Comparative Physicochemical and Pharmacokinetic Properties of Quetiapine and Its Active Metabolite Norquetiapine

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Quetiapine (QTP) is an atypical antipsychotic drug commonly used to treat several psychiatric disorders and is metabolized into the active metabolite norquetiapine (NQTP). This study was designed to evaluate and compare the physicochemical properties, metabolic stability, brain distribution, and pharmacokinetics of QTP and NQTP. Compared to QTP, NQTP had a higher pK_a, solubility, and rat liver microsomal stability, optimal logD and similar logP values. For pharmacokinetic evaluation, QTP and NQTP were administered orally and intravenously to rats at various doses. The plasma QTP and NQTP concentrations in rats were determined by a fully-validated liquid-chromatography tandem mass spectrometry (LC-MS/MS). Over the investigated dosing range, both QTP and NQTP showed linear pharmacokinetics. Following oral administration of the same dose, the area under the concentration–time curve (AUC_{0-∞}) and maximum serum concentration (C_{max}) were larger after NQTP administration compared to QTP administration. In addition, NQTP had a greater absolute oral bioavailability compared to QTP (15.6% vs. 0.63%, respectively). The brain-to-plasma concentration ratio was greater after NQTP administration compared to the QTP and NQTP ratios after QTP administration. NQTP administration results in increased systemic exposure and brain distribution compared to QTP administration. Future studies are needed to evaluate the pharmacologic and toxicologic effects of increased NQTP exposures.

Key words permeability; pharmacokinetics; quetiapine; norquetiapine; solubility

Quetiapine (QTP) is an atypical antipsychotic drug that has been commonly prescribed for treatment of schizophrenia and related psychotic disorders since its introduction in 1996. It belongs to the group of second-generation antipsychotics (SGAs) that were initially developed to have a lesser likelihood of precipitating extrapyramidal symptoms (EPS) and possibly tardive dyskinesia compared to the first generation antipsychotics (FGA).1,2 QTP is also a biopharmaceutics classification system (BCS) class II compound with high permeability and moderate solubility.3

QTP is extensively metabolized by hepatic CYP system, primarily by CYP3A, through sulfoxidation, N- and O-dealkylation, and, to a lesser degree, 7-hydroxylation.4,5 N-Desalkyl quetiapine, also known as norquetiapine (NQTP), is an important QTP metabolite produced by CYP3A4.5 According to a previous study, the pharmacokinetic variability of QTP was substantially greater in psychiatric patients compared to NQTP.7 Several factors may contribute to the inter-individual pharmacokinetic variability of QTP. Co-administered CYP3A inducers (e.g., carbamazepine, phenytoin) or inhibitors (e.g., ketoconazole, itraconazole, erythromycin, and fluvoxamine) may interact with QTP, resulting in the increased or decreased production of NQTP, respectively.8,9 Due to the lack of CYP3A4 genetic polymorphisms, genotypes may not be associated with the variability in QTP metabolism.

QTP is metabolized into more than 20 metabolites, but their pharmacological activities have not yet been clearly characterized.10 Compared to other metabolites, one of the QTP metabolites NQTP is slightly better characterized for its pharmacological properties. According to a previous study evaluating the pharmacodynamic properties of NQTP and QTP, NQTP shows higher affinity for many receptors involved in psychiatric disorders compared to QTP, requiring smaller doses than QTP for comparable pharmacodynamic effects.11 QTP and NQTP both show moderate affinity for dopaminergic D_2 receptor, but NQTP had slightly higher affinity.11 Also, compared to QTP, NQTP blocks 5-hydroxytryptamine (5-HT)_2A receptor with substantially higher affinity, resulting in its therapeutic effects with comparable or even better tolerability. In addition, NQTP binds to 5-HT_1A receptors with an approximately 10-times greater affinity and is more efficacious to elicit serotonergic activity (75% vs. 47% of maximum serotonin response) than QTP.11 Furthermore, in contrast to QTP, NQTP is a potent norepinephrine transporter (NET) inhibitor with similar affinity to other antidepressants, contributing to its antidepressant activity.11 Moreover, the presynaptic α_1 adrenergic receptors blockade of NQTP promotes the increased noradrenergic activity associated with its antidepressant effect. However, NQTP had much lower affinity for α_2 adrenergic receptors compared to QTP; therefore, NQTP is less likely to cause orthostatic hypertension.11 The higher binding affinity of NQTP for histamine H_1 receptor, compared to QTP, may be concerning because it may be associated with higher likelihood of antihistamine toxicities such as sedation and hypnotic effects although the clinical significance is unknown.11 Overall, these pharmacodynamic properties of NQTP may
contribute to the pharmacologic response of QTP, so NQTP itself may serve as a therapeutic alternative for the treatment of various psychiatric disorders. Also, the structural similarity among QTP, NQTP, and other antidepressants in clinical use, such as amoxapine and desipramine, further suggests the potential therapeutic use of NQTP acting on the central nervous system (CNS).

The pharmacologic activity of most drugs is highly associated with the magnitude of their systemic exposure in the body. In this context, bioavailability is one of the important factors determining the pharmacologic effects of drugs. For example, the bioavailability of paliperidone, the active metabolite of risperidone, has been shown to substantially differ compared to risperidone.\(^2\) Compared to the parental drug, administration of the active metabolite may have several advantages such as: 1) the metabolite may have an equivalent pharmacologic response with a smaller administered dose compared to that of the parent drug if the metabolite has favorable pharmacodynamic properties compared to the parent drug; 2) fewer inactive metabolites may be produced; 3) fewer drug–drug interactions may occur with other concurrently administered drugs; and 4) there could be less variability in the pharmacokinetic profiles including metabolism. However, since pharmacokinetic characteristics are typically described using plasma or blood drug concentration–time data, for some drugs, particularly the CNS-acting drugs including QTP and NQTP, the pharmacokinetic parameters estimated from blood or plasma concentration–time data may not be the best predictor of their pharmacologic activity. Actually, numerous additional factors influence the pharmacologic response of the CNS-acting agents, including blood–brain barrier (BBB) penetration, CNS accumulation, and receptor association–disassociation kinetics. To the best of our knowledge, no published data have compared the bioavailability and the relative proportion of brain exposure (i.e., concentration in brain–concentration in plasma) between QTP and NQTP. Therefore, in an effort to explore the suitability of NQTP as a novel therapeutic agent with improved pharmacokinetic and pharmacodynamic properties compared to QTP, we evaluated various physicochemical and pharmacokinetic properties of NQTP, including its distribution to brain, after its oral administration.

**Experimental**

**Reagents and Solvents** Quetiapine hemifumarate (2-(2-((4-dibenzo[b,f][1,4]thiazepine-11-yl-1-piperazinyl)ethoxy)ethanol hemifumarate) and norquetiapine (11-piperazin-1-yl-glucose-6-phosphate dehydrogenase, MgCl\(_2\), oxidized form (2-(4-dibenzo[b,f][1,4]thiazepine dihydrochloride) were obtained from Molcan in Canada. Megestrol acetate as an internal standard (IS), ammonium formate, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, MgCl\(_2\), oxidized form of nicotinamide adenine dinucleotide phosphate (NADP\(^+\)), and the reduced form of β-nicotinamide adenine dinucleotide phosphate (NADPH) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Methanol was obtained from J.T. Baker (Phillipsburg, NJ, U.S.A.). All other chemicals and solvents were of the highest analytical grades available.

**Animals** All procedures were approved by the Institutional Animal Care and Use Committee of Kyung Hee University (#KHP-2011-08-15). Male Sprague-Dawley rats weighing between 180 and 200 g for the in vivo pharmacokinetic study were obtained from Orient Bio (Gunsan, Gyeonggi, Republic of Korea). The animals were housed in an environmentally controlled animal room (20±2°C, relative humidity 40–60%) under 12 h dark/light cycle for at least two weeks, and only water was allowed 24 h prior to the initiation of any experimental procedure. In order to minimize any potential circadian variation of enzyme activity, animals were anesthetized at the same time of each experimental day (10:00–12:00 a.m.).

**Solubility** Equilibrium solubility was measured by Shake-flask method with water as solvent.\(^2\) Saturated solvent with QTP and NQTP was filtered through a 0.45-μm nylon filter and then assayed by ACQUITY UPLC\(^\text{®}\) system (Waters Ltd., Watford, U.K.).

**pK\(_a\) Determination** All measurements were performed using a Sirius T3 apparatus (Sirius Analytical Instruments Ltd., East Sussex, U.K.) at 25±1°C. pK\(_a\) titrations were conducted in ion-strength-adjusted water (0.15 M KCl) with 0.5 M KOH and 0.5 M HCl, respectively, under Argon atmosphere. Triplicate titrations were carried out at the pH ranging from 1 to 12 over the concentration range of 727.5 to 785.2 μM for QTP and 1181.2 to 1264.2 μM for NQTP, respectively. pK\(_a\) values were calculated based on the previously described method\(^4\) using ACD/Labs Software (Advanced Chemistry Development, Inc., Canada).

**Partition Coefficient** The logP and logD of QTP and NQTP were measured using the pH-metric method. Each compound was weighed (1 mg), dissolved in a two-phase water–octanol system, and titrated over a pH 1–12 range. The solution became opaque during stirring; however, the pH electrode was still able to measure the pH of the aqueous component of the solution. LogP and logD values were estimated using a complex computational process. The pH of each data point in the titration curve was calculated based on the previously described equations with pK\(_a\) and p\(\text{P}^\text{a}\) and the calculated points were fitted to the observations by manipulating the p value. The measured p was determined to be the p value that provided the best fit, and it was reported as the logarithm.

**In Vitro Liver Microsomal Metabolism** Pooled male rat liver microsomes were prepared as previously described.\(^6\) Ten livers were homogenized in three volumes of ice-cold buffer consisting of 0.154 M KCl, 50 mM Tris–HCl, and 1 mM ethylenediaminetetraacetic acid (EDTA) (pH 7.4). All subsequent steps were performed at 0–4°C. The homogenate was centrifuged at 10000×g for 20 min, and the supernatant was further centrifuged at 104000×g for 65 min. The microsomal pellet was suspended and centrifuged at 104000×g for 65 min. The microsomes were diluted to 1.0 g weight equivalent of liver/mL of buffer. The protein concentration was determined by the Bradford method with bovine serum albumin as the standard. Microsomal fractions were stored at −80°C until use. The incubation mixture contained 10 mM potassium phosphate buffer (pH 7.4), an NADPH-generating system (2 mM NADP\(^+\), 20 mM glucose-6-phosphate, 2 units/mL glucose-6-phosphate dehydrogenase, and 10 mM MgCl\(_2\)), 1 mg/mL microsomal protein from rat liver, and 1 mg/mL QTP or NQTP to make a final concentration of 100 ng/mL. The reaction mixture was incubated for 2 h at 37°C in shaking water bath. The control incubation without an NADPH-generating system was performed under otherwise similar conditions. Each reaction was terminated by the addition of ice-cold methanol (2 mL). After centrifugation at 10000×g for 10 min at 4°C, each supernatant was collected and evaporated under nitrogen gas to
the approximate final volume of 0.2 mL. The amount of the remaining parent drug QTP and the formed metabolite NQTP in the supernatant was measured by an LC-MS/MS method.

**Evaluation of QTP and NQTP Stability in Rat Plasma**

The rat plasma (100 μL) was pre-incubated at 37°C for 5 min. The reaction was initiated by adding 30 μL of QTP and NQTP solution (final concentrations as 1.5, 25, 400 ng/mL, respectively). The assay was performed at 37°C. At different time points (maximum incubation=2h), the reaction was ceased by placing the tube on ice, followed by adding 350 μL of 50 mM Tris–HCl buffer (pH 7.4). QTP and NQTP concentrations in these samples were measured by an LC-MS/MS method.

**Evaluation of QTP and NQTP Bioavailability in Rats**

The rats were fasted except with free access to water for 24 h prior to the experiments. Male Sprague-Dawley rats were randomly divided into one of the nine treatment groups: oral administration of QTP 5 mg/kg (group 1, n=13), 10 mg/kg (group 2, n=8), and 20 mg/kg (group 3, n=12); oral administration of NQTP 1 mg/kg (group 4, n=14), 5 mg/kg (group 5, n=15), 10 mg/kg (group 6, n=12), and 20 mg/kg (group 7, n=11); and intravenous administration of QTP 5 mg/kg (group 8, n=8) and NQTP 5 mg/kg (group 9, n=13). These QTP and NQTP doses were selected by converting the typical doses for patients (50 to 300 mg) to those for animals as previously described. Each QTP and NQTP dose was administered as 1 mL citrate buffer solution (0.1 M citric acid–0.1 M trisodium citrate=82:18, pH=3) to each animal. QTP and NQTP were administered orally using oral zoned needles and intravenously after sterilization using 0.22-μm membrane filter (Milipore), respectively.

The animals were surgically prepared at least 3 d prior to the study. On the study day, rats were anesthetized, and a cannula was inserted into the superior vena cava (SVC) via the jugular vein for intravenous administration and blood sampling. Serial blood samples (ca. 450 mL total) were collected from individual animals via the indwelling cannula at the following times: immediately prior to the drug administration and at 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 h after oral administration; immediately prior to the drug administration and at 0.033, 0.133, 0.25, 0.5, 1, 2, 3, 4, 6, and 8 h following intravenous administration. While collecting blood samples, sodium heparin (5001 U/mL) was used as an anticoagulant. The samples were stored on ice until centrifuged (10000×g for 5 min), and plasma was harvested for storage at −70°C until analysis.

**Brain and Blood Distribution Study**

The rats were randomly assigned into four groups (n=3 for each group). For groups 1 and 2, QTP 10 mg/kg was administered orally, and for groups 3 and 4, NQTP 10 mg/kg was given orally. Rats in group 1 were sacrificed at 20 min after QTP administration and those in group 2 at 45 min. Rats in group 3 were sacrificed at 60 min after NQTP administration and those in group 4 at 120 min. After oral administration of either QTP or NQTP, rats were anesthetized with isoflurane, and blood samples (500 μL) were collected from the epicantthic veins into heparinized tubes at specific time points at 20 min after drug administration (group 1), 45 min (group 2), 60 min (group 3), and 120 min (group 4). Blood samples were immediately centrifuged at 10000 rpm for 5 min, and the supernatant plasma samples were harvested and stored at −80°C until analysis. Immediately after collecting blood, rats were sacrificed as scheduled above, the whole brain was collected, rinsed with physiologic saline solution, blotted dry with filter paper, and homogenized in physiologic saline solution (1:2, v/v) with Superfine Homogenizer. The tissue homogenates were centrifuged, and aliquots of the supernatants were obtained and stored at −70°C until analysis.

**Determination of QTP and NQTP Concentrations in Rat Plasma, Liver, and Brain**

The quantification of QTP and NQTP for all samples was conducted using LC-MS/MS. Aliquots of 50 μL of rat plasma, brain homogenate (25 μg in 50 μL), and liver microsome (25 μg in 50 μL) were transferred into eppendorf tubes, and 20 μL of the working solution of IS (megestrol acetate, 1 μg/mL) was added. Afterwards, cold methanol (500 μL) was added to precipitate plasma proteins. The mixture was vortexed for 10 min and centrifuged at 14000 rpm for 10 min at 4°C. Each of sample supernatant (400 μL) was filtered through 0.22-μm syringe filter, and 2 μL aliquot of the filtered sample was injected into the LC-MS/MS system. Chromatographic separation was performed using Waters ACQUITY UPLC system (Waters, Milford, U.S.A.) with Waters ACQUITY UPLC® BEH C18 column (1.7 μm i.d.×100mm, 2.1 μm, Waters). The mobile phase was a binary mixture of 10 mM ammonium formate–methanol (12.5 : 87.5, v/v). The flow rate was 0.22 mL/min. The QTRAP 5500 mass spectrometer (AB sciex, Concord, Ontario, Canada) equipped with a TurboIonSpray® interface was used as a detector. The instrument was operated with an electrospray ionization interface (ESI) in positive ion modes to detect QTP at MS/MS transition (m/z) 384.3→253.2, NQTP at 296.2→210.1, and IS (megestrol acetate) at 385.4→267.3. The optimized TurboIonSpray® temperature was set at 650°C. Nitrogen gas was used for the curtain gas, collision gas and the values were 30 psi, medium. The declustering potential, entrance potential, and collision cell exit potential were: 70, 7, and 7 eV for QTP; 40, 7, and 17 eV for NQTP; and 80, 7, and 14 eV for IS (megestrol acetate). Calibration curves for plasma, brain, and liver microsomes were constructed from seven calibration samples over the concentration range of 0.5 to 500 ng/mL for both QTP and NQTP. Six blank sample without any analyte or IS and zero blank sample with only IS added were prepared to confirm the absence of interference between analytes and IS. Sample concentrations were calculated from the peak area ratio of each analyte against IS. The linearity of the calibration curve was estimated. The calibration curve had to have a correlation coefficient (r)≥0.99. A weighted (1/x²) least squares linear regression analysis was performed for calibration curves. The developed UPLC method was validated for selectivity, linearity, sensitivity, precision, and accuracy according to the U.S. Food and Drug Administration (FDA) guidance for bioanalytical method validation (Guidance for Industry: Bioanalytical Method Validation, U.S. FDA, 2001). The lower limit of quantification (LLOQ) was evaluated by analyzing five replicates of the lowest concentration standard on the calibration curve.

**Pharmacokinetic Data Analysis and Statistical Analysis**

Plasma concentration–time data for QTP or NQTP were analyzed using WinNonlin (version 4.1; Pharsight Co., Mountain View, CA, U.S.A.) to estimate the QTP and NQTP pharmacokinetic parameters. The area under the concentration–time curve (AUCt→∞) was determined using the trapezoidal method. The maximum serum concentration (Cmax) and the time to reach Cmax (Tmax) values were obtained by visual inspection.
of plasma drug concentration–time profiles of each animal. The absolute bioavailability ($F$) was calculated as the ratio between the oral and intravenous $AUC_{0→∞}$ at the equivalent dose. Descriptive statistics [mean±standard deviation (S.D.)] were used to summarize the pharmacokinetic parameters.

In order to evaluate the temporal variation of plasma–brain distribution, statistical tests were performed for the brain-to-plasma concentration ratios of QTP and NQTP after a single oral administration of QTP or NQTP in SPSS (SPSS Statistics for Windows, Version 22.0; SPSS Inc., IBM Corp., Armonk, NY, U.S.A.). Normality of the data distribution was tested using Kolmogorov–Smirnov test. When normality assumption was satisfied, differences in the brain-to-plasma concentration ratios of QTP or NQTP between different time points (i.e., 20 and 45 min after the administration of QTP, 60 and 120 min after the administration of NQTP) were tested using paired $t$-test. When normality assumption was violated, Wilcoxon signed-rank test was performed to test the differences in the brain-to-plasma concentration ratios between different time points. Statistical significance was defined as $p<0.05$.

**Results and Discussion**

The Physicochemical Properties of QTP and NQTP

NQTP is an active metabolite of QTP with better kinetic binding parameters on target receptors and transporters including $\alpha_2$, $5HT_2A$, $5HT_1A$, NET, and $\alpha 1b$ receptors\(^{(15)}\); as such, bypassing the metabolic activation process from QTP to NQTP may account for less pharmacokinetic variability with NQTP compared to QTP. In the present study, NQTP was investigated as a drug candidate with improved biopharmaceutical properties compared to QTP. The physicochemical properties of QTP and NQTP are shown in Table 1. The water-solubility value of NQTP (>3.68 mg/mL) was much higher than QTP (38.32±0.2 µg/mL). Our results suggested NQTP have more favorable physicochemical properties for intestinal absorption compared to QTP, which could increase the systemic exposure of NQTP. According to Fick’s law, when all other determinants of flux are similar, substances with higher concentration gradient have higher flux. Translating this into the absorption of QTP and NQTP, NQTP absorption may be increased compared to QTP due to higher concentration gradient in the intestine as a result of higher water-solubility as other factors of Fick’s law are comparable between QTP and NQTP.

**Metabolism of QTP and NQTP in Rat Liver Microsome in Vitro**

The metabolic rates of QTP and NQTP were determined in the rat liver microsome model (Table 2). QTP (100 ng/mL) was readily hydrolyzed to NQTP in rat liver microsomes within 10 min and disappeared almost completely in 120 min. In contrast, for NQTP, the relative NQTP concentrations remaining in 10 and 120 min were 40.51 and 25.89%, respectively. This may be explained by the longer half-life of NQTP in the rat liver microsomes compared to QTP. From our experiment with rat liver microsomes, the NQTP concentration remaining at 120 min was 39 times higher than QTP concentration for the same period (25.89 vs. 0.66% remaining), indicating a potentially higher systemic NQTP exposure after NQTP administration compared to QTP exposure after QTP administration. Thus, relatively more rapid biotransformation of QTP in vivo might be expected in comparison to NQTP. When comparing the stability of QTP and NQTP in rat plasma on a semi-logarithmic scale, QTP and NQTP were both stable at 37°C for 2 h (data not shown), suggesting that enzymatic hydrolysis, and probably not chemical instability, was involved in the half-life differences for the two compounds in the rat microsome assay.

**Assay Validation**

A rapid and simple procedure was developed for the simultaneous determination of QTP and NQTP concentrations in rat plasma using liquid chromatography coupled with ESI-tandem mass spectrometry (LC-ESI-MS/MS). Typical retention times for QTP, NQTP, and IS were 1.01, 0.95, and 1.03 min, respectively, and the peak shapes were sharp and symmetrical. The total runtime was approximately 3 min. Calibration curves were constructed daily with adequate linearity over the concentration range of 0.5–500 ng/mL for both QTP and NQTP. The correlation coefficients of the weighted ($1/x^2$) calibration curves were $\geq 0.995$ for both QTP and NQTP. The LLOQ of both compounds was 0.5 ng/mL by analyzing five replicates of the lowest standard on the calibration curve. The intra-day and inter-day accuracy ranged from 87.40 to 104.60% and 89.18 to 102.91% and precision ranged from 0.59 to 3.51% and 0.97 to 3.48%, respectively for QTP at QTP concentrations of 0.5, 5, 50, and 500 ng/mL. The intra-day and inter-day accuracy ranged from 89.20 to 101.85% and 94.72 to 102.87% and precision ranged from 0.91 to 5.26% and 1.68 to 5.02%, respectively, for NQTP at NQTP concentrations of 0.5, 5, 50, and 500 ng/mL. According to the Korean Ministry of Food and Drug Safety (KMFDS, former Korean Food and Drug Administration) guidance for bioanalytical applications, our results were within the acceptable criteria of $\leq 15\%$ deviation at LLOQ and $\leq 15\%$ deviation for three different concentrations above LLOQ.\(^{(20)}\) The mean extract recovery from rat plasma was 91.01% for the low (1.5 ng/mL), 91.90% for the medium (25 ng/mL), and 90.37% for the high (400 ng/mL) QTP quality control (QC) samples. For NQTP, the mean extract recovery was 97.32% for the low (1.5 ng/mL), 90.20% for the medium (25 ng/mL), and 88.67% for the high (400 ng/mL) QC samples. The mean extraction recovery for IS was 88.75%. The matrix effect for QTP, NQTP, and IS was more than 90%, indicating no co-eluting endogenous substances significantly influenced the ion suppression using

| Time (min) | QTP (ng/mL) | NQTP (ng/mL) |
|-----------|-------------|--------------|
| 0         | 100.00±0.00 | 100.00±0.00  |
| 10        | 14.44±3.75  | 40.51±2.96   |
| 30        | 2.08±0.13   | 36.80±3.52   |
| 60        | 1.15±0.28   | 32.27±2.42   |
| 120       | 0.66±0.53   | 25.89±2.47   |

Table 1. Physicochemical Properties of QTP and NQTP

| Items | QTP | NQTP |
|-------|-----|------|
| $pK_a$ | 6.87 | 8.6  |
| Log $P$ | 2.94 | 3.29 |
| Log $D$ | 2.85 | 2.11 |
| Permeability $(P_{app})$ | $\geq 5.64$ | $\geq 5.70$ |
| Solubility | $38.32$±$0.20$ µg/mL | $>3.68$ mg/mL |

Table 2. Liver Microsomal Stability of QTP (100 ng/mL) and NQTP (100 ng/mL)\(^{(a)}\)

\(^{(a)}\) Data are shown as the mean±S.D. concentration of each compound at each time point in the experimental rat liver microsome system.
our proposed analytical method. Accordingly, our analytical method was considered reliable with minimal matrix effect. The analytes were stable in rat plasma at room temperature, 4°C, and −20°C for 24 h and at −70°C for 60 d following three freeze-thaw cycles. Extracted samples were stable at 4°C for at least 24 h. Therefore, our analytical method to determine QTP and NQTP concentrations satisfied the current KMFDS and U.S. FDA guidelines for bioanalytical methods, suggesting acceptable precision and accuracy and the method using a positive ionization tandem MS detector allowed rapid, robust,
selective, and sensitive determination of QTP and its active metabolite NQTP in rat plasma. 18,20)

Pharmacokinetic Properties of QTP and NQTP The validated LC-MS/MS method was successfully used to determine the QTP and NQTP concentrations in rat plasma following a single-dose oral or intravenous administration of QTP and NQTP. After oral administration of QTP and NQTP, the mean ± S.D. plasma concentration–time profiles for QTP and NQTP are shown in Figs. 1 and 2, respectively. Following an intravenous administration of QTP and NQTP, Fig. 3 illustrates the mean ± S.D. plasma concentration–time profiles for QTP and NQTP. Tables 3 and 4 summarize the estimated pharmacokinetic parameters for QTP and NQTP, respectively, after oral and intravenous administration.

For the oral administration of QTP, NQTP $C_{\text{max}}$ values were 2.6-, 1.9-, and 2.4-fold higher than QTP $C_{\text{max}}$ at the QTP doses of 5, 10, and 20 mg/kg, respectively. Based on the plasma $AUC_{0-12\text{h}}$, systemic exposure to NQTP was 2.3-, 2.8-, and 4.2-fold higher compared to QTP after oral administration of QTP at 5, 10, and 20 mg/kg, respectively. The QTP $AUC_{0-\infty}$ increased in proportion to the QTP dose; the $AUC_{0-\infty}$ increased by 1.91 and 3.89 times as the dose increased from 5 to 10 mg/kg and 5 to 20 mg/kg, respectively. The $AUC_{0-\infty}$ of QTP was in a linear relationship with the administered QTP dose with a correlation coefficient ($r$) of $>0.99$. However, QTP $C_{\text{max}}$ values were less correlated with the QTP doses compared with the $AUC_{0-\infty}$. Regarding $T_{\text{max}}$ after oral administration of QTP, QTP $T_{\text{max}}$ was comparable over the evaluated dosing range (Table 3); however, NQTP $T_{\text{max}}$ appeared substantially prolonged as the QTP dose increased. This prolonged $T_{\text{max}}$ for NQTP may be caused by the different conversion rates from QTP to NQTP in the liver microsomes at different doses or concentrations of QTP. For bioavailability, the absolute oral bioavailability ($F$) of QTP based on the $AUC_{0-\infty}$ after intravenous and oral administration of QTP was 0.63%, indicating poor absorption following oral administration in rats. According to a previous study, the absolute oral bioavailability of QTP in humans was also low (9%), suggesting poor systemic absorption of QTP. 21) This low bioavailability of QTP after oral administration is primarily due to extensive first-pass metabolism, so various attempts including modifications of QTP formulation have been made to improve the oral bioavailability of QTP and ultimately, enhance the therapeutic effects of QTP.

Another way to improve the systemic exposure to QTP effects may be to administrate of NQTP directly, instead of QTP, and bypass the first-pass metabolism of QTP and take advantage of the pharmacologic activities of the active metabolite NQTP. In this study, we evaluated the pharmacokinetic characteristics of NQTP after oral and intravenous administration of NQTP to rats (Tables 3, 4). The absolute oral bioavailability ($F$) of NQTP based on the $AUC_{0-\infty}$ after intravenous and oral administration of NQTP to rats was 15.6%, which was approximately 25-fold higher than the $F$ of QTP (15.6 vs. 0.63%). This difference in $F$ between QTP and NQTP may be explained by the different involvement of transporters in the absorption of QTP and NQTP. QTP is a well-known substrate of P-glycoprotein, but NQTP is not. 22) The efflux of QTP, mediated by P-glycoprotein, may contribute to the lower bioavailability of QTP compared to NQTP. Currently, no transporters are known to facilitate the intestinal absorption of NQTP, and further studies are needed to evaluate the involve-

**Fig. 3.** Mean ± S.D. Plasma Concentration–Time Profiles for Quetiapine (QTP) and Norquetiapine (NQTP) after Intravenous (i.v.) Administration of QTP and NQTP. The solid line represents QTP concentration–time profile, and dashed lines represent NQTP concentration–time profile. The inset shows the mean ± S.D. plasma concentration–time profiles for NQTP after intravenous administration of QTP on an enlarged y axis. Error bars indicate standard deviations; only plus-side error bars are shown for visibility.
ment of transporters in the intestinal absorption of NQTP. Assuming NQTP absorption is solely mediated by passive diffusion as suggested by the currently available evidence, the difference in F between QTP and NQTP may be further accounted for by the different pKa and water-solubility values (Table 1). Compared with a previous study describing QTP concentration analysis method after oral administration of QTP (3.6 mg/kg) to rats, our study showed similar Cmax ratios of NQTP:QTP (approximately 2.5).23 However, compared to another study evaluating the pharmacokinetics of QTP after oral administration (25 mg/kg) to rats, our study showed a smaller Cmax and AUC0–∞ values (85.40 vs. 13.62 ng/mL; 137.10 vs. 24.09 ng·min/mL).24

Comparing NQTP pharmacokinetics after oral administration of QTP and NQTP, the AUC0–∞ and Cmax of NQTP were approximately 10 to 15 times and 9 times higher with the administration of NQTP than with QTP. Therefore, at the same dose, NQTP administration increases systemic exposure to NQTP compared to QTP administration, which may lead to enhanced pharmacologic effects with NQTP administration. Future studies are required to evaluate the pharmacologic and toxicologic effects of the increased systemic exposure to NQTP with the administration of NQTP compared to QTP.

**Brain Distribution** The extent of QTP and NQTP brain distribution was studied by assessing the brain-to-plasma concentration ratios for each compound (Table 5). Assuming that 1 g brain tissue is equivalent to 1 mL of plasma, the brain-to-plasma ratio of each compound was calculated as the average concentration in the brain tissue over the average concentration in the plasma, and this ratio may represent the ability of the compound to cross the BBB and distribute to the brain. At 20 min after oral administration of QTP (10 mg/kg), QTP levels decreased to approximately 20 and 120 min after oral administration of NQTP were also comparable (p>0.05). However, this lack of significant temporal variation may be due to the small sample size (n=3 per group) rather than true similarity. Therefore, future studies
with larger sample sizes at multiple time points are needed to determine the temporal variation of brain-plasma distribution of QTP and NQTP.

When comparing the brain-to-plasma concentration ratios of QTP and NQTP, the ratio for QTP was increased by 20% from 1.44 to 1.75 from 20 min to 45 min following oral administration of QTP although statistical significance was not reached. In contrast, the ratio for NQTP was maintained at almost the same level from 2.13 to 2.01 over the same time interval. This may indicate a relatively rapid distribution of NQTP from plasma to brain compared to QTP. Also, the higher NQTP brain-to-plasma concentration ratio compared to QTP indicates a more efficient distribution of NQTP to the brain from the plasma. Compared to a previous study evaluating the brain-to-plasma concentration ratios for QTP and NQTP in rats,25 our current study reported lower ratios for both QTP and NQTP. This difference may be because the ratio was estimated following a single oral dose in our study, but the previous study evaluated the ratio after a 60-d treatment with oral QTP (25 mg/kg/d). The higher brain-to-plasma concentration ratios with larger systemic exposure were further supported by our data after the oral administration of NQTP (10 mg/kg) [Table 5(B)]. Following NQTP administration, the brain-to-plasma concentration ratio for NQTP was at least 3-fold higher with much higher NQTP concentrations in both the brain and the plasma compared to the QTP administration [Table 5(B)]. Based on the physicochemical properties of QTP and NQTP (Table 1), although both compounds had similar logP values, logD of NQTP was close to more optimum levels (logD ca. 2) for BBB penetration,20 expecting higher BBB permeability. Indeed, our study showed increased NQTP brain distribution with higher systemic NQTP exposures as shown in Table 5, suggesting a possible exposure-dependent brain distribution of NQTP. This exposure-dependency of NQTP brain distribution may be at least partially explained by the involvement of transporters. A previous study evaluated single nucleotide polymorphisms (SNPs) of CYP3A4 and ABCB1 genes.22 As expected from the disposition pathway of QTP, both CYP3A4 and ABCB1 gene SNPs were significantly associated with the QTP concentrations in plasma and cerebrospinal fluid (CSF). In contrast, most of the CYP3A4 and ABCB1 gene SNPs were not significantly associated with NQTP disposition. Based on currently available published data, no transporters are yet known to affect NQTP disposition including brain distribution. Because our current study used only one single dose of QTP and NQTP to evaluate the brain distribution without any in vitro transporter data, our current study cannot identify the mechanisms of this apparent exposure-dependency of plasma-to-brain NQTP distribution and any specific transporters responsible for the brain distribution. Future studies are needed to determine the dose-dependency in the brain-plasma NQTP distribution and to identify any transporters involved in the NQTP distribution to the brain.

In summary, compared to QTP, NQTP had a higher brain-to-plasma concentration ratio suggesting a higher BBB permeability. This property may enhance the effects of NQTP on multiple neuropsychiatric processes in the CNS compared to QTP. Additional studies are required to evaluate the pharmacologic and toxicologic effects of enhanced brain permeability of NQTP.

### Conclusion

Compared to QTP, NQTP had better physicochemical properties as a drug candidate, such as pK_a, logP, and solubility. The in vitro stability test demonstrated that less amount of NQTP was degraded compared to QTP in the rat liver microsome over the same time period. Based on the NQTP pharmacokinetic characteristics estimated in our study, the AUC_0–12h and C_{max} values increased proportionally over the 1 to 20 mg/kg dose range. The oral bioavailability of NQTP was substantially improved by 26-fold with the administration of NQTP compared to QTP at the same dose (5 mg/kg). Additionally, NQTP administration resulted in a higher NQTP permeability through BBB compared to NQTP or QTP permeability following QTP administration, suggesting a higher proportion of NQTP was distributed to the brain after NQTP administration compared to QTP administration. Therefore, NQTP has better oral bioavailability and brain distribution compared to QTP. Based on the above data, our present study suggests NQTP is a promising, potential drug candidate for clinical development and use. Its substantially improved absorption and distribution to the site of action, mainly brain, makes it a promising druggable compound. Future preclinical studies evaluating the pharmacologic and toxicologic effects from enhanced systemic and brain exposure to NQTP may further provide the clini-

### Table 5. The Mean T/P Ratio (Brain/Plasma) of QTP and NQTP at Indicated Times after a Single Oral Administration (A) QTP (10 mg/kg) or (B) NQTP (10 mg/kg) to Rats (20 min, n = 3, 45 min, n = 3)

| Time (min) | Brain (ng/g) | Ratio (brain/plasma) |
|-----------|--------------|-----------------------|
|           | QTP          | NQTP                  |
| 20        | 29.34±14.04  | 20.40±10.91           | 1.44±0.13  | 2.13±0.05 |
| 45        | 18.29±5.82   | 20.39±4.42            | 1.75±0.35†| 2.01±0.63‡|

| Time (min) | Brain (ng/g) | Ratio (brain/plasma) |
|-----------|--------------|-----------------------|
|           | NQTP         |                        |
| 60        | 443.20±67.07 | 69.71±13.91           | 6.47±1.32 |
| 120       | 505.36±124.63| 74.60±13.13           | 7.05±2.65‡|

*p > 0.05 from paired t-test. †p > 0.05 from Wilcoxon signed-rank test.
cal development process of NQTP to a feasible drug product.

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Conflict of Interest The authors declare no conflict of interest.

References

1) Lieberman J. A., Stroup T. S., McEvoy J. P., Swartz M. S., Rosenheck R. A., Perkins D. O., Keefe R. S., Davis S. M., Davis C. E., Lebowitz B. D., Severe J., Hsiao J. K., Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) Investigators, *N. Engl. J. Med.*, 353, 1209–1223 (2005).

2) McIntyre R. S., Soczynska J. K., Woldeyohannes H. O., Alsuwaidan M., Konarski J. Z., *Expert Opin. Pharmacother.*, 8, 1211–1219 (2007).

3) Amidon G. L., Lennéräs H., Shah V. P., Crison J. R., *Pharm. Res.*, 12, 413–420 (1995).

4) Grimm S. W., Richtand N. M., Winter H. R., Stams K. R., Reele S. B., *Br. J. Clin. Pharmacol.*, 61, 58–69 (2006).

5) Grimm S. W., Stams K. R., Bui K., *Schizophr. Res.*, 24, 198–198 (1997).

6) Bakken G. V., Rudberg I., Christensen H., Molden E., Refsum H., Hermann M., *Drug Metab. Dispos.*, 37, 254–258 (2009).

7) Bakken G. V., Rudberg I., Molden E., Refsum H., Hermann M., *Theor. Drug Monit.*, 33, 222–226 (2011).

8) Winter H. R., Earley W. R., Hamer-Maansson J. E., Davis P. C., Smith M. A., *J. Child Adolesc. Psychopharmacol.*, 18, 81–98 (2008).

9) Prieto E., Mico J. A., Meana J. J., Majadas S., *Actas Esp. Psiquiatr.*, 38, 22–32 (2010).

10) DeVane C. L., Nemeroff C. B., *Clin. Pharmacokinet.*, 40, 509–522 (2001).

11) Jensen N. H., Rodriçuez R. M., Caron M. G., Wetzel W. C., Rothman R. B., Roth B. L., *Neuropsychopharmacology*, 33, 2303–2312 (2008).

12) Citrome L., *Expert Opin. Drug Metab. Toxicol.*, 8, 873–888 (2012).

13) Œglvgy G., Baka E., Box K. J., Comer J. E., Takács-Novák K., *Anal. Chim. Acta*, 673, 40–46 (2010).

14) Liao C., Nicklaus M. C., *J. Chem. Inf. Model.*, 49, 2801–2812 (2009).

15) Avdeef A., *J. Pharm. Sci.*, 82, 183–190 (1993).

16) Oh S. J., Choi J. M., Yun K. U., Oh J. M., Kwak H. C., Oh J. G., Lee K. S., Kim B. H., Heo T. H., Kim S. K., *Chem. Biol. Interact.*, 195, 173–179 (2012).

17) Reagan-Shaw S., Nihal M., Ahmad N., *FASEB J.*, 22, 659–661 (2008).

18) Food and Drug Administration, Center for Drug Evaluation and Research (CDER), “Guidance for Industry, Bioanalytical Method Validation,” U.S. Department of Health and Human Services, May, 2001.

19) Lopez-Muñoz F., Alamo C., *Front. Psychiatry*, 4, 102 (2013).

20) KFDA Guidance for Industry, “Statistical Approaches to Establishing Bioequivalence,” Bioequivalence Division, Pharmacology Department, National Institute of Toxicology Department: <http://ezdrug.kfda.go.kr/kfda2> 2003.

21) Narala A., Veerabrahma K., *J. Pharm. (Cairo)*, 2013, 265741 (2013).

22) Boulton D. W., DeVane C. L., Liston H. L., Markowitz J. S., *Life Sci.*, 71, 163–169 (2002).

23) Tu J. Y., Xu P., Xu D. H., Li H. D., *Chromatographia*, 68, 525–532 (2008).

24) Ezzeldin E., Asiri Y. A., Iqbal M., *Evid. Based Complement. Alternat. Med.*, 2015, 615285 (2015).

25) Yang X., Poddar I., Hernandez C. M., Terry A. V. Jr., Bartlett M. G., *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, 1002, 71–77 (2015).

26) Di L., Keras E. H., *Curr. Opin. Chem. Biol.*, 7, 402–408 (2003).