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Pharmacokinetics of Betamethasone Disodium Phosphate-Loaded Microparticle Following Pulmonary Delivery

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ABSTRACT

Betamethasone (BTM) is a therapeutic agent for the treatment of pulmonary diseases. Betamethasone disodium phosphate (BDP) is a prodrug of BTM and is rapidly converted into BTM after dosing. In this study, intratracheal administration of the dry powder (ITp) of BDP-chitosan microparticle (BDP-CM) was investigated to determine the drug absorption and disposition in New Zealand white rabbits. In addition, intravenous injection (IV) and intratracheal instillation (ITs) of BDP solution were also investigated. The investigated 10% drug-loaded BDP-CM had a narrow size distribution (2.76 ± 0.25 μm), positive zeta potential (40.23 ± 1.98 mV) and low tap density (0.257 ± 0.106 g/cm^3). There were no significant differences in AUC, half-life ($t_{1/2}$) and MRT (mean resident time) among the three delivery routes ($p > 0.05$), the pharmacokinetics of the three routes could be further evaluated to determine their effects. The $t_{1/2}$ of ITp, ITs and IV were 236 ± 6 (mean ± SEM), 153 ± 16 and 148 ± 12 min determined from plasma levels, and 220, 141, and 92 min, determined from lung levels, respectively. The MRT of ITp, ITs and IV were 376 ± 138, 231 ± 21 and 217 ± 18 min, determined from plasma levels, and 355, 227, and 150 min, determined from lung levels, respectively. The relative lung and absolute bioavailabilities of BTM for ITp were 222% (ITs as 100%) and 72% (IV as 100%), respectively.

Key words: betamethasone disodium phosphate, chitosan microparticle, half-life, particle characterizations, MRT and bioavailability

INTRODUCTION

Pulmonary delivery systems provide drugs to be rapidly absorbed in the lungs without causing potential systemic absorption by detouring the gastrointestinal tract[1-4]. These systems have been used to deliver steroids and antibiotics (e.g. triamcinolone, gentamicin, tobramycin and rifampicin) into the lungs in order to minimize unwanted systemic effects[5-8].

Chitosan is a cationic polyelectrolyte obtained after the deacetylation of chitin, a biopolymer with N-acetyl-d-glucosamine residue[9]. Owing to its biocompatibility, biodegradability, muco-adhesion and low toxicity, chitosan has been investigated for targeting delivery and controlled release of drugs in various formulations[10-15]. Chitosan-based microparticles (CMs) are also used in drug delivery *via* mucosal epithelia[16,17]. These microparticles might offer advantages in protecting the encapsulated active agent against degradation and controlling the release rate of the incorporated drug over a desired time. Microparticle characteristics, including density, size, morphology, surface charge, drug loading capacity and release kinetics, are important biopharmaceutic factors which contribute to drug availability and lung retention in pulmonary drug delivery[18-22].

Corticosteroids are the first-line drugs in the treatment of chronic obstruction pulmonary diseases[23]. Recently, these drugs have also played important roles in the treatment of severe acute respiratory syndrome (SARS)[24-26]. Betamethasone (BTM) is a highly potent anti-inflammatory drug and an important therapeutic agent for pulmonary diseases. Betamethasone disodium phosphate (BDP), a water-soluble prodrug of BTM, is often used to prepare aqueous injection. After intravenous administration of the BDP injection, BDP is rapidly converted into BTM with a half-life of about 10 min. In our previous studies, betamethasone disodium phosphate-loaded chitosan-based microparticles (BDP-CMs) had been prepared and shown to have a high cationic surface charge and good loading capacity of BDP up to 30%[27,28]. In the rat model, BDP-CMs significantly reduced pulmonary inflammation in lipopolysaccaride-induced rats[29]. The pharmacokinetics of drug-loaded microparticles following pulmonary delivery had been reported, including isoniazid-loaded microparticles[30], rifampin-lactose inhalable microparticles[8] and budesoide-chitosan microparticles[31]. Most of these studies evaluated important

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pharmacokinetic parameters, such as bioavailability and half-life from plasma profiles in a rat model. However, the relationship between the characterization of drug-loaded microparticles and lung pharmacokinetics (such as lung availability and retention) has not been fully investigated.

Rabbits have been used for assessing neonatal pulmonary function, as they have a body weight and size similar to that of a human newborn[32-34]. In this study, a BDP-CM containing 10% BDP was prepared and investigated in rabbits, following pulmonary delivery by intratracheal administration of the dry powder (ITp), which was compared with intravenous injection (IV) and intratracheal instillation (ITs) of BDP solution. The pharmacokinetic parameters estimated by a non-compartmental model were applied to evaluate the pulmonary absorption and disposition of the prepared BDP-CM. In addition, the particulate characterizations of the investigated BDP-CM were correlated to pharmacokinetics parameters.

MATERIALS AND METHODS

I. *Chemicals*

Betamethasone disodium phosphate (BDP) was purchased from Sicor (Milan, Italy). Betamethasone (BTM), hydrocortisone (HC), phosphate buffer saline (PBS), xylazine HCl, pluronic F68 and gelatin (type A) were from Sigma (St. Louis, MO, USA). Chitosan (MW 150,000, 87% deacetylation) was provided by Fluka BioChemika (Buchs, Switzerland). Ketamine HCl injection was purchased from Nang Kuang Pharmaceutical Co. (Tainan, Taiwan). Heparin sodium injection was purchased from Novo Nordisk (Bagsvaerd, Denmark). Other chemicals were of either analytical or reagent grade.

II. *Preparation of Betamethasone Disodium Phosphate Loaded Chitosan-Based Microparticle (BDP-CM)*

BDP-CM was prepared using a spray-drying process described previously[27,28]. Briefly, spray-drying was concurrently performed using a Buchi-190 spray dryer (Lab Plant, Switzerland). The operational conditions were set as follows: aspirator pressure: 30 mbar; atomizer pressure: 3.64 kg/cm^2 and flow rate: 5.0 mL/min. The inlet and outlet temperatures were controlled at 170 and 130°C, respectively. The feeding liquid of the spray dryer was prepared by dissolving BDP in 1% acetic acid with chitosan/Pluronic F68/gelatin at a ratio of 50/5/20 (w/w). The BDP-CM containing 10% (w/w) of BDP was prepared and investigated in the study.

III. *Determination the Characteristics of BDP-CM*

Particles were sized by a Coulter counter (LS 230, Coulter, USA) after dispersing the microparticles in deionic water using an ultrasonicator. The average particle size was expressed as volume mean diameter. Each sample was analyzed in triplicate.

The zeta potential of the investigated particle was recorded using Zeta Plus (Brookhaven Instruments, Holtsville, NY, USA), after the microparticle was prepared in KCl solution (1×10^{-3} M, pH 7) as 0.3% (w/v) suspension by dispersing the particles using ultrasonication. Each sample was analyzed five times to obtain an average and standard deviation. Tap density was determined by using a Vankel tap density meter (Cary, NC, USA). True density was measured by a gas pycnometer (Pycnometer1000, Quantachrome, Byton Beach, FL, USA).

IV. *Quantitative Analysis of BDP from BDP-CM*

Microparticles (10 - 15 mg, accurately weighed), were dissolved in 4.0 mL of 1% acetic acid solution. The samples were centrifuged and analyzed by HPLC to determine the concentration of BDP in the solution against a series of BDP standards prepared in 1% acetic acid solution. The HPLC system was connected to a fixed wavelength UV detector set at 254 nm and a photodiode array UV-VIS detector (Model SPD-M6A, Shimazu Corporation, Tokyo, Japan). A RP18 column (GL Science, Tokyo) was used for the separation. The mobile phase was a mixture of 60% CH_3OH and 40% 0.07 M K_2HPO_4 (v/v).

V. *In-vitro Release of BDP from BDP-CM*

The drug release characteristics of BDP-CM were determined using a dissolution apparatus (VK 7000, Vankel, USA) with a dissolution paddle assembly (USP apparatus 2) connected to an autosampling multichannel pump and a spectrophotometer set at a wavelength of 254 nm. Microparticles (10 - 20 mg) were suspended in 150 mL of pH 7.4 phosphate buffer (37°C) at an agitation rate of 50 rpm. Sampling times were 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110 and 120 min. The dissolution samples were also analyzed by HPLC to confirm the dissolution results. The release of BDP from the microparticles was described as first order kinetic, the release rate constant (k_r) was determined by the following equation: $X_{(t)} = X_\infty (1–e^{-k_r \cdot t})$, where $X_{(t)}$ was the cumulative release percentage at time t, and X_∞ was the maximum cumulative release percentage or 100%. In addition, the equation was transformed into $\log(\frac{X_\infty}{X_\infty - X_{(t)}}) = \frac{k_r t}{2.3}$ to obtain a linear line.

VI. *Calibration Curves of BTM in Plasma and Lung*

The calibration curve of BTM in plasma with concentrations of 50, 100, 200, 500, 1000, 3000 and 6000 ng/mL were established from analyzing spiked samples, which are obtained by the addition of known quantities of BTM and HC mixture to aliquots of plasma and prepared as described in previous study[35]. The calibration curve of BTM in lung with concentrations of 125, 250, 500, 750, 1000, 1250 and 2500 ng/g were established from analyzing spiked samples which were prepared by the addition of known quantities of BTM and HC mixture to lung tissue in test tube. The

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subsequent procedure for sample preparation was described in the method of *Pharmacokinetic Study*. The calibration curve was determined by weighted least square linear regression analysis using the weight of 1/concentration^2. The peak-height ratios of BTM to internal standard were plotted against the prepared concentrations.

Quality control (QC) samples at 125, 250, 1250, and 2500 ng/g for lung were prepared and analyzed to monitor the calibration curves.

VII. *Drug Treatment*

New Zealand white rabbits of either sex with a body weight of 2 - 3 kg were used in the study. The animals were handled according to a protocol approved by the Animal Care and Use Committee of the National Defense Medical Center (Taipei, Taiwan). Three treatments, IV, ITs and ITp, were conducted. For the IV treatment, the BDP solution was intravenously injected through the aural vein of the rabbit with a dose of 0.4 mg/kg. For the ITs treatment, the BDP solution was intratracheally delivered by using a spray-instillator (Microsprayer, IA-IC, Penn-Century, Philadelphia, PA, USA) at a dose of 0.4 mg/kg. For the ITp treatment, the BDP-CM was intratracheally delivered by using a powder insufflator (DP-4, Penn-Century, Philadelphia, PA, USA) at a dose of 0.25 mg/kg. The dose of ITp was less than those of other administrations, due to the low-volume capacity of the insufflator. Prior to the intratracheal administrations of ITs and ITp, the rabbit was anesthetized using ketamine HCl (70 mg/kg) and xylazine HCl (10 mg/kg), and the trachea of the rabbit was exposed through a longitudinal incision along the ventral aspect of the neck. The trachea was cut transversely, halfway through, between the second and third tracheal rings caudal to the thyroid cartilage. The spray-instillator and powder insufflator were inserted into the trachea from the incision, and the tips were positioned approximately 1 cm above the bifurcation of the trachea.

VIII. *Pharmacokinetic Study*

During the studies, the rabbits were maintained under anesthesia by the hourly intramuscular injection of ketamine HCl (70 mg/kg) and xylazine HCl (10 mg/kg). Blood samples (1 mL at each time point) were collected *via* a cannula in the femoral artery of rabbits, at predose, 5, 10, 15, 30, 60, 90, 120, 180 and 240 min for the three treatments. Plasma concentrations of BTM were measured by HPLC using HC as an internal standard. The analytical procedure for the HPLC plasma sample preparation was described in a previous report[35].

In the pharmacokinetic study of the lungs, lung tissues were sampled from sacrificed rabbits at 0 (predose), 30, 60, 90, 120, 180 and 240 min. Lung samples were obtained *via* the following procedure. Rabbits were exsanguinated and euthanized by injecting an overdose of sodium pentobarbital into the ear vein. After dissecting the chest chamber, the whole lung was collected and frozen at -20°C after weighing. The samples were analyzed within one week.

For the lung sample treatment, lung tissues (approximately 0.4 g) were sampled from four different regions of the lung after thawing the frozen lung at room temperature. The samples were weighed and cut into small blocks (1 × 2 mm) with a set of surgical micro-scissors, and put into a 10-mL test tube. HC was used as an internal standard for HPLC analysis, 30 μL of HC solution (50 μg/mL) was added to the tissue sample. The mixture was subjected to vortex for 10 s and left to stand for 5 min. Then, 2 mL of PBS was added. The sample mixture was homogenized for 10 s by using a homogenator in cold bath, repeating 3 times with 10-s rest intervals. Subsequently, the homogenator was washed with 0.5 mL of PBS and the washing was collected in the original tube. Then, 2.5 mL of the treated sample solution was mixed with 2 mL of acetonitrile by vortexing for 30 s (deproteinization). The mixture was centrifuged at 1,650 ×*g* at 4°C for 2 min (Sorvall RT7, Newton, CT, USA). The supernatant was transferred into another glass tube containing 2 mL of ethyl acetate. The following steps were processed according to a method for plasma treatment[35].

The absorption and disposition of BTM were evaluated by the use of a non-compartmental model and fit by the WinNonlin computer program (V3.0, SCI software, Mountain View, CA, USA). The pharmacokinetic parameters were determined from the plasma and lung data of BTM.

The pharmacokinetic parameters of BTM were obtained as follows:

C_{max}: maximum drug concentration in plasma or lung; T_{max}: the time for maximum drug concentration; AUC: area under curve of plasma (AUC_p) or lung (AUC_L) profiles determined by the trapezoidal method; $t_{1/2}$: half-life of terminal phase was estimated by 0.693/k, k was the elimination rate constant; MRT: mean residence time; F: the absolute bioavailability was determined by the formula: $F = [AUC_P \cdot dose_{(IV)}]/[AUC_{P(IV)} \cdot dose]$; F_{lung}: the relative lung bioavailability was estimated by the formula: $F_{lung} = [AUC_L \cdot dose_{(ITs)}]/[AUC_{L(ITs)} \cdot dose]$.

IX. *HPLC Analysis*

BTM levels in plasma and lung were determined by HPLC analysis. The method was modified from our previously reported method[35]. Briefly, separations were achieved using an Intersil C18 column (ODS-80Å, 5 μm, 250 × 4.6 mm, GL Science, Tokyo, Japan). The mobile phase consisted of 0.07 M KH_2PO_4 and CH_3OH in a ratio of 60/40 (v/v). The flow rate of the mobile phase was set at 1 mL/min and run at ambient temperature. The HPLC elute was monitored by an UV detector at 254 nm.

RESULTS

I. *Characteristics of BDP-CM*

The characterizations of BDP-CM are shown in Table 1.

The prepared BDP-CM contained 10% BDP. The investigated BDP-CM had a narrow particle size distribution (2.76 ± 0.25 μm), positive zeta potential (40.23 ± 1.98 mv) and low tap density (0.257 ± 0.106 g/cm^3). The scanning electron micrograph of BDP-CM indicated that the prepared microparticles were spheroid shaped with fairly smooth surfaces (Figure 1).

II. *In-vitro Release of BDP-CM*

During the initial 30 min, the release profile of BDP-CM showed zero order release (2.6%/min) in pH 7.4 buffer (Figure 2). The times corresponding to 50, 75 and 90% release of BDP-CM were 14, 26 and 50 min, respectively. In addition, the release profile could be described as $X_{(t)} = 100\% (1\text{-}e^{-0.0598t})$ and $\log(\frac{100\%}{100\%-X_{(t)}}) = \frac{0.0598t}{2.3}$, r = 0.9804. The release rate constant (k_r) of BDP was 0.0598 ± 0.0055 min^{-1} (n = 3) (Figure 2). The value of k_r was determined from the experiment at the 5 - 70 min time period, as the release profile from 80 to 120 min showed a plateau pattern.

III. *Chromatograms*

The HPLC chromatograms of BTM levels in plasma and lung tissues along with the internal standard of HC are shown in Figure 3. The retention times of HC and BTM were 8 and 11 min, respectively. The endogenous components of the lung did not interfere with the analysis of BTM (Figure 3C).

IV. *Calibration Curves of BTM in Plasma and Lung*

The calibration curves of BTM were established for determining BTM levels of plasma and lung tissues in the

Table 1. The characteristics of BDP-loaded chitosan microparticle

Characteristics	Values[a]
Particle size (μm)	2.76 ± 0.25
Zeta potential (mV)	40.23 ± 1.98
Tap density (g/cm^3)	0.257 ± 0.106
True density (g/cm^3)	1.55 ± 0.15

[a]mean ± SD (n = 6)

Table 2. Reproducibility of intra-day and inter-day analyses for BTM in the lung

Nominal concentration (ng/g)	Error (%)	CV (%)
Intra-day		
125	2.71	12.97
250	7.23	10.11
1250	5.15	7.31
2500	3.74	5.82
Inter-day		
125	-2.19	17.34
250	8.31	3.03
1250	-10.97	9.15
2500	6.98	13.77

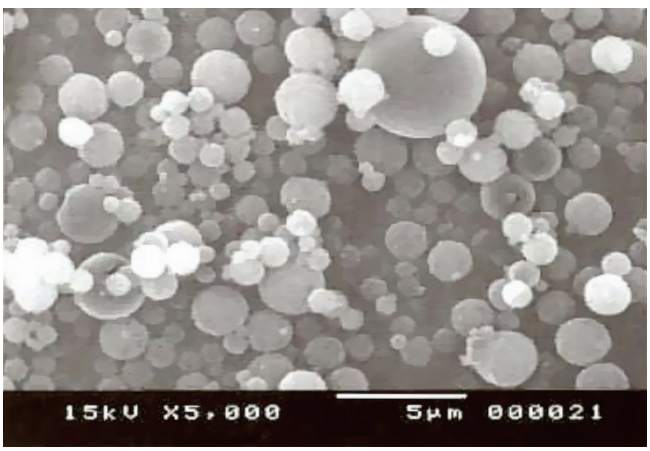

Figure 1. Scanning electron micrograph of betamethasone disodium phosphate-loaded chitosan microparticles prepared by spray-drying method.

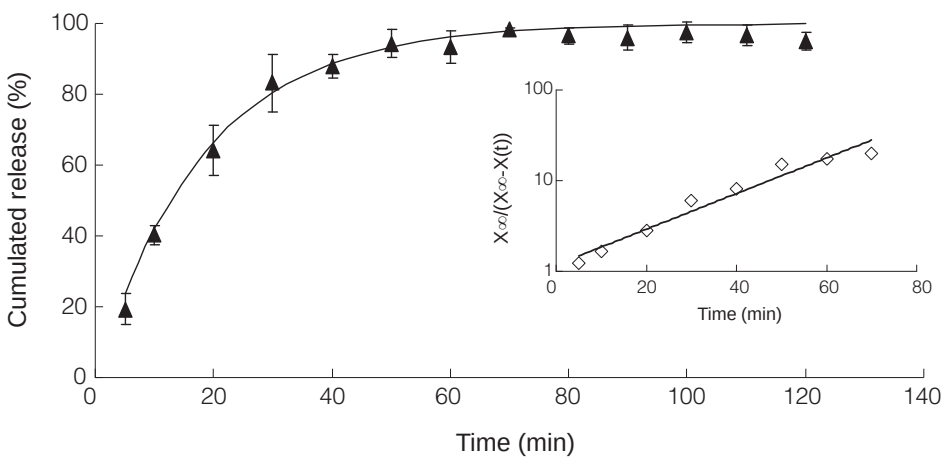

Figure 2. Release profile of betamethasone disodium phosphate-loaded chitosan microparticles. The inset graph is plotted by $X_\infty/(X_\infty\text{-}X_{(t)})$ vs. time.

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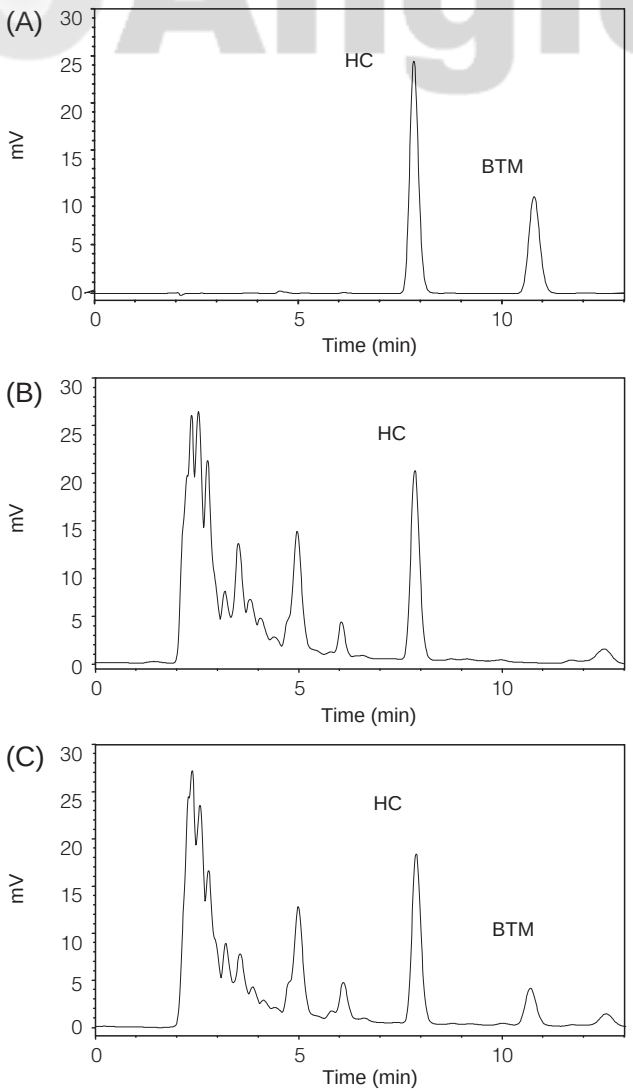

Figure 3. Chromatograms of betamethasone (BTM) and internal standard of hydrocortisone (HC). (A). Drugs spiked in mobile phase (BTM: 500 ng/mL); (B). HC in lung tissue sample at pre-dosing; and (C). Lung sample at 1 h after dosing betamethasone disodium phosphate solution by intratracheal administration (BTM concentration: 910 ng/g).

study. The regression lines had good linearity, ranging from 50 to 6,000 ng/mL for plasma and 125 to 2,500 ng/g for lung tissues:

BTM (plasma): $y = 0.000308\ x + 0.00294$ $(R^2 = 0.99997)$

BTM (lung): $y = 0.000081\ x - 0.00349$ $(R^2 = 0.99976)$

Reproducibility of the measurement was evaluated by intra-day and inter-day analyses and illustrated by the accuracy (error) and the precision (coefficient of variation, CV), as shown in Table 2. The accuracy and precision of BTM for lung were -10.97 - 8.31% and 3.03 - 17.34%, respectively.

V. *Pharmacokinetics*

The plasma profiles and pharmacokinetic parameters of BTM, which followed the administration of IV, ITs or ITp for dosing BDP in rabbits are shown in Figure 4 and listed in

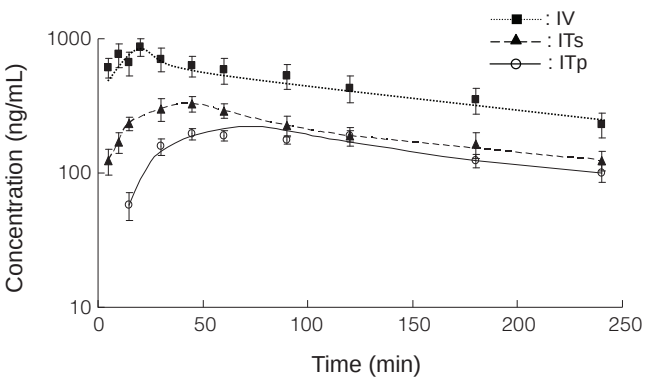

Figure 4. Time courses of plasma betamethasone (BTM) level in New Zealand white rabbits following three administrations. (Mean ± SEM) The three profiles are intravenous administration (IV) (■) (n = 6) and intratracheal administration (ITs) (▲) (n = 5) of betamethasone disodium phosphate (BDP) solution with a dose of 0.4 mg/kg, and intratracheal administration of BDP-chitosan microparticles (ITp) with a dose of 0.25 mg/kg (n = 6) (○).

Table 3. Non-compartmental pharmacokinetic parameters of betamethasone determined from the plasma data following the intravenous administration of betamethasone disodium phosphate (BDP) solution (IV) with a dose of 0.4 mg/kg, intratracheal instillation of BDP solution (ITs) with a dose of 0.4 mg/kg and intratracheal administration of BDP-loaded chitosan microparticle (ITp) with a dose of 0.25 mg/kg in New Zealand white rabbits

Parameters	Unit	IV (n = 6)	ITs (n = 5)	ITp (n = 6)
T_{max} **	min	19.2 ± 2.4	42.0 ± 5.6	75.0 ± 17.3
C_{max} *	ng/mL	816.3 ± 149.0	357.1 ± 35.0	223.7 ± 19.2
AUCP/dose	$x10^3$ ng min/mL	219.4 ± 40.3	97.7 ± 17.2	157.3 ± 41.5
$t_{1/2}$	min	148.4 ± 12.1	152.6 ± 15.7	237.0 ± 96.5
MRT	min	216.5 ± 18.4	230.7 ± 21.1	376.5 ± 138.8
F	%	100.0 ± 18.5	44.7 ± 7.8	71.9 ± 20.7

The results were expressed as mean ± SEM.
** T_{max}: one way ANOVA for IV, ITs and ITp treatments, $p < 0.01$.
* C_{max}: one way ANOVA for IV, ITs and ITp treatments, $p < 0.05$.

Table 3, respectively. In the IV treatment, the time course of BTM in plasma showed a remarkable peak which was related to the hydrolysis of BDP to BTM. In the ITs treatment, the plasma level of BTM increased from beginning until 45 min and decreased thereafter. In the ITp treatment, a plateau of plasma BTM profile was noticed during the 45 - 120 min interval. There were no statistically-significant differences among the AUC, half-life and MRT of the three delivery routes ($p > 0.05$). The absolute bioavailabilities (F_P) of the ITs and ITp treatments, determined from plasma data, were 44.7% and 71.9% (using IV as 100%), respectively.

Following the IV, ITs or ITp treatments for dosing BDP in rabbits, the lung profiles and pharmacokinetic parameters of BTM are shown in Figure 5 and listed in Table 4, respectively. In the IV treatment, the time course of BTM level in lung showed a steadily declining curve. The BTM relative bioavailabilities of lung (F_L) following ITp and IV treatments, were 222.1 and 88.9% (using ITs as 100%), respectively.

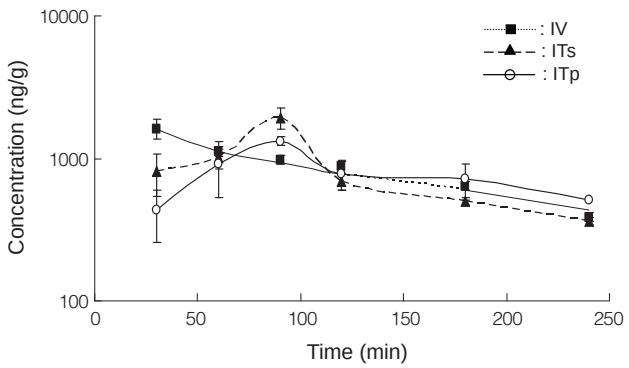

Figure 5. Time courses of betamethasone (BTM) level in the lung tissues of New Zealand white rabbits following three administrations. (Mean ± SEM).
Three profiles are intravenous administration (IV) (■) and intratracheal administration (ITs) (▲) of betamethasone disodium phosphate (BDP) solution with a dose of 0.4 mg/kg, and intratracheal administration of BDP-chitosan microspheres (ITp) with a dose of 0.25 mg/kg (○). (Each time point, n = 6)

DISCUSSION

In this study, the prepared BDP-CM achieved higher bioavailability and longer retention time in the lung in comparison with IV and ITs. As the physicochemical properties of pulmonary delivery systems are important factors that potentially affect the lung disposition of active drugs[36-38], the characteristics of the drug-loaded microparticle investigated could be further correlated to lung pharmacokinetic parameters. The lowest phagocytosis refers to the particle surface with a zeta potential of zero[39]. In the study, the investigated chitosan microparticle had a high zeta potential (40.23 mV) and suitable particle size, which might achieve rapid uptake by phagocytosis following pulmonary delivery during inflammation.

Naikwade *et al.* (2009) investigated the pharmacokinetics of budesonide-loaded microparticles in Wistar rats by administrating DPI formulations intratracheally[31]. The results indicated that the porous particle formulation of budesonide extended the release of the drug with a longer retention of budesonide in the lungs. In the present study, the investigated BDP-CM had a tap density of 0.257 g/cm^3. The granule density of BDP-CM was calculated from the tap density by deducting the values of porosity (30 - 50%) to be approximately 0.367 - 0.514 g/cm^3 which were remarkably lower than the true density of BDP-CM (1.55 g/cm^3). The results indicated that the prepared BDP-CM were porous particles and may improve efficacy by lowering particle agglomeration in pulmonary delivery.

The pharmacokinetic parameters of BTM were determined and evaluated from lung tissue samples. However, only one lung tissue sample or one time point was obtained in each rabbit. Thus, the parameters of AUC, MRT, F_{lung} and T_{max} for lung were listed as the calculated value without standard deviation in Table 4. The ITp treatment with BDP-CM obtained good lung bioavailability (2.5 fold of IV) and seemed to have good drug deposition in the lungs. The first-order rate constant of BDP-CM release was determined by *in vitro* study to be 0.0598 min^{-1}. The relationship between the peak time and drug absorption of BTM in

Table 4. Non-compartmental pharmacokinetic parameters of betamethasone determined from lung tissues following the intravenous administration of betamethasone disodium phosphate (BDP) solution (IV) with a dose of 0.4 mg/kg, the intratracheal administration of BDP solution (ITs) with a dose of 0.4 mg/kg and the intratracheal administration of BDP powder (ITp) with a dose of 0.25 mg/kg in New Zealand white rabbits (n = 6)

Parameters	Unit	IV	ITs	ITp
T_{max}	min	30.0	90.0	90.0
C_{max}[a]	ng/g	1703.3 ± 267.3	1801.4 ± 471.1	1561.2 ± 465.4
AUC_L/dose	x10^3 ng min/g	325.3	366.1	812.2
$t_{1/2}$	min	92.1	141.5	219.5
MRT	min	149.3	226.4	355.4
F_{lung}[b]	%	88.9	100.0	222.1

[a]The results were expressed as mean ± SEM.
[b]The relative lung bioavailability, the AUC/dose of ITs was regarded as 100%.

lung tissues might be further discussed. Both peak times of ITs and ITp treatments in lung tissues were about 90 min. For the *in vivo* study, BDP has been reported to be quickly hydrolyzed in blood by phosphatase with a half-life of about 10 min[(40)]. However, the hydrolysis rate of BDP in the lungs should be slower than in blood, as the bronchi and alveoli of lung components are mostly comprised of airspace with limited biological fluid. The proposed overall process of BDP-CM absorption following pulmonary delivery involved three steps: BDP release from the porous microparticle, BDP hydrolysis to BTM, and BTM absorption.

The pharmacokinetics of the investigated BDP-CM were associated with longer $t_{1/2}$ and MRT as well as good lung bioavailability following pulmonary delivery. These results might be explained by the prolonged effect of BDP-CM due to the slow release of BDP from the drug-loaded microparticles. This indicated that the investigated BDP-CM had the advantages of reducing dose, improving local efficiency and decreasing side effects for potential application in respiratory diseases.

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