

Pharmacokinetic characteristics of fluticasone, salmeterol and tiotropium after concurrent inhalation

Jung Sunwoo¹, Su-jin Rhee¹, SeungHwan Lee¹, Sang Won Lee¹, Jina Jung², Hankil Son² and In-Jin Jang^{1*}

¹Department of Clinical Pharmacology and Therapeutics, Seoul National University College of Medicine and Hospital, Seoul 03080, Republic of Korea, ²Hanmi Pharmaceutical Co., Ltd., Seoul 05545, Republic of Korea

*Correspondence: I. J. Jang; Tel: +82-2-2072-1910, Fax: +82-2-742-9252, E-mail: ijjang@snu.ac.kr



Received 10 May 2017

Revised 30 May 2017

Accepted 31 May 2017

Keywords

Fluticasone,
Salmeterol,
Tiotropium,
Pharmacokinetics,
Chronic Obstructive
Pulmonary Disease

pISSN: 2289-0882

eISSN: 2383-5427

Chronic obstructive pulmonary disease (COPD) is a type of progressive, obstructive lung disease characterized by long-term poor airflow. The symptoms of COPD may be relieved and its progression delayed by fluticasone (FTS), salmeterol (SM), and tiotropium (TTP). The aim of this study is to investigate pharmacokinetic (PK) characteristics of inhaled FTS, SM, and TTP after co-administration. An open-label, single-arm, three-period, simple ascending dose study was conducted in 10 healthy male subjects. A single dose of FTS/SM (250/50 µg) and TTP (18 µg) were concomitantly inhaled in period 1, and the dose of each drug was escalated to two- and three-fold in periods 2 and 3, respectively, with a 2-week washout between periods. Activated charcoal was co-administered before and after inhalation to block gastrointestinal absorption. Blood samples for PK analysis were collected up to 24 hours. PK parameters were obtained by non-compartmental analysis. FTS, SM, and TTP rapidly reached maximum plasma concentration after inhalation (0.08–3.00 h, 0.03–0.10 h and 0.03–0.10 h, respectively) and were eliminated with mean half-lives of 9.29–10.44 h, 6.09–12.39 h and 0.25–47.42 h, respectively. PK assessment of the lowest dose of TTP was limited due to relatively low systemic exposure compared to the lower limit of quantification. In conclusion, PK characteristics of FTS, SM, and TTP by pulmonary absorption were evaluated after concurrent inhalation. FTS and SM showed dose-proportional PK profiles between 250–750 µg and 50–150 µg, respectively, while TTP presented dose-proportionality in the early phase exposure between 18–54 µg.

Introduction

Chronic obstructive pulmonary disease (COPD) is a type of progressive, obstructive lung disease characterized by long-term poor airflow due to pulmonary and systemic inflammation. Common signs and symptoms of COPD include mucinous cough, shortness of breath, wheezing, and chest tightness. The cause of COPD is usually the long term exposure to lung irritants that damage the lungs and the airways such as cigarette smoke. COPD is currently ranked as the 4th leading cause of mortality worldwide and projected to be the 3rd leading cause of death by the year 2020.[1]

There is no definite cure for COPD, but the symptoms are

treatable and its progression could be delayed by using inhaled drugs. Orally inhaled fluticasone (FTS), salmeterol (SM), and tiotropium (TTP) are widely used to relieve the symptoms of COPD. FTS, an inhaled corticosteroid, prevents COPD exacerbation through its anti-inflammatory action. SM and TTP, which are a long-acting β_2 adrenergic receptor agonist and a long-acting antimuscarinic bronchodilator, respectively, inhibit parasympathetic activity, which is followed by bronchial smooth muscle relaxation and a decrease in the release of mucus into the airway lumen.[2,3]

Many different combinations among FTS, SM, and TTP have been studied lately. In particular, The Canadian Optimal Therapy of COPD trial undertaken in 2004 revealed better improvements in lung function, quality of life, and hospitalization rate in moderate to severe COPD patients treated with a three drug combination (FTS + SM + TTP) than the improvements in patients treated with a two-drug combination (TTP + SM

Copyright © 2017 Translational and Clinical Pharmacology
© It is identical to the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>).
© This paper meets the requirement of KS X ISO 9706, ISO 9706-1994 and ANSI/NISO Z.39.48-1992 (Permanence of Paper).

or TTP + placebo). Consequently, a combination of TTP and a drug formulation combining FTS and SM is commonly used to treat COPD in clinical situations.[3]

Although diverse pharmacokinetic (PK) data for FTS, SM, and TTP have been reported, there are no PK data after concurrent inhalation of those three drugs. In this study, we aimed to investigate the PK characteristics of FTS, SM, and TTP after concomitant inhalation of TTP (18 μg) and a combination formulation of FTS/SM (250/50 μg).

METHODS

Study design and subjects

The present study was designed as an open-label, single-arm, three-period, single ascending dose study. Korean male volunteers were eligible for the study if they were 19–45 years of age, weighed at least 55 kg, and had a body mass index of 18.0–27.0 kg/m^2 . Subjects were excluded if they had a history of or current clinically significant abnormalities based on medical history, physical examination, and clinical laboratory tests. In addition, subjects who smoked within the 6 months before the first dose were also excluded. The study protocol was reviewed and approved by the Institutional Review Board of Seoul National University Hospital (ClinicalTrials.gov registry number: NCT02441114). Written informed consent was obtained from subjects before any study-related procedure was performed, and the study was conducted according to the ethical principles of the Declaration of Helsinki and the Korean Good Clinical Practice Guidelines.

The subjects who met the screening criteria were admitted to the Clinical Trials Center at Seoul National University Hospital for 3 days in each treatment period, with a 14-day washout between periods. The study drugs were Seretide 250 Diskus® (GlaxoSmithKline, South Korea) which is a combination formulation of FTS/SM 250/50 μg (72.5 μg as salmeterol xinafoate), and Spiriva® HandiHaler® (Boehringer Ingelheim,

South Korea) which contains 18 μg TTP (22.5 μg as tiotropium bromide). Single dose of Seretide (FTS/SM 250/50 μg) and Spiriva (TTP 18 μg) were concomitantly inhaled in treatment period 1, and the dose of each drug was escalated by two-fold (FTS/SM 500/100 μg and TTP 36 μg) and three-fold (FTS/SM 750/150 μg and TTP 54 μg) in periods 2 and 3, respectively. The time taken for total inhalation was less than 2 min. In addition, Charcodote® (activated charcoal /sorbitol 50/140 g per 250 mL, Pharmascience, Canada) was orally administered to block the gastrointestinal absorption of the inhaled drugs as previously described: 5 g of Charcodote was administered at 2 min before and after inhalation, and a further 10 g was given at 1, 2, and 3 h after inhalation.[4] Blood samples for PK assessments were collected at 0, 0.03, 0.08, 0.17, 0.33, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24 h after inhalation (Fig. 1).

Quantification of plasma fluticasone, salmeterol, and tiotropium

Plasma concentrations of FTS, SM, and TTP were determined by using liquid chromatography with tandem mass spectrometry (LC/MS-MS): Shimadzu Prominence LC API 5000 (Shimadzu, Japan) coupled with TA5500, 5500 QTRAP MS (AB SCIEX, USA) for FTS and SM; Prominence UFLC XR (Shimadzu, Japan) coupled with 5500QTRAP, TQ5500 MS (AB SCIEX, USA) for TTP.

The internal standard material (IS) of FTS was fluticasone propionate-d3. The LC column was C_{18} (2.1 mm i.d. \times 50 mm; particle size 1.7 μm) and ion mode of MS/MS was APCI (atmospheric pressure chemical ionization; positive ion mode). The IS of SM was salmeterol-d3. The LC column was C_{18} (2.1 mm i.d. \times 100 mm; particle size 3 μm) and the MS/MS condition was ESI (electrospray; positive ion mode). The FTS or SM sample (500 μL) along with IS standard solution (10 μL ; 50 ng/mL) and ethyl ether (3 mL) were placed in a polypropylene tube. After vortexing for 5 min, the mixture was centrifuged at 3,000 rpm for 5 min. When the organic layer was fully dried, it was dissolved by

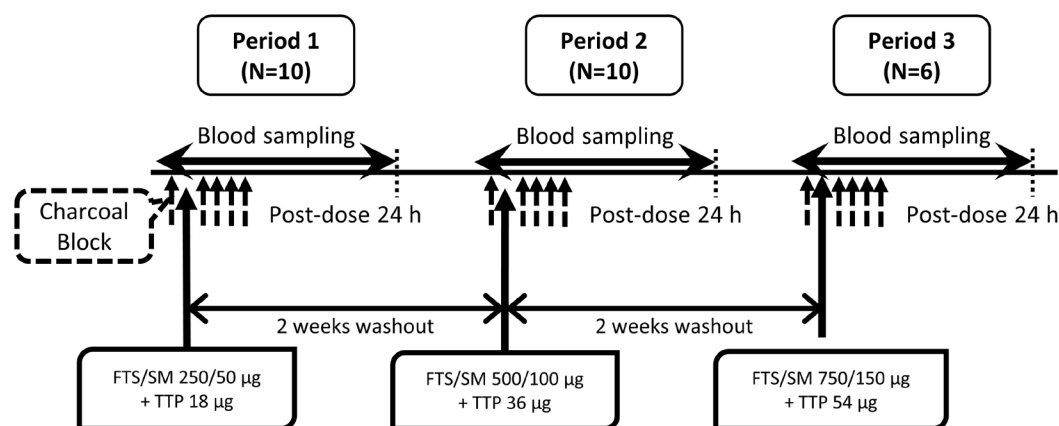


Figure 1. Schematic diagram of study flow. FTS, fluticasone propionate; SM, salmeterol; TTP, tiotropium.

adding 150 μL of 50% acetonitrile solution and then centrifuged at 13,000 rpm for 5 min. Product ion m/z for FTS was selected as 293.25 and that for SM was 380.4.

The IS for TTP was tiotropium-d3 bromide. The LC column was phenyl (2.1 mm i.d. \times 100 mm; particle size 1.8 μm) and the MS/MS condition was ESI (positive ion mode). The TTP sample (400 μL) along with IS standard solution (10 μL ; 10 ng/mL) and acetonitrile (1.2 mL) were added to a polypropylene tube. After mixing for 1 min, the mixture was centrifuged at 3,000 rpm for 5 min. The upper layer was separated by adding 2 mL of dichloromethane followed by 1 min of mixing and centrifuging at 4,000 rpm for 5 min. When the organic layer was fully dried, it was dissolved by adding 100 μL of 50% acetonitrile and then centrifuged at 13,000 rpm for 5 min. Product ion m/z for TTP was selected as 155.1.

Calibration curves showed linearity from the lower limit of quantification (LLOQ) to the upper limit of quantification in all three drugs: 2–200 pg/mL for FTS and SM; and 1–100 pg/mL for TTP. Coefficients of variation (CV) for precision and accuracy of quality control samples were below 15%, while that of the quantification control samples was below 20% for the LLOQ. These results indicate that the bioanalytical method was accurate and precise.

PK analysis

The PK parameters were calculated by using Phoenix[®] Win-Nonlin[®] (Version 6.3; Pharsight, Mountain View, CA, USA) with the non-compartmental method. Maximum plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) were obtained directly from the observed values. Area under the plasma concentration versus time curve from dosing to the last quantifiable concentration (AUC_{last}) was calculated using the linear trapezoidal method for ascending concentrations and the log trapezoidal method for descending concentrations. Extrapolation beyond the last plasma concentration was performed to gain AUC from the time zero to infinity (AUC_{inf}). For additional information on the early phase exposure of TTP, the AUC from the time zero to 30 min ($\text{AUC}_{0-30\text{min}}$) was calculated. The elimination half-life ($t_{1/2}$) was calculated as the natural logarithm of 2 divided by λ_z , where λ_z is the terminal elimination rate constant estimated from a regression line of log-transformed plasma concentrations versus time over the terminal log-linear portion. Apparent clearance (CL/F) was calculated as dose divided by AUC_{inf} . In addition, dose-normalized C_{max} (i.e., $C_{\text{max}}/\text{Dose}$) and AUC (i.e., $\text{AUC}_{\text{last}}/\text{Dose}$ for FTS and SM, and $\text{AUC}_{0-30\text{min}}/\text{Dose}$ for TTP) were calculated and compared among doses in each drug.

Safety and tolerability assessments

Safety and tolerability was assessed for all subjects who underwent test drug administration at least once. The frequency and aspect of adverse events (AEs) were reviewed generally by integrating the physical examination, vital sign, electrocardiogram,

and laboratory test results. Furthermore, the severity of AEs and their relationship to the test drugs were assessed.

Statistical analysis

Statistical analyses were performed by using the SAS program (Version 9.4; SAS Institute, Cary, NC, USA). Dose proportionality analysis on C_{max} , AUC_{last} , AUC_{inf} , and $\text{AUC}_{0-30\text{min}}$ were carried out by performing log-transformed data regressions (linear regression using a power model).[5] When the 95% confidence interval (CI) for the slope of the graph included 1, the drug was considered to have dose-proportional PK characteristics.[6]

Results

Subjects

Sixteen healthy Korean male volunteers were screened, and 10 subjects were enrolled in the study. Subject age ranged from 21 to 38 years, and mean height and weight ranges were 162.3–185.2 cm and 55.5–87.7 kg, respectively. Four subjects withdrew consent before admission to the third dosing period for private reasons; the other six subjects completed the study as per protocol. Consequently, the PK analysis was conducted for total 10 subjects for period 1 (FTS/SM 250/50 μg + TTP 18 μg) and period 2 (FTS/SM 500/100 μg + TTP 36 μg), whereas 6 subjects were included in the analysis for period 3 (FTS/SM 750/150 μg + TTP 54 μg).

PK results

Concentrations of FTS, SM, and TTP were detectable in plasma within 2 min after concomitant inhalation (Fig. 2). FTS was rapidly absorbed resulting in a T_{max} of 0.08–3.00 h and a mean $t_{1/2}$ ranged of 9.29–10.44 h, which were similar for each dose. The plasma concentrations of SM increased rapidly with a T_{max} of 0.03–0.10 h followed by a decline in a multi-phasic manner with a mean $t_{1/2}$ of 6.09–12.39 h for each dose (Table 1). The T_{max} of TTP was achieved within 0.03–0.05 h after inhalation and the plasma concentrations declined rapidly, resulting in a level below the LLOQ at 24 h in all dosages. Especially for the first dose (18 μg), plasma concentrations of TTP was lower than LLOQ in all subjects after 1.5 h.

For FTS and SM, systemic exposure levels (i.e., C_{max} and AUC) via pulmonary absorption increased in dose-proportional manners as the inhaled dose increased from 250 to 750 μg for FTS and 50–150 μg for SM, respectively (Fig. 3). The estimates of curve slopes in the power model for C_{max} and AUC were not significantly different from 1 for both FTS and SM (Table 2). For TTP, however, AUC_{last} and AUC_{inf} did not satisfy the dose-proportionality criteria. Nevertheless, TTP exposure in the early phase showed a dose-proportional increase in dose range of 18–54 μg , which was confirmed by the 95% CI of slope in power model for C_{max} and $\text{AUC}_{0-30\text{min}}$ including 1. In addition, dose-normalized PK parameters were similar among doses (Table 1 and Supplementary Fig. 1).

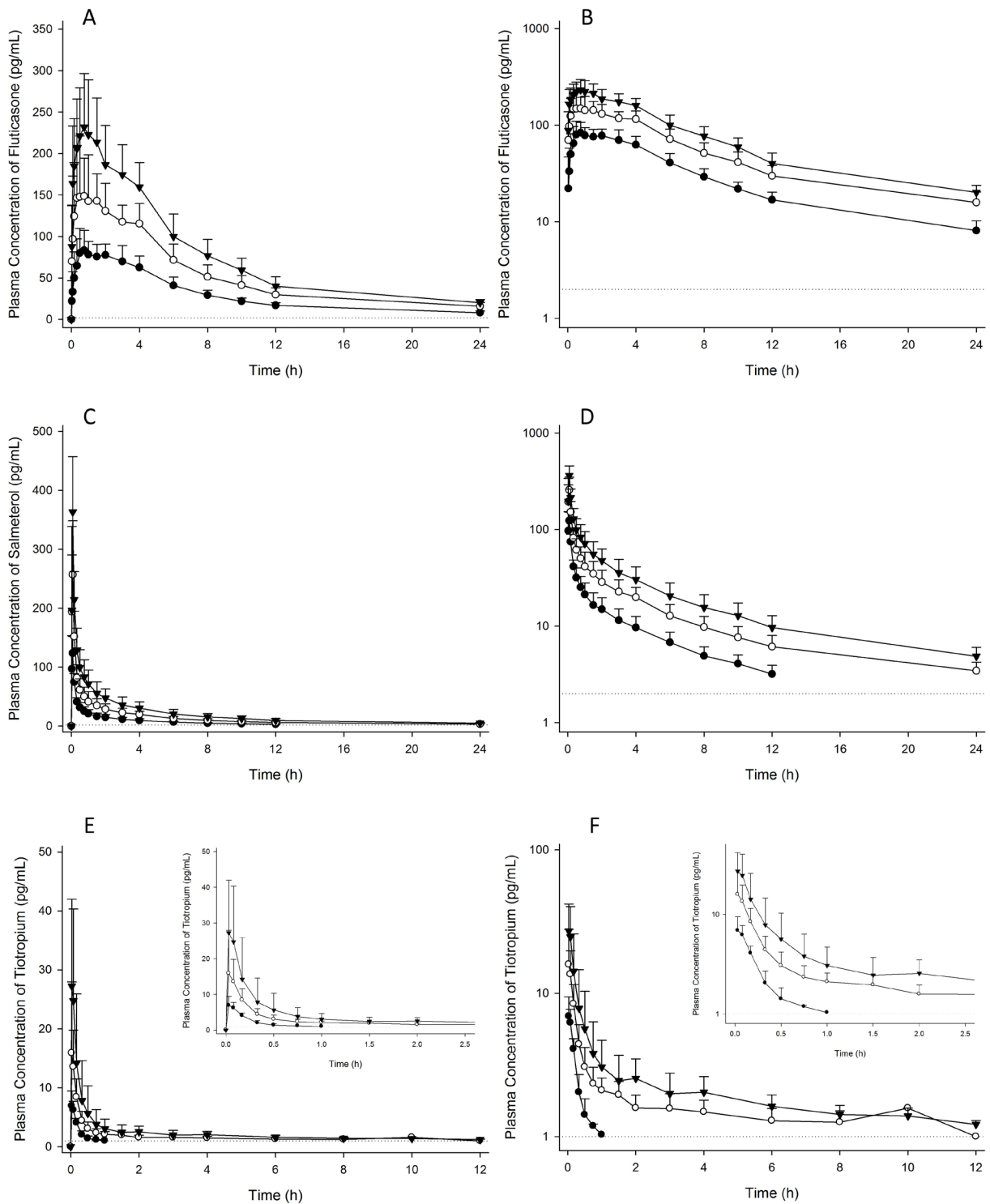


Figure 2. Mean plasma concentration-time profiles of FTS (A, B), SM (C, D) and TTP (E, F) after concomitant inhalation (A, C, and E in linear scale; and B, D, and F in log-linear scale). The closed circles represent FTS/SM 250/50 µg + TTP 18 µg (n=10), the open circles represent FTS/SM 500/100 µg + TTP 36 µg (n=10), and the closed triangles represent FTS/SM 750/150 µg + TTP 54 µg (n=6). The dotted lines represent lower limit of quantification. FTS, fluticasone; SM, salmeterol; TTP, tiotropium.

Table 1. Summary of pharmacokinetic parameters for fluticasone (FTS), salmeterol (SM), and tiotropium (TTP) after concomitant inhalation

Drug	Parameters	FTS 250 µg/SM 50 µg + TTP 18 µg (N=10)	FTS 500 µg/SM 100 µg + TTP 36 µg (N=10)	FTS 750 µg/SM 150 µg + TTP 54 µg (N=6)
Fluticasone	T _{max} (h)	0.76 [0.33 – 3.00]	0.50 [0.08 – 1.50]	0.88 [0.33 – 1.50]
	C _{max} (pg/mL)	95.70 ± 20.14	173.56 ± 44.32	246.77 ± 57.30
	C _{max} /Dose (pg/mL/µg)	0.38 ± 0.08	0.35 ± 0.09	0.32 ± 0.08
	AUC _{last} (h*pg/mL)	686.98 ± 111.36	1247.63 ± 276.51	1769.58 ± 370.80
	AUC _{last} /Dose (h*pg/mL/µg)	2.75 ± 0.45	2.50 ± 0.55	2.36 ± 0.49
	AUC _{inf} (h*pg/mL)	806.04 ± 131.46	1486.65 ± 324.23	2039.70 ± 406.90
	t _{1/2} (h)	9.86 ± 1.98	10.44 ± 1.60	9.29 ± 1.15
	CL/F (L/h)	318.83 ± 60.02	355.64 ± 102.14	381.80 ± 85.91
Salmeterol	T _{max} (h)	0.08 [0.03 – 0.10]	0.08 [0.03 – 0.10]	0.08 [0.03 – 0.10]
	C _{max} (pg/mL)	130.74 ± 36.06	265.22 ± 107.14	362.96 ± 94.11
	C _{max} /Dose (pg/mL/µg)	2.61 ± 0.72	2.65 ± 1.07	2.42 ± 0.63
	AUC _{last} (h*pg/mL)	128.62 ± 28.77	310.27 ± 89.39	480.96 ± 147.46
	AUC _{last} /Dose (h*pg/mL/µg)	2.57 ± 0.58	3.10 ± 0.89	3.21 ± 0.98
	AUC _{inf} (h*pg/mL)	156.49 ± 33.00	371.23 ± 97.09	554.04 ± 155.37
	t _{1/2} (h)	6.09 ± 1.63	12.39 ± 1.56	10.61 ± 1.96
	CL/F (L/h)	331.72 ± 65.76	284.25 ± 66.04	288.27 ± 76.00
Tiotropium	T _{max} (h)	0.05 [0.03 – 0.08]	0.03 [0.03 – 0.10]	0.04 [0.03 – 0.08]
	C _{max} (pg/mL)	7.17 ± 2.26	16.97 ± 11.29	27.31 ± 14.87
	C _{max} /Dose (pg/mL/µg)	0.40 ± 0.13	0.47 ± 0.31	0.51 ± 0.28
	AUC _{last} (h*pg/mL)	1.80 ± 0.59	12.11 ± 6.64	21.38 ± 14.59
	AUC _{inf} (h*pg/mL)	2.22 ± 0.74	84.27 ± 176.34	41.31 ± 26.75
	AUC _{0-30min} (h*pg/mL)	1.63 ± 0.35	3.54 ± 1.53	6.19 ± 4.57
	AUC _{0-30min} /Dose (h*pg/mL/µg)	0.09 ± 0.02	0.10 ± 0.04	0.11 ± 0.08
	t _{1/2} (h)	0.25 ± 0.12	47.42 ± 120.95	11.23 ± 13.32
CL/F (L/h)	8912.64 ± 2698.25	2950.02 ± 4735.09	2177.89 ± 1824.59	

Pharmacokinetic parameters are represented as mean±SD except for T_{max}, which is represented as median [minimum-maximum]. T_{max}, time to achieve C_{max}; C_{max}, maximum plasma concentration; C_{max}/Dose, dose normalized C_{max}; AUC_{last}, area under the plasma concentration-time curve from time zero to the last observed time point; AUC_{last}/Dose, dose normalized AUC_{last}; AUC_{inf}, AUC from time zero to infinity; t_{1/2}, elimination half-life; CL/F, apparent clearance; AUC_{0-30min}, AUC from time zero to 30 minute; AUC_{0-30min}/Dose, dose normalized AUC_{0-30min}.

Safety profiles and tolerability

There were 37 reports of AEs among the 10 subjects, and all AEs were assessed as not being- related to the test drug. The most common AE was diarrhea (*i.e.*, 27 cases among the 10 subjects), and those AE were assessed to be related to the sorbitol that was contained in the Charcodote®. There was no clinically significant abnormality in physical examinations, vital signs, electrocardiograms or laboratory test results during the study.

Discussion

Although there are many reports on studies comparing the effects of FTS, SM, and TTP[7-9] there are no reports assessing PK characteristics when all three drugs are administered con-

currently. To describe the pharmacokinetic-pharmacodynamic relationship of drug, PK studies need to be performed. This study is the first pilot study to evaluate PK characteristics via lung absorption when FTS, SM, and TTP were inhaled concomitantly. In this study, the plasma concentrations of drugs were measurable allowing estimation of lung exposure through systemic exposures.

One of the main characteristics of COPD is chronic lung inflammation and airway remodeling,[10] and it has recently been shown that airway inflammation resides not only in the larger airways but also in the peripheral airways.[11] Therefore, elucidating the local effects of drugs in the lung are important in the treatment of COPD. For the accurate estimation of local effects

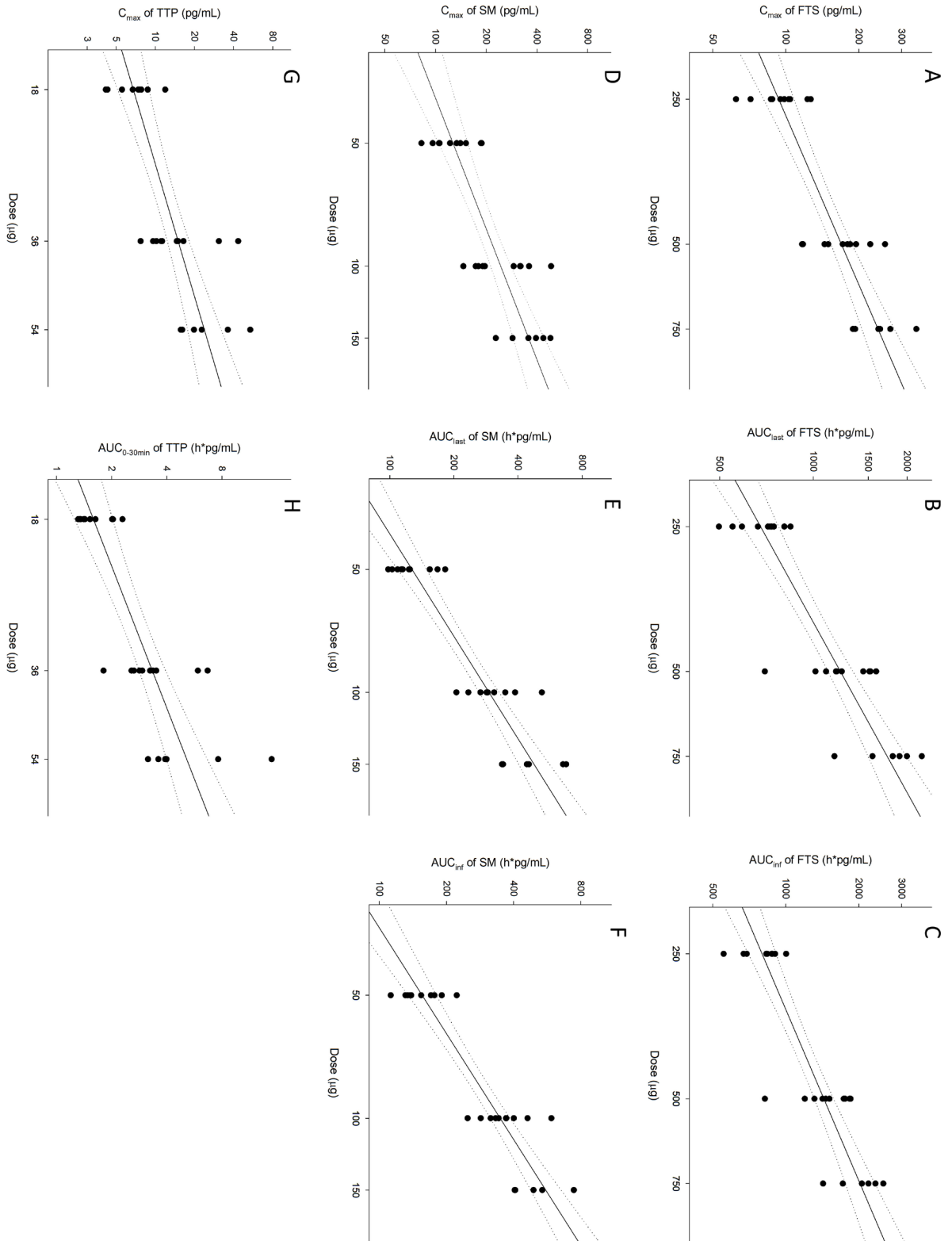


Figure 3. Scatterplot of dose-proportionality assessment for fluticasone (FTS), salmeterol (SM), and tiotropium (TTP). The circles represent pharmacokinetic parameters of each subject. The solid line denotes the predicted line from the power model. The dotted line indicates 95% confidence intervals for the predicted line. C_{max} : maximum plasma concentration; AUC_{last} : area under the plasma concentration-time curve from time zero to the last observed time point; AUC_{inf} : AUC from time zero to infinity; $AUC_{0-30min}$: AUC from time zero to 30 minute.

Table 2. Assessment of dose-proportionality for pharmacokinetics of fluticasone, salmeterol, and tiotropium using power model regression

Study drug	Parameters	Slope (95% confidence interval)
Fluticasone	C _{max} (pg/mL)	0.8584 (0.6458 – 1.0710)
	AUC _{last} (h*pg/mL)	0.8513 (0.6562 – 1.0463)
	AUC _{inf} (h*pg/mL)	0.8466 (0.6528 – 1.0403)
Salmeterol	C _{max} (pg/mL)	0.9410 (0.6467 – 1.2353)
	AUC _{last} (h*pg/mL)	1.2010 (0.9709 – 1.4311)
	AUC _{inf} (h*pg/mL)	1.1616 (0.9457 – 1.3776)
Tiotropium	C _{max} (pg/mL)	1.1452 (0.7355 – 1.5548)
	AUC _{0-30min} (h*pg/mL)	1.0674 (0.7143 – 1.4206)
	AUC _{last} (h*pg/mL)	2.1977 (1.6239 – 2.7716)
	AUC _{inf} (h*pg/mL)	2.7928 (1.8118 – 3.7739)

Power model: $\ln(Y) = (\text{Intercept}) + (\text{Slope}) \times \ln(\text{Dose})$, Y: pharmacokinetic parameter. C_{max}, maximum plasma concentration; AUC_{last}, area under the plasma concentration-time curve from time zero to the last observed time point; AUC_{inf}, AUC from time zero to infinity; AUC_{0-30min}, AUC from time zero to 30 minute.

of drugs, there is a need to evaluate the pulmonary exposure of the drug. However, there is no appropriate method for evaluating pulmonary exposure directly. Regardless, systemic exposure of orally inhaled drug is probably related to the drug passing through the alveolar-capillary interface and into the mucosal and submucosal vessels.[2] Thus, systemic exposure could reflect pulmonary exposure, and it is appropriate to evaluate pulmonary exposure by measuring plasma concentrations. Orally inhaled drugs can also be absorbed by the gastrointestinal (GI) tract. In this study, to block GI tract absorption of drugs, we co-administered activated charcoal. In general, charcoal co-administration is important if the objective of a trial is to evaluate the relative contributions of oral and pulmonary absorption to the total systemic exposure of a drug.[12] Therefore, concomitant administration of activated charcoal was an appropriate method to block the GI tract absorption of the test drugs in this study.

The results of this study revealed a TTP t_{1/2} of up to 47.4 h, which is notably shorter than the previous reported TTP t_{1/2} of 5–6 days.[13,14] However, the previous reported t_{1/2} was estimated from the calculated geometric mean TTP concentrations which were standardized to dose of 10 µg assuming dose-proportional PK of TTP. Although the LLOQ was lowered in this study (*i.e.*, 1 pg/mL) compared to that of previous study (*i.e.*, 4.8 pg/ml), the concentrations of TTP after 12 h in all subjects were below the LLOQ resulting in a lack of information about the elimination phase. Therefore, further assessment with a lower LLOQ is required to investigate more accurate elimination characteristics of TTP.

The PK characteristics of FTS, SM and TTP could have some differences in COPD patients. Systemic availability of FTS is

reported to be substantially lower in moderate to severe asthma patients than in healthy controls.[15] When COPD patients inhaled 500 µg of FTS, its t_{1/2} was shorter and T_{max} was larger than those in this study. Furthermore, when COPD patients inhaled 50 µg of SM, t_{1/2} was longer and T_{max} was larger than those observed in the present study.[7,16] Based on these results, direct extrapolation of this study to COPD patients may have limitations. Nonetheless, it is important to evaluate the inherent PK characteristics in healthy subjects, an approach that excludes drug-drug interactions with concomitant medications taken by patients. Although FTS, SM, and TTP were concomitantly used in this pilot study, there was no control group for each drug, which makes it difficult to evaluate the exact drug-drug interactions.

In conclusion, PK evaluation of systemic drug exposure was possible when FTS, SM, and TTP were orally inhaled concurrently. FTS and SM showed dose-proportional PK profiles between 250-750 µg and 50-150 µg, respectively. In addition, TTP exhibited dose-proportionality in early phase exposure (*i.e.*, AUC_{0-30min}) between 18-54 µg.

Acknowledgements

This study was supported by Hanmi Pharmaceutical Co., Ltd., Seoul, 05545, Republic of Korea.

Conflict of interest

The authors have no conflicts of interest to disclose.

References

- Koumis T, Samuel S. Tiotropium bromide: A new long-acting bronchodilator for the treatment of chronic obstructive pulmonary disease. *Clin Ther* 2005;27:377-392. doi:10.1016/j.clinthera.2005.04.006.
- Cazzola M, Testi R, Matera MG. Clinical pharmacokinetics of salmeterol. *Clin Pharmacokinet* 2002;41:19-30.
- Jung KS, Park HY, Park SY, Kim SK, Kim YK, Shim JJ, et al. Comparison of tiotropium plus fluticasone propionate/salmeterol with tiotropium in COPD: a randomized controlled study. *Respir Med* 2012;106:382-389. doi: 10.1016/j.rmed.2011.09.004.
- Borgström L, Nilsson M. A method for determination of the absolute pulmonary bioavailability of inhaled drugs: terbutaline. *Pharm Res* 1990;7:1068-1070.
- Gough K, Hutchison M, Keene O, Byrom B, Ellis S, Lacey L, et al. Assessment of dose proportionality: Report from the statisticians in the pharmaceutical industry/pharmacokinetics UK joint working party. *Ther Innov Regul Sci* 1995;29:1039-1048.
- Smith BP, Vandenhende FR, DeSante KA, Farid NA, Welch PA, Callaghan JT, et al. Confidence interval criteria for assessment of dose proportionality. *Pharm Res* 2000;17:1278-1283.
- Daley-Yates PT, Mehta R, Chan RH, Despa SX, Louey MD. Pharmacokinetics and pharmacodynamics of fluticasone propionate and salmeterol delivered as a combination dry powder from a capsule-based inhaler and a multidose inhaler in asthma and COPD patients. *J Aerosol Med Pulm Drug Deliv* 2014;27:279-289. doi: 10.1089/jamp.2013.1040.
- Mehta R, Montembault M, Warren F, Gupta A, Brealey N, Moore A. Systemic exposures of fluticasone propionate and salmeterol following inhalation via metered dose inhaler with the mini spacer compared with aerochamber plus spacer. *J Aerosol Med Pulm Drug Deliv* 2016;29:386-392. doi: 10.1089/jamp.2015.1236.
- Aaron SD, Vandemheen KL, Fergusson D, Maltais F, Bourbeau J, Goldstein R, et al. Tiotropium in combination with placebo, salmeterol or fluticasone-salmeterol for treatment of chronic obstructive pulmonary disease: a randomized

- trial. *Ann Intern Med* 2007;146:545-555.
10. Dentener MA, Vermooy JH, Hendriks S, Wouters EF. Enhanced levels of hyaluronan in lungs of patients with COPD: relationship with lung function and local inflammation. *Thorax* 2005;60:114-119. doi: 10.1136/thx.2003.020842.
 11. Meijer RJ, Kerstjens HA, Arends LR, Kauffman HF, Koëter GH, Postma DS. Effects of inhaled fluticasone and oral prednisolone on clinical and inflammatory parameters in patients with asthma. *Thorax* 1999;54:894-899.
 12. Hochhaus G, Möllmann H, Derendorf H, Gonzalez-Rothi RJ. Pharmacokinetic/Pharmacodynamic aspects of aerosol therapy using glucocorticoids as a model. *J Clin Pharmacol* 1997;37:881-892.
 13. Disse B, Speck GA, Rominger KL, Witek TJ Jr, Hammer R. Tiotropium (Spiriva™): Mechanical considerations and clinical profile in obstructive lung disease. *Life Sci* 1999;64:457-464.
 14. Durham MC. Tiotropium (Spiriva): a once-daily inhaled anticholinergic medication for chronic obstructive pulmonary disease. *BUMC Proc* 2004;17:366-373.
 15. Brutsche MH, Brutsche IC, Munawar M, Langley SJ, Masterson CM, Daley-Yates PT, et al. Comparison of pharmacokinetics and systemic effects of inhaled fluticasone propionate in patients with asthma and healthy volunteers: a randomized crossover study. *Lancet* 2000;356:556-561.
 16. Singh SD, Whale C, Houghton N, Daley-Yates P, Kirby SM, Woodcock AA. Pharmacokinetics and systemic effects of inhaled fluticasone propionate in chronic obstructive pulmonary disease. *Br J Clin Pharmacol* 2003;55:375-381.