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Simultaneous Determination of Plasma Betamethasone Disodium Phosphate and Betamethasone in Rabbit by High Performance Liquid Chromatography

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ABSTRACT

Betamethasone disodium phosphate (BDP), a hydrophilic prodrug of betamethasone (BTM), was rapidly converted into betamethasone in blood following intravenous administration. In this study, we established an analytical method of high performance liquid chromatography (HPLC) to simultaneously determine BDP and its free base BTM in plasma samples. The calibration curves demonstrate good linearity (BDP, $R^2 = 0.99999$; BTM, $R^2 = 0.99997$) and reproducibility within a range from 50 ng/mL to 6000 ng/mL. The analytical method was applied to determine the pharmacokinetics of BTM and BDP following intravenous bolus injection of BDP in New Zealand white rabbits (0.4 mg/kg). The determined pharmacokinetic parameters were listed as follows: for BDP, half-life: 13.69 ± 2.70 min (Mean ± SEM, n = 6), clearance: 3.96 ± 0.45 mL/min/kg and distribution volume: 72.9 ± 9.67 mL/kg; for BTM, half-life: 228.58 ± 72.9 min, clearance: 3.50 ± 1.18 mL/min/kg, and distribution volume: 327.57 ± 69.9 mL/kg. The established HPLC analytical method and related pharmacokinetic parameters of BDP and BTM might be potentially applied to use these drug administration in the treatment of respiratory relevant diseases.

Key words: betamethasone disodium phosphate (BDP), betamethasone (BTM), high performance liquid chromatography (HPLC)

INTRODUCTION

Corticosteroids are the first-line drugs in the treatment of chronic obstruction pulmonary diseases[(1)]. Recently, these drugs also play important roles in the treatment of severe acute respiratory syndrome (SARS) [(2-4)]. Three dosage forms of corticosteroids, including oral, parenteral and inhalation preparations, are widely used in clinical therapy. Respiratory distress syndrome (RDS) is one of the most common causes of morbidity and mortality in premature newborns especially in twins. In the last stages of pregnancy, 34 to 37 gestational weeks, type II alveolar cells of fetus normally produce surfactant for reducing the surface tension of alveolar lining fluid, and the surfactant is important for neonate at birth to expand air sacs and breathe normally. In the past, betamethasone (BTM), a highly potent anti-inflammatory corticosteroid, has been shown to reduce the incidents and severity of RDS in premature neonates when it is administrated antenatally to women who are at risk of delivering prematurely[(5)]. Consequently, BTM is further applied to prevent neonatal RDS[(6)]. In addition, postnatal corticosteroid therapy significantly facilitates extubation and shortens the duration of intubation in preterm/extremely low birth weight infants[(7)]. The ester derivatives of BTM, including benzoate, dipropionate and valerate, are poorly water-soluble agents, and commonly used in powder and suspension dosage forms. Betamethasone disodium phosphate (BDP) is a hydrophilic prodrug of BTM and is rapidly hydrolyzed to BTM in blood for further distributing into target tissues to achieve therapeutic effects. BDP is a water soluble agent which had been investigated and prepared as BDP-loaded microparticles for pulmonary delivery to extend the resident time of BDP in lung[(8,9)]. However, BTM also causes severe adverse effects in human infant such as impairing growth and increasing incidence of central nervous system abnormalities. Thus, a rapid and convenient analytical method for measuring BDP and BTM concentrations in plasma would provide important information to the drug regimen of BDP for adjusting dose and minimizing side effects.

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Recently, high performance liquid chromatography (HPLC) has been employed to measure BTM level in biological fluids[10,11]. In these methods, authors assumed that BDP is rapidly hydrolyzed to BTM in plasma so that BDP and BTM are not separated during sample preparation in their HPLC analysis. The approach often overestimates the blood levels of BTM, especially in initial time points. Although BDP can also be analyzed by HPLC methods[12,13], BDP and BTM analyses usually involve two separate sample preparations associated with individual HPLC determinations. Thus, the analytical method for BDP and BTM in plasma sample need to be improved for rapid and efficient measurement of their concentrations.

Rabbit has body weight and size similar to human newborn. Rabbit model has been frequently used for investigating neonatal pulmonary function[14-16]. Thus, rabbit could be a suitable animal model for investigating BDP and BTM pharmacokinetics, which in turns can be applied in the prevention or treatment of neonate RDS. In this study, we intended to establish an HPLC analytical method for simultaneously determining plasma BDP and BTM concentrations. In addition, the established analytical method was applied to investigate the pharmacokinetics of BDP and BTM following intravenous (IV) administration of BDP in rabbits. The specific aims of this study were to evaluate the established HPLC method including precision and reproducibility. In addition, the pharmacokinetic model of BDP and BTM was further proposed to measure their pharmacokinetic parameters.

MATERIALS AND METHODS

I. *Chemicals*

Betamethasone disodium phosphate (BDP) was purchased from Sicor (Milan, Italy). Betamethasone (BTM), hydrocortisone (HC) and xylazine HCl were from Sigma (St. Louis, MO, USA). Prednisolone sodium phosphate (PSP) was gifted from China Chemical Pharmaceutical Incorporation (Taipei, Taiwan). Ketamine HCl was from Nang Kuang Pharmaceutical Co. (Tainan, Taiwan). Heparin sodium injection was from Novo Nordisk (Bagsvaerd, Denmark). Other chemicals were of either analytical or reagent grade.

II. *Liquid Chromatography*

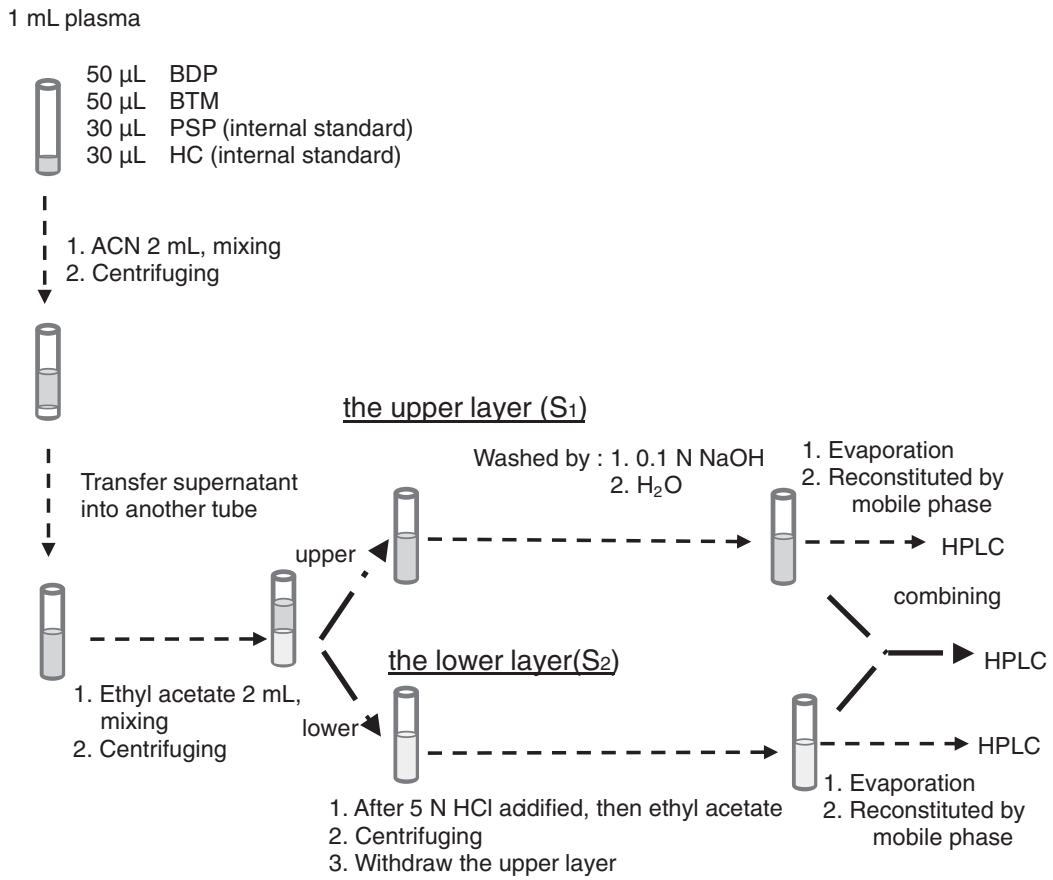
BDP and BTM were determined by an HPLC system (Jasco Inc., Tokyo, Japan), including a pump (PU-980), an autosampler (851-AS) associated with a sample loop (50 μL), and a UV detector (UV-975). Analyte peaks were determined using an integration software (SISC 32, Scientific Information Service Corporation, Taipei, Taiwan). 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 with a ratio of 60/40 (v/v) which was filtered through a 0.45 μm membrane filter (HA, Millipore Corporation, Bedford, MA, USA) and degassed by sonication. The flow rate of the mobile phase was set at 1 mL/min and run at ambient temperature. The HPLC elute was monitored by absorbance at 245 nm.

III. *Plasma Obtainment*

New Zealand white rabbits, weighing 2 - 2.5 kg, either sex, were used in the study. Animals were handled according to a protocol approved by the Animal Care and Use Committee, National Defense Medical Center (Taipei, Taiwan). Rabbits were anesthetized using ketamine HCl (70 mg/kg) and xylazine HCl (10 mg/kg) via intramuscular injection. Blood sample was withdrawn from the ear central vein of rabbit via a cannula and collected into a test tube (rinsing with 10 μL of 100 IU/mL heparin sodium) with Teflon-lined screw cap. The blood sample was immediately mixed with 1 M sodium arsenate (20 μL for 1 mL blood sample) and centrifuged at 2800 rpm for 20 min at 4°C (Sorvall RT7, Newton, CT, USA). The plasma supernatant was then collected and frozen at -20°C. Samples were analyzed within one week in the same day.

IV. *Sample Preparation*

Plasma concentrations of BDP and BTM were measured by HPLC using PSP and HC as internal standards. The procedure of sample preparation is briefly described in Scheme 1. PSP and HC solutions, each 30 μL (concentration: 50 μg/mL), were added to 1 mL plasma in a glass tube and mixed with 2 mL of acetonitrile for deproteinization by vortex 10 s. After mixing, the mixture was centrifuged at 2800 rpm for 2 min in a centrifuge (Sorvall RT7, Newton, CT, USA) at 4°C. The supernatant was transferred into another glass tube containing 2 mL of ethyl acetate. This tube was capped with a Teflon-lined screw cap and mixed for 1 min. After centrifuging at 2800 rpm for 2 min at 4°C, the mixture was separated into two portions, organic layer (S1) on top and aqueous layer (S2) at the bottom. S1 was transferred into another tube. Then, 0.1 N NaOH (250 μL) was added into the tube and mixed for 10 s, the supernatant was immediately transferred into a new tube after standing 5 s. Following, 500 μL of water was added to wash the alkalized liquid by vortex 10 s and standing 5 s. The upper layer was withdrawn and evaporated to obtain dried residue by using a centrifuging vapor condenser (Eyela, Tokyo, Japan). For S2, 5 N hydrochloric acid solution (40 μL), sodium chloride (300 mg) and ethyl acetate (5 mL) were added into the tube and completely mixed by vortex 10 s. After centrifuging for 3 min, the upper layer was withdrawn and evaporated



Scheme 1. The procedure for the treatment of plasma sample
PSP: Prednisolone sodium phosphate
BDP: Betamethasone disodium phosphate
HC: Hydrocortisone
BTM: Betamethasone
S_1: For determining BTM.
S_2: For determining BDP.

to obtain dried residue. The dried residues of S1 and S2 were separately reconstituted in 200 µL of mobile phase which were analyzed either alone or combined together in HPLC analyses.

V. *Calibration Curve and QC Preparation*

A calibration curve was established from analyzing spiked samples, which are obtained by the addition of known quantities of BDP, BTM, HC and PSP mixture to aliquots of plasma and prepared as described above for sample preparation. The concentrations for BDP and BTM were 50, 100, 200, 500, 1000, 3000 and 6000 ng/mL. The peak-height ratios of BDP or BTM to internal standard were plotted against the prepared concentrations. Linearity was determined from all concentrations (50-6000 ng/mL). The calibration curve of each compound was determined by weighted least-square linear regression analysis using the weight of 1/ concentration^2.

Quality control (QC) samples at 4 levels, 50, 100, 1000, and 5000 ng/mL were prepared and analyzed to monitor the calibration curves. Sample extraction and HPLC analysis for QC samples were carried out as described above.

VI. *Stability Studies*

Two spiked plasma samples with concentrations of BDP, 100 and 1000 ng/mL, were used as low and high levels for stability studies. The spiked samples including high and low levels were divided into four groups as A, B, C, and D, each group was run triplicate. Group A samples were analyzed immediately to determine the initial drug concentration of spiked samples. Group B samples were analyzed after standing at room temperature for 4 hrs. These samples were used to mimic the required time in the preparation of the HPLC sample during analytical process. Group C samples were stored at -20°C for 6 days and thawed at room temperature

before analysis. Group D samples were stored at -20°C for 24 h and thawed at room temperature, this freeze-thaw cycle (FTC) was repeated another twice before analysis. The stability studies of BTM in plasma were conducted as the similar process of BDP.

VII. *Pharmacokinetic Study*

New Zealand white rabbits, 2-2.5 kg either sex, were used in the study of pharmacokinetics. Rabbits were anesthetized using ketamine HCl (70 mg/kg) and xylazine HCl (10 mg/kg). BDP with a dose of 0.4 mg/kg was intravenously injected through the ear margin vein of rabbit. Blood samples were collected via a cannula in the femoral artery of rabbits, at predose, 5, 10, 15, 30, 60, 90, 120, 180 and 240 min. Blood samples were treated as the same procedure described in sections of *Plasma obtainment* and *Sample preparation*. Plasma concentrations of BDP and BTM were determined by the established HPLC method. The time courses of BDP and BTM plasma levels were fit by a proposed pharmacokinetic model as Scheme 2. In this model, BDP was considered to be rapidly and completely converted into BTM in blood, two compartment open model was proposed for the disposition of BTM. Then, the time course of BDP concentration (C_{BDP}) in plasma could be described as follows:

$$C_{BDP} = C_{BDP0}e^{-k_h t} \quad (1)$$

Where C_{BDPo} represents the initial concentration of BDP in plasma and k_h is the hydrolysis rate constant of BDP. The time course of BTM concentration (C_{BTM}) was proposed as a three exponential-term equation:

$$C_{BTM} = Ae^{-\alpha t} + Be^{-\beta t} + Ne^{-k'_h t} \quad (2)$$

Eq. 1 and Eq. 2 were fit by using WinNonlin computer program (V3.0, SCI software, Mountain View, CA, USA) in order to obtain the pharmacokinetic parameters of BDP and BTM in rabbit.

The pharmacokinetic parameters of BDP were obtained as follows:

k_h = hydrolysis rate constant (or elimination rate constant)

half-life = $0.693/k_h$

$V = \text{Dose}/k_h\ AUC_{0\sim\infty}$

$$AUC_{0\sim\infty} = \int_0^\infty C_{BDP}dt = \frac{C_{BDP0}}{k_h}$$

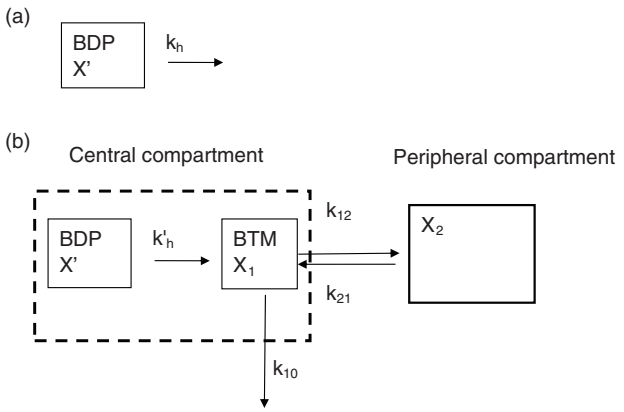
Clearance = $\text{Dose}/AUC_{0\sim\infty}$

In addition, the pharmacokinetic parameters of BTM were also obtained as follows:

k'_h = input rate constant

α = distribution rate constant

β = elimination rate constant



$$\frac{dX'}{dt} = -k_h X' \quad (1)$$

$$\frac{dX'}{dt} = k'_h X' + k_{21} X_2 - (k_{12} X_1 + k_{10} X_1) \quad (2)$$

Scheme 2. Pharmacokinetic models of betamethasone sodium phosphate (BDP) and betamethasone (BTM) following intravenous administration of BDP in rabbits.
(a) BDP and (b) BTM.

$$k_{10} = \frac{\alpha\beta(A+B)}{A\beta+B\alpha}$$

$$k_{12} = \frac{AB(\beta-\alpha)^2}{(A+B)(A\beta+B\alpha)}$$

$$k_{21} = \frac{A\beta+B\alpha}{A+B}$$

$V_d = \text{Dose}/\beta AUC_{0\sim\infty}$

$$AUC_{0\sim\infty} = \int_0^\infty C_{BTM}\,dt = \frac{A}{\alpha} + \frac{B}{\beta} + \frac{N}{k'_h}$$

RESULTS

I. *Chromatograms*

The chromatograms of BDP and BTM associated with their two internal standards, PSP (for BDP) and HC (for BTM) are illustrated in Figure 1. The HPLC retention times of PSP, BDP, HC and BTM were 5.3, 6.5, 8.3 and 11.8 min, respectively. Four compounds were completely separated in the established HPLC method. The endogenous components of plasma did not interfere with the analyses of BDP and BTM. (Figure 1C)

II. *Calibration Curves of BDP and BTM*

The regression lines had good linearity ranging from 50 to 6000 ng/mL for BDP and BTM. The linear regression equations of BDP and BTM calibration curves were constructed in triplicate determinations and listed as follows:

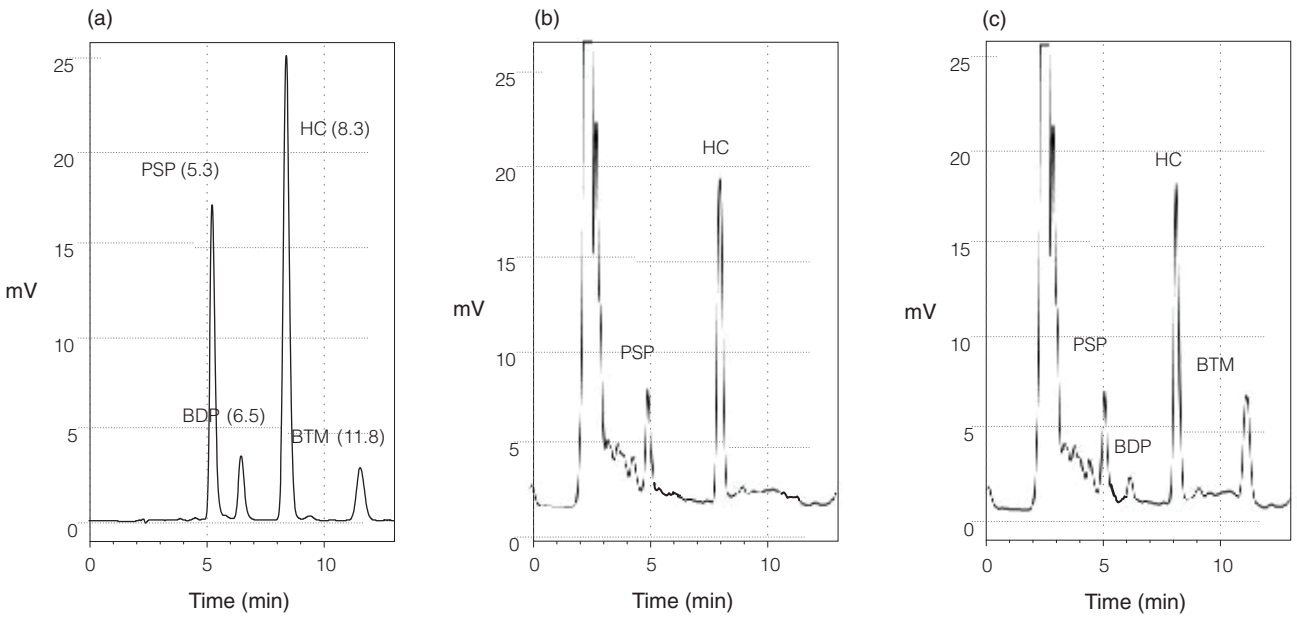


Figure 1. Chromatograms of betamethasone disodium phosphate (BDP), betamethasone base (BTM) and two internal standards: prednisolone sodium phosphate (PSP) and hydrocortisone (HC). (a). Drugs in mobile phase (BDP: 500 ng/mL, BTM: 500 ng/mL, PSP: 50 μg/mL, HC: 50 μg/mL); (b). pre-dosing; and (c). 1 hr post-dosing (BDP: 540 ng/mL; BTM: 1090 ng/mL).

Table 1. Reproducibility of inter-day and intra-day analyses for BTM and BDP

BTM			BDP		
Nominal concentration (ng/mL)	Error (%)	CV (%)	Nominal concentration (ng/mL)	Error (%)	CV (%)
Intra-day*					
50	2.11	17.97	200	-5.18	12.21
100	-6.58	13.18	500	-3.94	13.16
1000	6.78	3.85	1000	-0.64	7.32
5000	3.52	8.91	5000	-2.18	10.35
Inter-day*					
50	0.23	8.21	200	-12.12	14.57
100	13.38	11.46	500	-8.12	11.90
1000	14.35	9.39	1000	-8.89	13.65
5000	8.42	6.38	5000	-9.72	12.28

*n = 5

BDP: $y = 0.000393 x + 0.001055$ ($R^2 = 0.99999$)
BTM: $y = 0.000308 x + 0.00294$ ($R^2 = 0.99997$)

III. *Reproducibility*

Reproducibility of the measurement was evaluated by within-day and between-day analyses and illustrated by the accuracy (error) and the precision (coefficient of variation, CV), as shown in Table 1. The determined concentrations had variations within ± 15%, except 50 ng/mL of BTM in within-day associated with a higher CV (17.97%), but the value is less than 20%[17]. The results of the reproducibility were within an acceptable range.

IV. *Stability*

Two levels (100 and 1000 ng/mL) of BDP and BTM were investigated for drug stabilities during sample storage or sample preparation. For FTC experiments, we

observed a significant reduction of HPLC peak areas for BDP, about 30% in 100 ng/mL and 10% in 1000 ng/mL by comparing the initial peak area of BDP (Group A). During the storages of 4 h at room temperature and 6 days at -20°C, BDP peak areas of two levels did not show any significant reduction. Nearly 5% reduction was observed for 100 ng/mL of BTM in the FTC experiment. There were no any significant reductions of BTM concentrations in other stability studies (data not shown).

Table 2. Pharmacokinetic parameters of BDP in New Zealand white rabbits following the intravenous administration of BDP with a dose of 0.4 mg/kg.

Parameter	unit	mean[a]	SEM[a]	CV (%)
V[b]	mL/kg	72.92	9.67	29.66
k_h	min^{-1}	0.0597	0.0114	42.68
half-life	min	13.69	2.70	44.05
Clearance[c]	mL/min/kg	3.96	0.45	25.61
$AUC_{0\sim\infty}$[d]	min ng/mL	0.107 x 10^6	11952	25.06

[a]sample size = 6; [b]$V = Dose/k_h$ $AUC_{0\sim\infty}$; [c]$Clearance = Dose/AUC_{0\sim\infty}$; [d]$AUC_{0\sim\infty} = \int_0^\infty C_{BDP}dt = \frac{C_{BDP_0}}{k_h}$

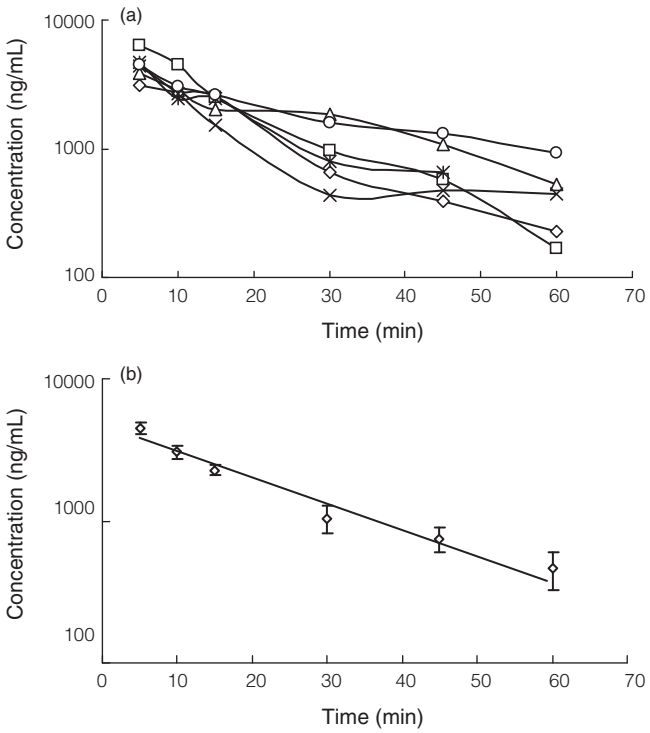


Figure 2. Time course of betamethasone disodium phosphate (BDP) plasma level in New Zealand white rabbits following the intravenous administration of BDP with a dose of 0.4 mg/kg. (a) Individual data and (b) fit curve and mean data (Mean ± SEM, n = 6).
□, ◇, ○, △, × and ∗ represented different rabbits.

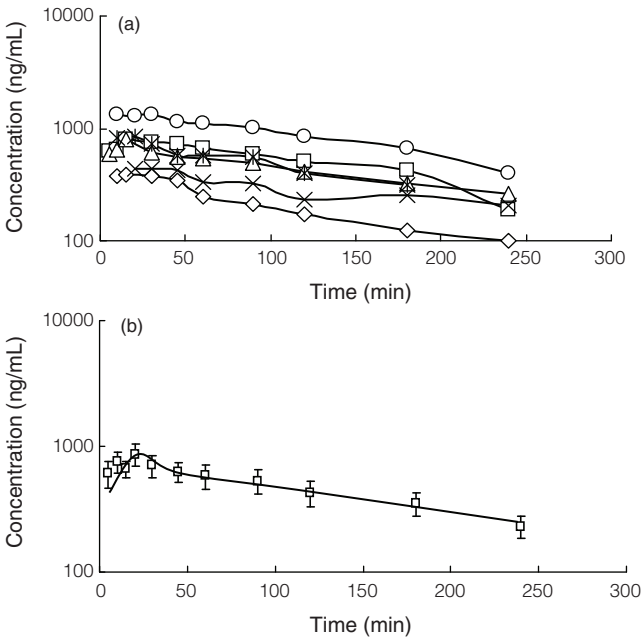


Figure 3. Time course of betamethasone plasma level following the intravenous administration of betamethasone disodium phosphate with a dose of 0.4 mg/kg. (a) Individual data and (b) fit curve and mean data (Mean ± SEM, n = 6)
□, ◇, ○, △, × and ∗ represented different rabbits.

V. *Pharmacokinetics*

Following the intravenous administration of BDP in rabbits, the plasma profiles and pharmacokinetic parameters of BDP are illustrated in Figure 2 and listed in Table 2, respectively. After 60 min, BDP plasma levels could not be determined due to below the quantitative limit. In addition, BDP levels rapidly declined following first-order kinetics with a mean rate constant of 0.0597 ± 0.0114 min^{-1} (Mean ± SEM, n= 6) and a mean elimination half-life of 13.69 ± 2.70 min. *In vivo* study, BDP has been reported to be quickly hydrolyzed by phosphatase associated with a half-life about 10 min which is consistent with our study results[18]. The volume distribution of BDP was 72.9 ± 9.67 mL/kg which was close to the volume of blood.

Following the intravenous administration of BDP, the plasma profile and pharmacokinetic parameters of BTM are illustrated in Figure 3 and listed in Table 3, respectively. The proposed three exponential-term equation [$C=A\exp(-\alpha t) + B\exp(-\beta t)+N\exp(-k'_h t)$] was fitted with BTM experimental points very well. The BTM plasma profile was noticed with a peak concentration and time as 816 ± 162 ng/mL (Mean ± SEM, n = 6) and 19.17 ± 2.62 min, respectively. The fit rate constant for hydrolyzing BDP to BTM (0.0474 ± 0.022 min^{-1}) was slightly less than the elimination rate constant of BDP. The distribution and elimination rate constants of BTM were 0.1558 ± 0.023 and 0.0040 ± 0.00094 min^{-1},

Table 3. Pharmacokinetic parameters of BTM following intravenous administration of BDP with a dose of 0.4 mg/kg in New Zealand white rabbits.

Parameter*	unit	mean	SEM
k'_h	min^{-1}	0.0474	0.022
α	min^{-1}	0.1558	0.023
β	min^{-1}	0.0040	0.00094
k_{10}	min^{-1}	0.0124	0.0051
k_{12}	min^{-1}	0.0593	0.016
k_{21}	min^{-1}	0.0882	0.038
α-$t_{1/2}$	min	5.01	0.957
β-$t_{1/2}$	min	228.58	72.9
V_d	mL/kg	327.57	69.9
Clearance	mL/min/kg	3.50	1.18
AUC	min nmol/mL	479.58	121.99

*pharmacokinetic parameters were determined by the proposed model in Scheme 2.

respectively. Due to its short half-life, the distribution phase was almost finished as the peak time of BTM. Thus, a linear line was obtained in semi-log scale after 50 min. Furthermore, the terminal elimination half-life and total body clearance of BTM were obtained to be 228.58 ± 72.9 min and 3.50 ± 1.18 mL/min/kg, respectively.

DISCUSSION

In this study, we established an HPLC method for simultaneously determine BDP and BTM concentrations in plasma using two internal standards. This method demonstrated good sensitivity and specificity for BDP and BTM measurements. The lower limit of quantitation for both analytes was 50 ng/mL. Petersen *et al.* previously reported HPLC methods for determining dispositions of betamethasone and its phosphate ester by analyzing plasma levels of BTM and BDP separately, and using different sample preparation methods associated with two different HPLC analyses[6,12]. In this study, we modified their methods by applying dual internal standards, PSP for BDP and HC for BTM, to achieve a simple and more convenient HPLC determination. In sample preparation, two treated samples either from organic (containing BTM) or aqueous (containing BDP) layer extractions of plasma sample were combined together for single injection to simultaneously determine both BDP and BTM levels in once. Although the established HPLC analytical method involved several steps in sample preparation, the yields of BDP and BTM were good (BDP: 79.68% ± 1.98% and BTM: 78.37% ± 1.47%, for 100 ng/mL concentration, n = 6) with acceptable precision and accuracy. Furthermore, the HPLC method was further applied in pharmacokinetic study of BTM and BDP.

Recently, analytical methods of liquid chromatography/tandem mass spectrometry (LC/MS/MS) are also established for measuring BTM concentration in plasma sample. The analytical methods demonstrate excellent sensitivity (0.1~2 ng/mL) for BTM[11,19]. However, LC/MS/MS methods usually involve complicate solid-phase extraction for sample preparation and can only determine BTM concentration in plasma. In addition, the approach of LC/MS/MS demands higher expense for instrument maintenance. Thus, our established analytical method with UV detection might be more suitable for routine measurement of BTM and BDP in plasma samples. Furthermore, our analytical method also demonstrated good sensitivity to enable measuring BTM levels at initial time points for characterizing the absorption peak of BTM following intravenous administration of BDP. Samtani *et al.*, also reported an HPLC method for measuring BDP and BTM plasma concentrations[13]. Although this method has an advantage for using small sample volumes (50 μL of sample) in analysis, the analytical method is less sensitive with quantitative limits of 300 ng/mL for BTM and 500 ng/mL for BDP. Consequently, the HPLC method could not be applied for determining the complete pharmacokinetic parameter easily due to some important data points below detection limits.

According to the studies of Tamvakopoulos *et al.*[11] and Samtani *et al.*[19], the terminal half-lives of BTM in rat and ewe (body weight about 70 kg) were 2.02 and 3.95 hr, respectively. In comparison to our HPLC study results, the terminal half-life of BTM in rabbit was 3.8 hr (228 min) similar to that in ewe but different from rat. The difference might be due to different species of animals or other factors. The body weight of ewe is similar to a pregnant woman so that it is frequently used as a suitable animal for research related to pregnant woman. It is interesting to obtain about equal half-lives for BTM in ewe and rabbit.

Pharmacokinetics of BTM have been studied in healthy adults and pregnant women[6,12], BTM with a mean terminal half-life and clearance as follows: 6 h and 178 mL/min for healthy adults; 4 h and 287 mL/min for pregnant women. In our study, we determined the terminal elimination half-life and clearance of BTM in rabbits to be 3.8 hr and 3.5 mL/min/kg, respectively. The results were close to the reported pharmacokinetic parameters of pregnant women[12]. In pregnant woman study, the average body weight of 13 subjects at 76 kg obtained the clearance of BTM as 3.8 mL/min/kg (287 mL/min divided 76 kg). In addition, Trenque *et al.* also investigated BTM disposition in rabbits and obtained the elimination half-life and clearance of BTM to be 2 hr and 2.1 mL/min[10], respectively, which are significantly lower than those of our study results. It was possible that BTM concentrations in plasma

included a significant portion of BDP in Trenque *et al.* study, which led to overestimate the BTM plasma levels at early time points due to no further separating BDP and BTM in HPLC analysis[10].

According to our proposed pharmacokinetic model, *in vivo* hydrolysis rate constant for converting BDP to BTM was calculated from the slope by plotting logarithm BDP concentration versus time to be 0.0579 min^{-1}. The value was slightly higher than the input rate constant of BTM (0.0474 min^{-1}). The decline of BDP level in plasma was not only via the hydrolysis pathway, but a small portion of BDP (about 18%) might also be eliminated by renal excretion due to the hydrophilicity of BDP. Both BTM and BDP formulations have been used in clinical trials to improve precocious fetal lung maturation[19]. The severity of fetal growth restriction increases as the number of maternal BTM injections increases. Therefore, the pharmacokinetics of BTM in the fetal circulation is important for reducing the side effects of fetal growth restriction. Because the body weight and size of rabbit are similar to human fetal, the BDP and BTM pharmacokinetics of rabbits might be further investigated to apply in human fetal for optimizing antenatal glucocorticoid treatment.

In conclusion, we established an HPLC analytical method for simultaneously determining BDP and BTM in plasma samples. The method was capable of measuring BDP and BTM at early time points to characterize the pharamacokinetics of BDP and BTM following intravenous injection. The analytical method was simple and more convenient for routine measurement of BDP and BTM in plasma which might be applied to optimize the glucocorticoid treatment in respiratory relevant diseases.

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