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ISSN 0946-1965

DOI 10.5414/CP201644
e-pub: February 27, 2012

A positron emission tomography microdosing study with sertraline in healthy volunteers

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Key words

microdosing – CNS drug
– positron emission
tomography – pharma-
cokinetics – sertraline

Abstract. Objective: This study explored microdosing methods for evaluating the distribution and pharmacokinetics (PK) of a central nervous system (CNS) drug candidate. **Methods:** We used sertraline as a model drug. In this open-label, one-arm, three-period, multiple-dosing study, 10 healthy male volunteers received 6-day administrations of sertraline at doses of 5, 25 or 50 mg/d in three different periods. Before the first dose of Period 1, and 24 h after the last dose of each period, an intravenous bolus of [¹¹C]sertraline was injected for positron emission tomography (PET) scanning. After the sixth dose in each period, serial blood samples were collected at scheduled intervals over 48 h; then serum sertraline concentrations were determined with liquid chromatography-tandem mass spectrometry (LC-MS/MS). **Results:** Sertraline was distributed in the brain within 20 min, and it was highly distributed in the putamen, cingulate, and thalamus. Linearity in steady-state C_{max} and AUC_{last} were observed in the 5 – 50 mg dose range. The results suggested that microdosing with PET was a useful method for exploring the blood-brain-barrier penetration and distribution of a candidate CNS drug. **Conclusions:** This study described a microdosing method that combined PET with LC-MS/MS for determining the brain distribution and PK characteristics of a CNS drug candidate.

dose is intentionally small to avoid producing a pharmacological effect or adverse reactions in humans [1]. Previous studies have proposed methods for microdosing that employed highly sensitive techniques, including accelerator mass spectrometry (AMS) or positron emission tomography (PET) [8, 12]. Guidelines for these exploratory clinical trials have been issued by regulatory authorities; the European Medicines Agency issued the “Position paper on non-clinical safety studies to support clinical trials with a single microdose” in 2004 [1]; the Federal Drug Administration issued guidelines for “Exploratory IND studies” in 2006 [4]; and the International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH) issued the M3 guideline “Guidance on nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals” in 2008 [3].

Several microdosing studies have aimed at evaluating the predictability of these microdose methods for determining the pharmacokinetic and/or pharmacodynamic characteristics of known drugs [6, 26, 29, 31]. They were conducted with doses that ranged from a microdose to a therapeutic dose. Those studies used PET scanning with a radio-labeled drug, AMS, or liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods. They reported “lower limits of quantification (LLOQ)” that were lower than those reported in other studies. More recently, microdosing studies have been conducted for newly developed drugs [7, 14]. For example, an underdeveloped drug for Alzheimer’s disease was investigated in a microdos-

Received
August 16, 2011;
accepted
October 19, 2011

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Introduction

In drug development, human microdosing studies are conducted at an early stage to evaluate the basic pharmacokinetics or distribution characteristics of a novel drug. A microdose is defined as 1/100th of the pharmacological dose determined from modeling studies, or 100 µg, whichever is smaller. The

ing study with PET imaging to explore drug biodistribution and characteristics [7].

Drugs that target areas in the central nervous system (CNS) must penetrate the blood brain barrier (BBB) and reach the appropriate brain region to provide sufficient brain exposure. Over 98% of small molecules do not effectively pass the BBB, which results in inadequate brain exposure [23] and failure in drug development [27]. Therefore, CNS drug development would benefit from a microdosing approach for predicting the biodistributions and pharmacokinetic profiles of novel drugs in early phase clinical trials [7].

Sertraline (Zoloft[®], Pfizer, New York, NY, USA), an antidepressant, is a selective serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitor (SSRI) that targets receptors in the CNS. Many studies have characterized the pharmacokinetics and pharmacodynamics of sertraline. Sertraline reached maximum concentration at 4–8 h after oral administration and was eliminated with a 22–36 h half-life. A linear pharmacokinetic profile was reported for the 50–200 mg dose range [30]. The therapeutically effective dose of sertraline was 50 mg/d, which inhibited serotonin uptake by 76–80% [11, 28].

The present study explored the potential of the microdose method for predicting the distribution and pharmacokinetic characteristics of CNS drug candidates. Since this study was intended as a method exploration study rather than an actual study in a new drug candidate, we selected a compound which is already authorized for marketing and of which the pharmacological properties are relatively well known. Among the conventional drugs, we used sertraline as a model CNS drug for purposes of this study.

Subjects and methods

Subjects

Within 3 weeks of the first drug administration, all subjects were evaluated with a physical examination that included a neurological examination, measurement of vital signs, a 12-lead electrocardiogram (ECG), serology, a urine screen for drug abuse and routine clinical laboratory tests. Subjects

were excluded when they met the following exclusion criteria: history of significant clinical illness that required medical caution, including cardiovascular, immunologic, hematologic, neuropsychiatric, respiratory, gastrointestinal, hepatic or renal disease or other chronic disease; a history or evidence of drug abuse; use of any prescription medication or OTC medication. The subjects' medical and alcohol/medicine abuse histories were accessed in an interview conducted by investigators and by the subject's self-report. And additional urinary drug testing was performed within 3 weeks of the first administration of the study drug.

Eleven eligible healthy Korean male volunteers were enrolled. Each volunteer gave written informed consent before enrollment. The study protocol was approved by the Institutional Review Board of Seoul National University Hospital. All procedures were performed in accordance with the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use-Good Clinical Practices [2] and the recommendations of the Declaration of Helsinki on biomedical research involving human subjects [5].

Study design

This was an open-label, three-period, single sequence, multiple-dosing study. The study consisted of three consecutive treatment periods of 7 days each, with no wash-out in between doses. Each treatment period comprised a daily, oral, single dose of sertraline for 6 days. On the 7th day of each period, no dose was given, and the PET scan was performed. The doses increased each treatment period, starting with 5 mg in the first, 25 mg in the second and 50 mg in the third period. Subjects were prohibited from using any drugs within 7 days prior to the study.

On the 6th day of each study period, sertraline was given after overnight-fasting, and the subject maintained the semi-fowler position and a fasting state until 4 h after drug administration. Each dose was administered with 240 ml of water. Serial blood samples were drawn over the next 48 h for pharmacokinetic analyses.

Before the first oral sertraline administration of Period 1 (baseline), and 24 h after the last dose of each period, an intravenous, microdose bolus of [^{11}C]sertraline was administered, and the PET scan was performed.

Synthesis of [^{11}C]sertraline

[^{11}C]sertraline was prepared in the Department of Nuclear Medicine, Seoul National University Hospital, according to a published method [19]. Briefly, [^{11}C]carbon dioxide was produced by irradiating nitrogen gas with 13 MeV of protons with the TR13 cyclotron (EBCO. Co., Richmond, BC, Canada). [^{11}C]methyl iodide was produced from lithium aluminum hydride and hydroiodide [10]. Distilled [^{11}C]methyl iodide was passed through a preheated (230 °C) silver triflate-graphac gas chromatography column to produce [^{11}C]methyl triflate. Then, the [^{11}C]methyl triflate was reacted with 1 mg of norsesraline (3 μM) and 1 M sodium hydroxide (3 μM) to produce [^{11}C]sertraline. The [^{11}C]sertraline was purified by high-performance liquid chromatography (column: Waters XTerra RP-8, 10 \times 250 mm, 10 μm ; Waters, Milford, MA, USA; eluent: EtOH 55% in 10 mM phosphate buffer at pH 7; flow rate: 3 ml/min). The specific activity of [^{11}C]sertraline was 577.2 ± 181.3 MBq. The mean (range) amount of radiotracer injected into subjects was 690.42 (569.8 – 832.5) MBq, and it contained less than 12.8 nmol of sertraline.

PET scan and serotonin transporter SUV measurement

PET scans were performed in 2D mode on the whole body scanner, ECAT Exact 47 (Siemens, Knoxville, TN, USA). Radiotracer was injected via a catheter placed in the vein of the subject's arm before the scan started. After 690.42 MBq (range 569.8 – 832.5 MBq) of [^{11}C]sertraline was administered, the scan was acquired for 90 min with a variable frame duration (15 s \times 8, 30 s \times 16, 60 s \times 10, 240 s \times 10, 300 s \times 12). With a filtered back projection, scanned images were reconstructed as 128 \times 128 \times 47 matrices of 2.57 \times 2.57 \times 3.375 mm voxels. Eight

regions of interest were analyzed according to a method previously reported [16]. These included the cerebellum, thalamus, caudate nucleus, cingulate, hippocampal formation, putamen, temporal lobe and occipital lobe. The standardized uptake values (SUV) in the striatum were calculated and compared to the reference region as a ratio (SUV_r), as follows: $\text{SUV}_r = (\text{SUV of the specific region}) / (\text{SUV of the reference region})$. SUV_r measurements were obtained between 50 and 90 min after [^{11}C]sertraline injection.

To obtain accurate delineation of the brain regions for data analysis, each subject underwent magnetic resonance imaging (MRI; GE 3.0T VH/i SIGMA EXCIE E2M4, T1-weighted, 3D, SPGR). MRI images were 0.94 \times 0.94 \times 1.00 mm pixel in size.

Pharmacokinetic analysis

On the 6th day of each treatment period, an indwelling cannula was inserted into a forearm vein, and blood samples (8 ml) were collected at scheduled times: before (0 h), and 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24 and 48 h after the oral sertraline dose.

Individual pharmacokinetic parameters were determined with the non-compartmental methods used in Phoenix[®] software (Pharsight Corporation, St. Louis, MO, USA). The terminal elimination half-life ($t_{1/2}$) was calculated from a linear regression of log-transformed serum concentrations over the time course for each individual, where $t_{1/2} = \ln(2) / \lambda_z$. The area under the plasma concentration-time curve over the dosing interval (24 h) at steady-state ($\text{AUC}_{\tau, \text{ss}}$) was calculated with the linear-up and log-down trapezoidal method on serum concentration-time curves. λ_z is the terminal elimination rate constant. The accumulation ratios were calculated as $1 / (1 - e^{-\lambda_z \tau})$.

Measurement of drug concentrations

A rapid, sensitive, specific method was employed for quantification of plasma sertraline. Fluoxetine was used as the internal standard (IS). The sample preparation involved a simple liquid-liquid extraction procedure [17, 25]. The extract was analyzed with high

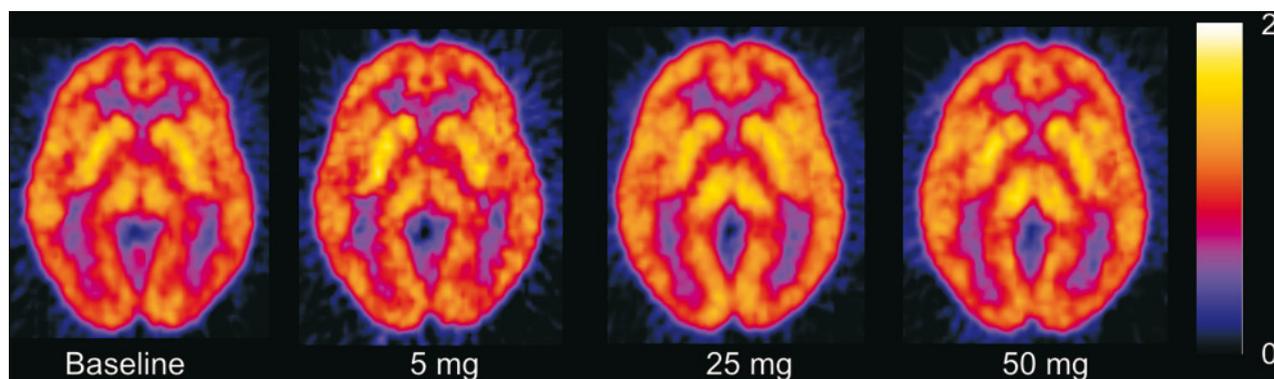


Figure 1. Representative [^{11}C]sertraline PET images of the human brain, before (baseline) and 24 h after 6-day administrations of sertraline at different doses in a typical subject. Bar represents a color-coded scale of the range of standardized uptake values ratios (SUVr); (SUV of the specific region)/(SUV of the reference region).

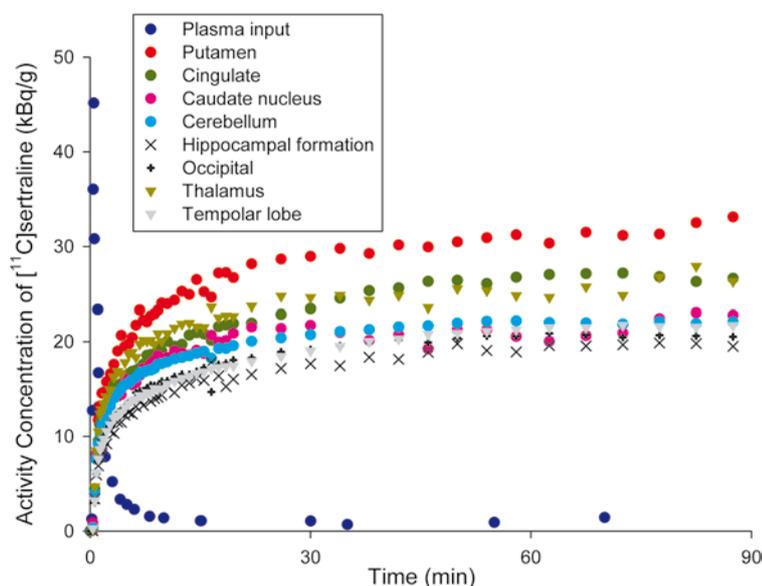


Figure 2. Activity concentration of [^{11}C]sertraline over time in the regions of interest at baseline in a typical subject.

performance liquid chromatography coupled to electrospray tandem mass spectrometry (LC-MS/MS). The analyte and IS were separated on a C18 reversed phase column (Luna, $5\ \mu\text{m} \times 50\ \text{mm} \times 2.0\ \text{mm}$, Phenomenex Inc., Torrance, CA, USA), with a mobile phase composed of acetonitrile (B) and 10 mM ammonium acetate (A) with 0.1% formic acid. Separation proceeded by gradient elution (10 – 45% B from 0 to 1.4 min, 90% B from 1.5 to 2.0 min, 10% B to 3 min). Quantitation was performed in positive ion and multiple reaction monitoring (MRM) mode. We monitored protonated precursor product ion transitions of $m/z\ 306.2 \rightarrow 275.2$ for sertraline and $310.6 \rightarrow 148.4$ for the IS. The method was fully validated for selectivity, linearity,

accuracy, precision and stability. The sertraline concentration was a linear function over the range from 0.05 to 50 ng/ml, and the lower limit of quantification (LLOQ) in serum was 0.05 ng/ml. The accuracy for the within- and between-run values ranged from 87.17 to 110.05% and from 87.91 to 101.99%, respectively. The precision levels for the within- and between-run values were below 14.71 and 9.64%, respectively.

Statistical analysis

Dose linearity was tested for $C_{\text{max,ss}}$ and $\text{AUC}_{\tau,ss}$ by linear regression analysis to evaluate whether sertraline demonstrated dose-independent behavior [9]. Analysis of variance (ANOVA) tests were used for comparisons among dose-normalized $C_{\text{max,ss}}$ and $\text{AUC}_{\tau,ss}$. The Kruskal-Wallis test was performed for comparisons among SUVr values in the putamen, cingulate, and caudate at baseline and sertraline dose of 5 mg, 25 mg and 50 mg dosing. All significance levels ($p < 0.05$) were obtained from two-sided tests. Statistical analyses were performed with SPSS[®] 17.0 software (SPSS Inc., Seoul, Korea) and SAS[®] 9.2 (SAS Institute Inc., Cary, NC, USA).

Results

Subjects

Eleven volunteers were enrolled, and 1 subject dropped out the study; that subject withdrew informed consent before drug administration. Ten volunteers completed the study with a mean age (range) of 27.5 (21.0 – 43.0) years,

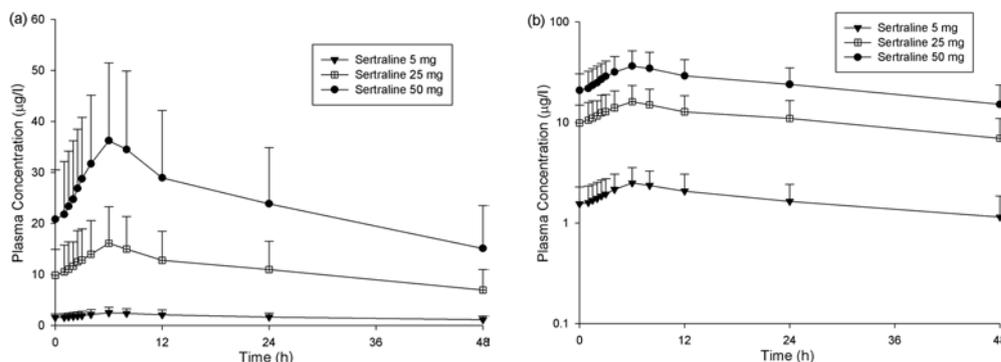


Figure 3. Mean serum concentration (SD) of sertraline in 10 healthy volunteers after 6-day oral administrations of sertraline at doses of 5, 25, or 50 mg/day. a: linear scale; b: semi-logarithmic scale.

Table 1. Standardized uptake value ratios (SUVr) in 10 healthy volunteers in the putamen, cingulate and caudate nucleus after [^{11}C]sertraline administration.

Regions of interest	Baseline	Sertraline 5 mg	Sertraline 25 mg	Sertraline 50 mg	χ^2 *	p value*
Putamen	1.40 (0.17)	1.36 (0.23)	1.36 (0.16)	1.41 (0.17)	1.574	0.665
Cingulate	1.19 (0.13)	0.96 (0.71)	1.18 (0.14)	1.19 (0.13)	0.824	0.844
Caudate nucleus	1.09 (0.20)	1.09 (0.22)	1.10 (0.17)	1.11 (0.19)	0.072	0.995

Values are mean (SD). * The Kruskal-Wallis test was performed among baseline, sertraline 5 mg, 25 mg and 50 mg treatment.

Table 2. Pharmacokinetic results for 5, 25 and 50 mg doses of sertraline.

Parameters	Sertraline 5 mg	Sertraline 25 mg	Sertraline 50 mg	F value	p value*
$C_{\max,ss}$ (ng/ml)	2.55 ± 1.03	16.27 ± 7.20	37.28 ± 16.02	29.66	< 0.001
$C_{\max,ss}/\text{dose}$ (ng/ml/mg)	0.51 ± 0.21	0.65 ± 0.27	0.75 ± 0.32	1.84	0.179
$AUC_{\tau,ss}$ (ng × h/ml)	47.55 ± 21.03	305.17 ± 139.82	681.62 ± 301.03	27.58	< 0.001
$AUC_{\tau,ss}/\text{dose}$ (ng × h/ml/mg)	9.51 ± 4.21	12.21 ± 5.59	13.63 ± 6.02	1.54	0.232
CL_{ss}/F (l/h)	135.38 ± 88.41	143.15 ± 75.43	92.82 ± 55.98	1.08	0.353
t_{\max} (h) [†]	6.0 (3.98 – 8.00)	6.0 (4.0 – 8.0)	6.0 (4.0 – 8.0)	0.70	0.503
$t_{1/2}$ (h)	36.7 ± 12.1	37.8 ± 8.34	39.1 ± 11.1	0.13	0.353
Accumulation ratio	2.75 ± 0.71	2.81 ± 0.49	2.89 ± 0.65	0.13	0.881

$C_{\max,ss}$ = maximum concentration at steady state; $C_{\max,ss}/\text{dose}$ = dose normalized $C_{\max,ss}$; $AUC_{\tau,ss}$ = area under the plasma concentration-time curve during the dosing interval ($\tau = 24$ h) at steady-state; $AUC_{\tau,ss}/\text{Dose}$ = dose normalized $AUC_{\tau,ss}$; CL_{ss}/F = apparent clearance at steady-state; t_{\max} = time at maximum concentration; $t_{1/2}$ = terminal elimination half-life, values (except t_{\max}) represent the arithmetic mean ± SD among 10 subjects. *ANOVA test was performed among 5 mg, 25 mg, 50 mg. t_{\max} values are presented as the median (minimum – maximum).

mean weight (range) of 66.3 (64.4 – 79.5) kg, and individual body weight values were within 80 – 120% of ideal body weight.

PET-standardized uptake values

Figure 1 shows the brain PET images of a representative subject taken at baseline and at the end of Periods 1 – 3. Further analysis (Figure 2) showed that the putamen had the highest uptake of [^{11}C]sertraline, and the

cingulate had the next highest uptake. These results were consistent with results from a previous study [24]. The SUVr values were referenced to the SUV of the cerebellum [21]. The main SUVr findings are presented in Table 1.

Pharmacokinetics

All three doses achieved a mean peak concentration at 6 h (range 3.98 – 8.0 h). For

5, 25 and 50 mg doses, the mean terminal elimination half-lives were 36.7, 37.8 and 39.1 h, respectively (Table 2) (Figure 3); the mean (SD) dose normalized $C_{\max,ss}$ values were 0.51 (0.21), 0.65 (0.29) and 0.75 (0.32) mg/l/mg, respectively ($p = 0.179$); and the dose normalized $AUC_{\tau,ss}$ values were 9.51 (4.21), 12.21 (5.59) and 13.63 (6.02) mg \times h/l/mg, respectively ($p = 0.232$). Dose linearity in the $C_{\max,ss}$ and $AUC_{\tau,ss}$ was assessed with linear regression and expressed in terms of 95% confidence intervals (CIs) of the intercept. For $C_{\max,ss}$ and $AUC_{\tau,ss}$, the CIs were -8.54 to 4.64 (intercept: -1.95) and -156.4 to 92.3 (intercept: -32.0), respectively. The mean accumulation ratios were between 2.75 and 2.89.

Safety

All subjects who received at least one dose of the study drug were included in the safety assessment ($n = 10$). A total of 12 adverse events (AEs) was reported in 6 of 10 subjects. All AEs were transient, and the subjects recovered spontaneously without intervention. All AEs were of mild intensity, and the subjects showed no significant changes in vital signs, ECGs, laboratory tests, or physical examinations.

Discussion

We explored the potential of the microdose method by testing its predictions of sertraline biodistribution and pharmacokinetic characteristics. We demonstrated that PET analysis could predict the brain distribution and that LC-MS/MS could predict the pharmacokinetics of low doses of sertraline. This study described a microdosing method that combined PET with a LC-MS/MS-based pharmacokinetic assessment. The results showed that sertraline was rapidly distributed in the brain and slowly eliminated. The region with the highest concentration was the putamen and the region with the lowest concentration was the cerebellum. The results were consistent with other PET studies of sertraline [11, 28]. There was no significant trend in the mean SUVr values measured at steady state for dose increments of 5, 25 and

50 mg sertraline. A linear pharmacokinetic profile was observed in the dose range of 5 – 50 mg of sertraline.

The PET results demonstrated that the drug penetrated the BBB and reached the expected region. Sertraline was distributed throughout the brain within 20 min, and it was highly distributed in the putamen, cingulate, and thalamus. Sertraline and other SSRIs primarily target the 5-HT_{2A} receptors, which are highly expressed in the striatum which includes putamen and caudate in brain [20].

Our results also showed that little drug was eliminated during the 90 min PET scan. This might be related to physicochemical properties of the radio-labeled ligand. Slow elimination may result from a high affinity or non-specific binding; for example, some radioligands exhibit amine binding. SSRIs, including fluoxetine, paroxetine, and sertraline have relatively high affinity for 5-HT transporters; thus, they have been radio-labeled with ¹¹C or ¹⁸F for use as radiotracers for PET. However, these tracers have shown extensive lipophilicity, which resulted in high levels of nonspecific binding and slow clearance rates. Among the mentioned SSRIs, sertraline has the highest affinity to sigma receptors ($K_i = 57$ nM) [22], which are known to be present at high density in the human cerebellum [18]. This may have contributed to our observations of high nonspecific binding of sertraline and its slow elimination. Thus, the interpretation of the PET results requires an understanding of the physicochemical properties of the radioligand. Moreover, the chemical structure and metabolism of the drug should be considered in the interpretation of the PET images.

The SUVr did not show a typical pattern in this study. This may have been due to the relatively high steady-state levels of sertraline, which may have saturated the transporters. On the other hand, down-regulation of transporters may have resulted from the consecutive sertraline doses. In a previous study, after 4 days of multiple citalopram administration in rats, the transport rate (V_{\max}) and ligand binding sites (B_{\max}) of serotonin transporters were reduced by 38% and 53%, respectively, compared to the control group [15].

In a previous study, sertraline showed linear kinetics for a dose range of 50 – 200 mg,

and a 0.1 ng/ml LLOQ value was achieved with LC-MS/MS [13]. In the current study, we obtained a lower LLOQ (0.05 ng/ml) for sertraline. The pharmacokinetic analysis for 5–50 mg of sertraline demonstrated a linear relationship between the doses and serum concentrations. This linear PK profile, from a low-dose to therapeutic doses supported the validity of the microdosing approach, because a linear PK is crucial for extrapolating the PK profile.

This study had some limitations. First, we used a 90 min PET scan for acquiring measurements for each period. This short measurement period only provided limited information on the distribution kinetics of the drug and its time course. PET scans were conducted at baseline and at 24 h after the last dose was administered, because the study aimed at investigating the distribution at steady state and observe changes in brain distributions with different dosages. We did not perform more PET scans, because we wanted to avoid exposing the volunteers to excessive radiation, which might be incurred with frequent PET scans. Second, the plasma concentration of [¹¹C]sertraline could have been more accurately measured by AMS. However, because the AMS method is costly to setup and requires a radio-labeling process, current studies are increasingly using the LC-MS/MS approach [31, 32]. Thus, development of a study design with LC-MS/MS for microdosing was relevant in terms of general accessibility.

In conclusion, this study provided information on the brain disposition and BBB penetration of a CNS drug in healthy volunteers. We obtained linear PK characteristics, which supported the extrapolation of the PK profile from a low dose to a therapeutic dose for microdosing. Sertraline was used as a model of a CNS drug candidate, and the current results indicated that this method can be applied to drug development. It will be important in future studies to consider several factors that affect the interpretation of microdosing study results, including the expected mechanism of action, the physicochemical properties of the drug that might affect the disposition pattern, the LLOQ of the assay method, the safety and the accessibility of the method. This study provided support for using PET microdosing as a tool to investi-

gate the distribution of radio-labeled CNS drug candidates. Further studies are needed to establish more efficacious microdosing strategies.

Acknowledgments

This study was supported by a grant from the Korea Healthcare technology R&D Project, Ministry of Health and Welfare Affairs, Republic of Korea (A070001). Kwang-Hee Shin was supported by a training program grant of the Korea Healthcare Technology R&D Project, Ministry for Health and Welfare Affairs, Republic of Korea (A070001). The authors thank Jin-Woo Park, Jun-Yul Bae, Seoul National University College of Medicine, for assistance in manuscript preparation.

Financial disclosure

This study was supported by a grant from the Korean Healthcare Technology R&D Project, Ministry of Health and Welfare Affairs, Republic of Korea (A070001). The authors declare no conflict of interest to disclose.

Clinical trial registry: <http://clinicaltrials.gov>, NCT00969852.

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