. (Fig. 2). For the subsequent experiments, we used ER-290 for the SDC formulation because it exhibited the least leaked amount.

**Characterization of SDC/MAN Microparticles and SDC Nanoparticles**  From the obtained SEM images (Fig. 3), the sizes of the four spray-dried microparticles were approximately 1–3 \mu m, which were much smaller than OCT313 original powder. The sizes of the SDC nanoparticles encapsulated within the SDC MAN microparticles were subsequently investigated after dissolution in water. From dynamic light-scattering analysis, the mean diameters of the SDC nanoparticles and encapsulated SDC MAN microparticles were submicron size (Table 2; 233.3 ± 152.1 nm, calcein–SDC nanoparticles; 277.9 ± 56.1 nm, OCT313–SDC nanoparticles). The weight percent of drug contained in the microparticles was then determined (Table 3). The final drug content varied between the drug-MAN microparticles as controls and the calcein–SDC/MAN microparticles because the preparation method and the amount of drug used to prepare the microparticles formulations were different.

**Effect of SDC Formulations on the Cellular Uptake in Macrophage-Like Cells**  To evaluate the effects of SDC formulations on cellular uptake against macrophages, a macrophage-like cell line, Raw264.7, was used for an in vitro study. The intracellular distribution of calcein was observed by con-
focal microscopy (Fig. 4). Calcein–SDC/MAN microparticles and Calcein–MAN microparticles as a control were compared. Calcein–MAN microparticles which were dissolved in medium and behave like a calcein solution in medium showed slight uptake at 1 h (Fig. 4b) and 4 h after the treatment (Fig. 4c). By contrast, the calcein–SDC/MAN formulation which was expected to be dissolved into SDC nanoparticles and to be taken up as SDC nanoparticles into cells exhibited remarkable calcein uptake at 1 h (Fig. 4d) and at 4 h after the treatment (Fig. 4e).

To confirm the improved uptake of calcein against cells, cellular uptake was assessed using flowcytometry. As shown in Fig. 5, calcein–SDC/MAN microparticles exhibited significantly higher calcein uptake compared with calcein–MAN (9.3-fold, at 1 h after the treatment; 7.4-fold, at 4 h after the treatment).

**In Vivo Evaluation of Delivery Efficiency in Alveolar Macrophages of Rats after Administration of SDC/MAN Microparticles** To evaluate the delivery efficiency of the SDC/MAN microparticles in vivo, the formulation was intratracheally administered to rats, and then the alveolar macrophages were collected and the amount of OCT313 was analyzed. The amount of OCT313 in alveolar macrophages fraction isolated after the administration of OCT313–SDC/MAN was significant and much higher than OCT313–MAN (3.7-fold, at 1 h after the treatment; 9.1-fold at 4 h after the treatment) (Fig. 6).
alveolar macrophages. The aerosol performance of particles may affect the lung deposition and the results of cellular uptake; however, we did not conduct experiments to investigate this. We consider that that the optimized SDC/MAN microparticles (1–3 μm) reached the alveoli present in the deeper lung tissue more efficiently and that MAN was then dissolved in the alveoli because it is believed that alveolar air is believed to be 100% saturated with water vapor (100% relative humidity). Consequently, the SDC nanoparticles released from the MAN were probably recognized by the alveolar macrophages; therefore, relatively high concentrations of calcein–SDC nanoparticles could be ingested by the alveolar macrophages, whereas the calcein–MAN microparticles became a simple calcein solution. An advantage of the use of nanocarriers involves the expectation that the drugs in nanoparticles have longer retention times in lung tissue. For example, it has been reported that the inhalation of liposomal drugs can increase retention in the lungs. Therefore, SDC nanoparticles may have the ability to retain drugs in lung tissue.

Alveolar macrophages preferably ingest lipophilic and submicron size nanoparticles. Particularly, liposome formulations have been extensively investigated and characterized for pulmonary delivery; consequently, their sizes and lipophilic properties have been developed to be suitable for uptake by alveolar macrophages. Another group reported that mannose-modified liposome formulations enhanced accumulation in alveolar macrophages. Oh et al., showed that silica-based nanoparticles accumulated in mouse alveolar macrophage cells (J774A.1) more than breast cancer cells (SK-BR-3). They suggested that the increased cellular uptake of nanoparticles is probably due to phagocytosis. In our study, the SDC nanoparticles prepared using the water-in-oil method exhibited high encapsulation efficiency, and our formulation approach is of priority in development at this point. Moreover, the SDC/MAN formulation could be handled under dry conditions, and appears to provide an advantage with regard to storage and stability. In future studies to improve our formulation, we propose the preparation of carriers that have the ability to retain drugs in lung tissue.

DISCUSSION

Recently, TB drug formulations and vaccines for pulmonary delivery have been under active development because they can be efficiently delivered to the targeted site. In the present study, we developed microparticles containing hydrophobic surfactant-coated drug nanoparticles, termed SDC/MAN microparticles, to deliver concentrated hydrophilic drugs efficiently to alveolar macrophages, which are known targets for the treatment of existing TB, and characterized these microparticles both in vitro and in vivo.

The accumulated amount of calcein in macrophage-like cells was much higher after the treatment of calcein–SDC/MAN microparticles than after the treatment of calcein–MAN microparticles in vitro (Figs. 4, 5). Additionally, we found that OCT313–SDC/MAN microparticles exhibited much higher drug accumulation in alveolar macrophages than did OCT313–MAN microparticles in vivo (Fig. 6). These results suggest that the SDC/MAN microparticles were more efficiently delivered to and/or ingested at higher amounts by the alveolar macrophages.
cations. Several articles have reported sucrose ester (Ryoto sugar ester series)-incorporated carriers containing small and large molecules, including pDNA and proteins, although the characteristics of drug release vary depending on the carrier. We investigated a formulation for pulmonary administration, and prepared the SDC/MAN microparticle formulation was prepared using spray drying. These SDC formulations, except for the use of O170, were stable during preparation (Fig. 2). Although a detailed reason for why this was true has not been elucidated, we assume that the length of the fatty acid chain in the surfactant, viscosity of the surfactant, and impurity of this compound may have affected the ability to form micelles and contributed to the stability of the SDC formulations. The size of the SDC nanoparticles was relatively smaller, which can be an appropriate range for stable presentation of the nanoparticles by themselves as a nanoeulsion in contrast to the stability shown for the particles as a microemulsion. These results also suggest that the SDC nanoparticles were stably present in the lung tissue after pulmonary administration, which resulted in increased uptake by the alveolar macrophages (Fig. 6). In addition, sugar esters, which have natural surfactants and biodegradable properties, are relatively useful compounds. Although a detailed investigation of the safety of the formulation is necessary, these results indicate that the SDC/MAN microparticles may be a suitable formulation for pulmonary delivery.

CONCLUSION

In this study, we found that SDC/MAN microparticles significantly enhanced drug delivery efficiency for a macrophage-like cell line (Raw264.7 cells) in vitro and alveolar macrophages in vivo compared with drug solution alone. As the SDC formulation whose surface has hydrophobic properties can be preferably taken up by macrophages, the fabrication of the SDC formulation allows hydrophilic drugs, including OCT313 to be delivered to cells at high concentrations. In addition, microparticles containing SDC nanoparticles were successfully prepared using a unique spray-drying technique through a two-solution, mixing-type nozzle. This composite formulation is useful for good handling and preservation, and the appropriate particle size prepared by the spray dryer may also be useful for inhalation formulation. Although further studies are needed to understand the mechanism of uptake and the intracellular behavior of the SDC nanoparticles, our findings suggest that the SDC/MAN microparticles can be a novel approach for the efficient delivery of hydrophilic drugs to alveolar macrophages for TB therapy.

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Conflict of Interest The authors declare no conflict of interest.

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