Pharmacokinetic Models Scaled-up from Humanized-liver Mouse Data Can Account for Drug Monitoring Results of Atomoxetine and Its 4-Hydroxylated and N-Demethylated Metabolites in Pediatric Patients Genotyped for Cytochrome P450 2D6

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Running title: Exposures of atomoxetine prescribed to CYP2D6*10 patients

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Abbreviations

P450, cytochrome P450

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Abstract

Atomoxetine is a cytochrome P450 (P450) 2D6 probe substrate and an approved medicine for attention-deficit/hyperactivity disorder. In this humanized-liver mouse study, interactions between atomoxetine and the P450 2D6 probe drug paroxetine were observed. Human physiologically based pharmacokinetic (PBPK) models were established by scaling up humanized-liver mouse data obtained in the absence or presence of paroxetine. These models could explain the drug monitoring results of atomoxetine and its primary 4-hydroxylated and N-demethylated metabolites in Japanese children aged 8–14 years and could be used to help establish the correct dosage and for the evaluation of clinical outcomes. The results of simple PBPK models (using input parameters that reflected the subjects’ small body size and normal or reduced P450 2D6-dependent clearance) were in general agreement with one-point measured plasma concentrations of atomoxetine and its 4-hydroxylated and N-demethylated metabolites in 13 pediatric participants. Unexpectedly high hepatic exposure, possibly in intermediate-metabolizer patients harboring CYP2D6*10 or 2D6*36 alleles, might in part explain the adverse effects of atomoxetine prescribed alone recorded in a Japanese adverse-event database. The steady-state, one-point drug monitoring data from the participants indicated extensive biotransformation of atomoxetine to 4-hydroxyatomoxetine under individually prescribed doses of atomoxetine. These results also suggest that a relatively narrow range of 4-hydroxyatomoxetine and N-desmethylatomoxetine concentration ratios in spot urine and/or plasma samples from pediatric patients could be a simple semiquantitative determinant factor for P450 2D6 intermediate metabolizers, compared with the wide range of concentrations of the two primary metabolites and substrate in extensive metabolizers.

Keywords: Humanized-liver mouse, Mechanism-based inhibitor, Paroxetine, Intermediate metabolizer.
Significance Statement

Validated simple pharmacokinetic models are able to predict steady-state plasma concentrations of the approved medicine atomoxetine and its primary metabolites in the majority of pediatric patients. The package insert advises careful dose escalation, especially for poor metabolizers; however, no simple way exists to determine P450 2D6 phenotypes. A relatively narrow range ratio of 4-hydroxyatomoxetine and N-desmethyloxetine in spot urine/plasma samples could be a simple semi-quantitative determinant factor for P450 2D6 intermediate metabolizers to optimize or confirm the correct dosage.
Introduction

Atomoxetine is a selective serotonin reuptake inhibitor approved for the treatment of attention-deficit/hyperactivity disorder (ADHD) in children (≥6 years) (Yu et al., 2016; Brown et al., 2019; Smith et al., 2023). High systemic exposure to atomoxetine leading to hepatotoxicity has been suggested both in drug interactions strongly perpetrated by cytochrome P450 (P450 or CYP) 2D6 probes/inhibitors, such as paroxetine (Venkatakrishnan and Obach, 2005), and in impaired pharmacokinetics mediated by polymorphic P450 2D6 variants, thereby validating the results of pharmacokinetic models for adults (Dinh et al., 2016; Huang et al., 2017; Kim et al., 2018). Detailed but complex metabolic pathways of atomoxetine have been identified as a result of clinical and basic research (Ring et al., 2002; Sauer et al., 2003; Matsui et al., 2012; Brown and Bishop, 2015; You et al., 2021). Highly polymorphic P450 2D6 and 2C19 mainly mediate atomoxetine 4-hydroxylation and demethylation, respectively (Choi et al., 2014; Byeon et al., 2015; Smith et al., 2023). The primary 4-hydroxylated metabolite is promptly transformed into its inactive glucuronide (Brown et al., 2016).

Paroxetine, another selective serotonin reuptake inhibitor, is a potent perpetrator of drug interactions with P450 2D6 substrates (Bertelsen et al., 2003) because it causes the loss of human P450 2D6 activity in a time-dependent manner (Rougee et al., 2016). Caucasian subjects have clear differences in frequencies of extensive and poor P450 2D6 metabolizers compared with other ethnicities (Ingelman-Sundberg et al., 2007; Pridgeon et al., 2022), e.g., Asian subjects have a higher frequency of intermediate P450 2D6 metabolizers (Kiyotani et al., 2010; Okubo et al., 2016). The package inserts for atomoxetine (approved label in Japan) recommend careful dose escalation (with caution) according to the patient’s P450 2D6 phenotype. Unfortunately, patient CYP2D6 genotyping is not routinely covered by the general insurance system in Japan,
and, consequently, the P450 2D6 phenotype of pediatric patients is generally not known (Matsui et al., 2012; Notsu et al., 2020). The aim of the present study was to establish pharmacokinetic models scaled up from humanized-liver mouse data obtained in the absence or presence of paroxetine; it was then investigated whether such models could reproduce the drug monitoring data of atomoxetine and its primary 4-hydroxylated and N-demethylated metabolites in Japanese children aged 6–14 years with a diagnosis of ADHD who had been treated with atomoxetine. Our focus was the investigation of events recorded in the Japanese Adverse Drug Event Report (JADER) database in patients being prescribed single drugs (Adachi et al., 2023a; Adachi et al., 2023b). Various mechanisms have been suggested as the cause of adverse events related to the prescription of atomoxetine or paroxetine alone, but differences in the in vivo intrinsic clearance of the drug, mediated mainly by P450 2D6, in these patients may be an important causal factor. It has been proposed that the concentrations of atomoxetine in saliva or sweat can be used to predict the pharmacological response to atomoxetine (Marchei et al., 2013; Papaseit et al., 2013; Alsmadi et al., 2022). Herein, we report that validated simple physiologically based pharmacokinetic (PBPK) models scaled up from humanized-liver mouse data obtained in the absence or presence of paroxetine can be used to predict steady-state plasma concentrations and possibly urinary excretion of atomoxetine and its primary metabolites in the vast majority of Asian pediatric patients with polymorphic P450 2D6 variants.
Materials and Methods

Numbers of Adverse Events with Atomoxetine or Paroxetine Prescribed Alone

The numbers of patients recorded in the Japanese Adverse Drug Event Report (JADER) database between April 2004 and March 2022 who experienced adverse drug events associated with atomoxetine or paroxetine administered alone were examined using the same approach as that previously employed for atorvastatin (Adachi et al., 2023b) and celecoxib or diclofenac (Adachi et al., 2023a). The number of drug dosage modifications that were documented as "reduced" or "discontinued" and the number of patients suffering adverse events were investigated. The time to the onset of relevant events, e.g., hepatic disorders after administration, was evaluated as previously communicated (Adachi et al., 2023a; Adachi et al., 2023b). Although a relatively high rate of non-fatal suicidal behaviors has been reported for patients being administered atomoxetine (Thomas et al., 2014), such adverse events were excluded from this study. The cumulative incidence of adverse events was analyzed using the Kaplan–Meier method on JMP Pro 13.2.1 (SAS Institute, Cary, NC) and Prism 9 (GraphPad Software, La Jolla, CA).

Inactivated Liver Microsomal Enzymes and Humanized-liver Animals

Sets of control pooled human liver microsomes and liver microsomes with selectively inactivated P450 2D6 [Silensomes pretreated with the mechanism-based inhibitor paroxetine, 50 µM (Parmentier et al., 2019)] were obtained from Biopredic International (Rennes, France). Recombinant human P450 2D6.1, 2D6.10, and 2C19 proteins in bacterial membranes were prepared as previously described (Yamazaki et al., 2002; Matsunaga et al., 2009; Yamazaki et al., 2010). Atomoxetine (10 µM) was incubated with sets of control liver
microsomes and inactivated human liver microsomes (0.20 mg/mL) or with recombinant P450 2D6 and 2C19 (2.0 pmol) at 37°C for 10 min in a total volume of 200 μL of 50 mM phosphate buffer (pH 7.4). After incubation, the reactions were terminated by treatment with 200 μL acetonitrile and then centrifuged at 20,000 × g for 10 min at 4°C. The levels of metabolites in the supernatants were determined using liquid chromatography–tandem mass spectrometry, according to previously reported methods (Notsu et al., 2020). The fraction of substrate metabolized ($f_m$) was calculated using the following equation: $f_{m, \text{P}450} = 1 - \frac{\text{activity in liver microsomes selectively inactivated for P450 isoform/activity in control liver microsomes}}{}$ (Murayama et al., 2018).

An updated TK-NOG mouse (NOG-TKm30, Central Institute for Experimental Animals, Kawasaki, Japan) was employed in the current study (Uehara et al., 2022) with the approval of the Animal Ethics Committee of the Central Institute for Experimental Animals ( Permit Number: 20060A). Human donor hepatocytes HUM4282 (genotyped as CYP2D6*1/*4) were previously transplanted into NOG-TKm30 mice (Uehara et al., 2023). Humanized-liver NOG-TKm30 mice (six males weighing approximately 20–30 g) were administered a single intravenous dose of atomoxetine (3.0 mg/kg) or a single oral administration of paroxetine (8.0 mg/kg). In a different set of experiments, humanized-liver mice were orally pretreated for 3 days with paroxetine at 8.0 mg/kg to achieve metabolic inactivation of P450 2D6 in liver; this dosage was based on the reported inhibitory dose (MacLeod et al., 2017) that resulted in an approximately 30% decrease in the area under the concentration–time curve (AUC) of the tamoxifen metabolite endoxifen. On day 4, humanized-liver mice were intravenously infused with a single dose of atomoxetine (3.0 mg/kg). Plasma samples were obtained from control (untreated) and pretreated humanized-liver mice at 0.25, 0.5, 1, 2, 4, 7, and 24 h after administration. Urinary samples (0–24 h, ~ 1 mL) were also collected from the humanized-liver
mice. Plasma and urine samples (10 µL) underwent deproteinization by the addition of 10 and 90 µL acetonitrile, respectively, and were then centrifuged at 20,000 \( \times g \) for 10 min at 4°C. The changes in plasma concentration over time for each humanized-liver mouse were investigated using non-compartmental analysis in Phoenix WinNonlin 8 (Pharsight, Mountain View, CA) to determine pharmacokinetic parameters for individual mice. Statistical analyses of these pharmacokinetic parameters in both humanized-liver mice treated with paroxetine and in untreated mice were performed using GraphPad Prism 9.

**Pediatric Patients Treated with Atomoxetine**

Modeling of the drug monitoring data for of atomoxetine in thirty-three Japanese pediatric patients genotyped for cytochrome P450 2D6 and enrolled between April 2017 and February 2019 was previously reported (Notsu et al., 2020). For the current study, 13 new patients orally treated with atomoxetine at the Nara Prefecture General Medical Center between March 2019 and July 2023 were recruited. The ethics committee of the Nara Prefecture General Medical Center approved this study, and written informed consent was supplied by the legal guardians of the patients. All 13 participants (ten boys and three girls in the 8–14 years age range) were Japanese. These subjects were receiving atomoxetine at doses ranging from 30 to 90 mg/day, administered orally in one or two divided daily doses. Blood samples were taken after steady-state conditions had been sustained for a minimum of 4 weeks after the prescription of atomoxetine had begun, and no notable side effects had been reported. Urine samples were taken from only three boys and one girl. Genomic DNA was isolated using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). \( CYP2D6 \) (*1, *2, *5, *10, and *36) genotypes and copy numbers of \( CYP2D6 \) were determined according to previously described methods (Okubo et al., 2012; Notsu et al., 2020). Diplotypes, whole-gene deletions (\( CYP2D6\)**5), and
duplications (CYP2D6*2-*2, CYP2D6*36-*10, and CYP2D6*36-*36) were detected (Kiyotani et al., 2010; Notsu et al., 2020). The subjects were classified into the impaired allele group (n = 3, 2D6*10, *36, or *36-*10) and the normal allele group (n = 8, 2D6*1 or *2) (Gaedigk et al., 2008).

Patient plasma and urine samples were treated with one volume of methanol, and the aqueous supernatant was centrifuged at 2 × 10^4 g for 10 min at 4°C. The supernatant was then analyzed for atomoxetine, 4-hydroxyatomoxetine and its glucuronide conjugate, and N-desmethylatomoxetine using liquid chromatography–tandem mass spectrometry according to previously reported methods (Notsu et al., 2020). Atomoxetine, 4-hydroxyatomoxetine and its glucuronide conjugate, and N-desmethylatomoxetine were quantified based on standard curves of commercially available atomoxetine, 4-hydroxyatomoxetine and its glucuronide conjugate, and N-desmethylatomoxetine supplied by Toronto Research Chemicals (North York, Ontario, Canada).

**Pharmacokinetic Simulations**

The current simple PBPK models consisted of gut, liver, kidney, and central compartments and were modified from a previous system (Notsu et al., 2020). The humanized-liver mouse PBPK model input parameters, i.e., volumes of the systemic circulation (V₁) and hepatic intrinsic clearances (CLh,int), were generated using a nonlinear least-squares algorithm (simplex and modified Marquardt methods) to ensure that the model results were consistent with the experimental plasma substrate concentrations measured in this study. The resulting differential equations were solved to model the substrate concentrations. To establish simplified human PBPK models based on these humanized-liver mouse models, V₁ and CLh,int values, along with the absorption rate constants (kₐ) for humans, as shown in Table 3, were estimated using
scale-up strategies from humanized-liver mice to human adults. Half-reduced values of the volume of the liver ($V_h$), $V_1$, and $CL_{h,inf}$ and a reduced value of blood flow rates of the systemic circulation to the liver ($Q_h$, 60.0 L/h) based on Simcyp pediatric system information were adopted on the basis that subjects had half the body weight of an adult as described previously (Notsu et al., 2020). Although body size matrices (such as $V_1$ per kg body weight) are among the most important parameters, the present modeling for pediatric subjects used a single value for $V_1$. 
Results

Adverse Events with Atomoxetine or Paroxetine Prescribed Alone

Only adverse events in patients in which atomoxetine (n = 58) or paroxetine (n = 651) was administered alone were considered in the current study (Fig. 1A and 1B). As documented in the JADER database, the drug dose was decreased or discontinued as a result of adverse events in 16 and 81 patients treated with atomoxetine or paroxetine, respectively. Because the information available with respect to dosage was limited, the time to onset of adverse events (n = 14) for atomoxetine was combined for doses of 10–100 mg/day and for cases in which the dose was not recorded. The number of days (median with interquartile range) to adverse event onset was 10 (2–319) for daily doses of atomoxetine in patients aged <20 years (Fig. 1C). In contrast, for patients receiving daily doses of paroxetine, the number of days (median with interquartile ranges) to adverse event onset was 5 (1–21) and 3 (0–15) for 10-mg and 20-mg doses of paroxetine, respectively; however, there was no significant difference in the time to onset between the two dosage groups (p = 0.15, Fig. 1D). Among the factors underlying these adverse events, the intrinsic drug clearance rate may be a contributing factor.

Animal Model for Decreased P450 2D6 Activity

The in vitro fraction of atomoxetine metabolized to 4-hydroxylated and N-demethylated metabolites mediated by P450 2D6, as assessed in vitro using human liver microsomes selectively inactivated by paroxetine, were 0.93 and 0.39, respectively (Fig. 2A). Recombinant human P450 2D6.1/2D6.10 and 2C19 mainly mediated the biotransformation of atomoxetine into 4-hydroxylated and N-demethylated metabolites, respectively, in the in vitro system (Fig. 2B). In vivo pharmacokinetics were analyzed after intravenous administration of atomoxetine
(3.0 mg/kg) in humanized-liver mice previously treated with daily oral doses of paroxetine (8.0 mg/kg) for 3 days (Fig. 3). Fifteen minutes after atomoxetine administration, 4-hydroxyatomoxetine and its glucuronide were detected in the plasma samples from humanized-liver mice (Fig. 3A, 3B). 4-Hydroxyatomoxetine glucuronide was also detected as the main urinary metabolite, along with a minor amount of N-desmethyloxatomoxetine in the 24-h urine samples (Fig. 3C, 3D). The maximum plasma concentrations ($C_{\text{max}}$) and the areas under the plasma concentration-versus-time curves (AUC) are summarized in Table 1. The $C_{\text{max}}$ and AUC values for the plasma levels of total 4-hydroxyatomoxetine (unconjugated form plus glucuronide, Fig. 3F) in mice treated with paroxetine were significantly lower (0.24-fold and 0.52-fold, respectively) than those in untreated mice (Table 1), whereas the mean AUC value of atomoxetine in the plasma (Fig. 3E) was 4.4-fold higher in the treated mice. The expected concentration of the probe substrate (atomoxetine) increased, and the level of total hydroxylated metabolites decreased as a result of this modulator. Under the current conditions, the plasma and urinary levels of N-desmethyloxatomoxetine increased tenfold in the treated mice (Fig. 3B, 3D, and 3G). The in vivo pharmacokinetics of paroxetine were also analyzed after oral administration (8.0 mg/kg) in humanized-liver mice (Fig. 4) to confirm its clearance from the body, as reported in mice humanized for P450 2D6 (MacLeod et al., 2017). On the 4th day, the mean half-life of paroxetine in the one-compartment model in humanized-liver mice intravenously administered atomoxetine was extended 2.5-fold (21 ± 7 h from 8.5 ± 3.7 h) in those treated with a single dose of paroxetine, probably because of its mechanism-based time-dependent inhibitory effects on P450 2D6 in humanized-liver mice.

Subsequently, plasma concentration-versus-time curves of atomoxetine and its two primary metabolites (4-hydroxyatomoxetine and N-desmethyloxatomoxetine) after virtual administration in humanized-liver mice were generated using simple PBPK models (Fig. 5A, 5B) with the in
silico physiological properties and input parameters shown in Tables 2 and 3. Under the present conditions, the estimated $C_{\text{max}}$ and AUC values of atomoxetine in humanized-liver mouse plasma after virtual administration were within two- or threefold errors, respectively, compared to those determined experimentally in untreated mice (Table 4). When humanized-liver mice were treated with paroxetine, a reduced value for the hepatic intrinsic clearance for atomoxetine (0.309 L/h) was used in the model because of the suppression (~40%) in plasma $C_{\text{max}}$ (24%) and AUC (52%) values of atomoxetine and changes to the fraction metabolized by P450 2D6 (0.9) and the unchanged parts ($0.671 \times 0.9 \times 0.4 + 0.671 \times 0.1$). The original metabolic ratio of 4-hydroxyatomoxetine and $N$-desmethylatomoxetine (0.95 and 0.05) from atomoxetine by hepatic clearance was also modified to 0.70 and 0.30 in the presence of paroxetine in humanized-liver mice. Urinary concentrations of atomoxetine and the two primary metabolites over 24 h were computed using humanized-liver mouse PBPK models. The output AUC values for urinary excretions divided by 24 h, i.e., the mean urinary concentrations (Table 4), were roughly similar to the observed urinary concentrations of atomoxetine, total hydroxylated metabolites, and $N$-desmethylatomoxetine in humanized-liver mice (Fig. 5C, 5D), with the exception of atomoxetine and $N$-desmethylatomoxetine in the untreated mice.

**Single-point Plasma/urine Concentrations of Atomoxetine and Metabolites at Steady State**

Single-point plasma concentrations of atomoxetine, the total concentration of 4-hydroxyatomoxetine and its glucuronide conjugate, and $N$-desmethylatomoxetine at steady state were measured in the 13 pediatric patients enrolled in this study (Table 5) and are shown after dose normalization (Fig. 6A, 6B). Human PBPK models for atomoxetine and its two primary metabolites were established based on humanized-liver mouse PBPK models by utilizing allometric scaling methods without the application of interspecies factors between in
vitro liver clearances. A reduced value of the hepatic intrinsic clearance of atomoxetine was adopted for the impaired P450 2D6 phenotype group of pediatric patients (n = 3). The estimated hepatic concentrations of atomoxetine and its two primary metabolites were also calculated (Fig. 6C, 6D). Urinary concentrations of atomoxetine and the two primary metabolites over 14 days were computed using human PBPK models based on humanized-liver mouse PBPK models for virtual oral administration. No apparent accumulation of urinary excretion of atomoxetine or of the two primary metabolites was observed (Fig. 6E, 6F) because of their rapid clearance from human blood. In fact, under the present conditions in which only four urine samples were available, the output values of the human PBPK model were reasonable (Fig. 6F).
Discussion

Validated simple PBPK models scaled up from humanized-liver mouse data obtained in the absence or presence of paroxetine were proposed in this study to predict steady-state plasma concentrations and possibly urinary excretion of atomoxetine and its primary metabolites in the vast majority of Asian pediatric patients with polymorphic P450 2D6 variants. In the current humanized-liver mouse study, interactions between atomoxetine and paroxetine were observed (Figs. 3-5). The mechanism-based and competitive inhibitory effects of paroxetine were deemed significant in a humanized-liver mouse model (Fig. 3). The importance of P450 2D6 in this interaction was underlined by the reduced activity of P450 2D6-inactivated human liver microsomes under the current conditions, and genetic effects were indicated by comparison of the activities of recombinant P450 2D6.1 and impaired P450 2D6.10 (Fig. 2). Various mechanisms have been suggested as the cause of adverse events in the clinical patients (Fig. 1), but differences in the in vivo intrinsic clearance of the solely-prescribed drug mediated mainly by P450 2D6 may be an important causal factor. We monitored the plasma/urinary concentrations at steady state of individual pediatric patients in a clinical setting during atomoxetine treatment (Fig. 6); these patients required a variety of individualized doses, and the per-dose plasma concentrations could be predicted using the validated simple human PBPK models established in this study based on humanized-liver mouse models. The introduction of less complex and less time consuming methods to predict the pharmacokinetics of atomoxetine and its metabolites would facilitate its general implementation in the clinical setting.

These models can be utilized to perform forward dosimetry simulations in individual patients prescribed with atomoxetine. In the current study, the focus was on a simple prediction method; however, no poor metabolizer subjects harboring CYP2D6*5/*5 were enrolled over the several
years of the study entry period. The present PBPK model for impaired P450 2D6 activity may be applicable to intermediate metabolizers, i.e., patients with CYP2D6*10 or 2D6*36. Although the age range for pediatric patients is comparatively broad (Table 5), and their body weight increases greatly at this stage of development, it appeared to be acceptable to apply a single value for the distribution volume when predicting the pharmacokinetics of atomoxetine in pediatric patients (Table 3 and Fig. 6). Among the various factors underlying the potential mechanisms of liver damage, the metabolic activation of atomoxetine has been suggested as a possible cause of liver impairment (You et al., 2021); consequently, unexpectedly high hepatic exposure, possibly in intermediate-metabolizer patients harboring CYP2D6*10 or 2D6*36 (Fig. 6), might be a contributory cause of adverse effects such as those recorded for atomoxetine prescribed alone in the JADER database (Fig. 1). It should be noted that N-desmethyloatomoxetine was not detected in urine samples from four extensive-metabolizer subjects (healthy volunteers) who had received atomoxetine twice daily over 6 days at 20 mg, whereas N-desmethyloatomoxetine was detected in urine samples from three poor metabolizers (Sauer et al., 2003). Against these background findings, output AUC values for mean daily urinary excretion may be a simple tool for estimating the P450 2D6 phenotype in pediatric patients; moreover, because the ranges of 4-hydroxyatomoxetine and N-desmethyloatomoxetine concentration ratios in spot urine samples from pediatric patients would be relatively narrow, such tests could constitute a simple semiquantitative determinant factor for P450 2D6 intermediate metabolizers (Fig. 6E, 6F). This kind of urine sampling is now being planned for Japanese pediatric patients to facilitate a simple evaluation following initial atomoxetine administration, because only four (two and two) urinary samples taken from extensive- and intermediate-metabolizer patients, respectively, harboring CYP2D6*1/*1 and CYP2D6*10 or 2D6*36 were currently available.
In conclusion, to evaluate treatment outcomes and to facilitate establishment of the precise dosage, validated one-compartment models and simplified PBPK models for the ADHD drug atomoxetine could be readily applied to the extrapolation of steady-state plasma concentrations of the parent drug and/or its primary metabolites (metabolized by polymorphic P450 2D6) in Japanese pediatric patients. We also propose straightforward estimation procedures that indicate a range of pharmacokinetic profiles. Validated simplified PBPK models can be used to predict the steady-state plasma concentrations and possibly urinary excretion of atomoxetine and its primary metabolites in the vast majority of pediatric patients. In Japan, the package insert of atomoxetine advocates careful dose escalation, especially for poor metabolizers; however, the determination of a patient’s P450 2D6 phenotype is not currently covered by the general insurance system in Japan. The atomoxetine PBPK models presented in this study are informative and offer promise for clinical application, although prospective validation and refinement of the model in a clinical setting involving a larger number of pediatric atomoxetine patients is required.
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Data Availability Statement

The authors declare that all the data supporting the findings of this study are contained within the paper.

Authorship Contributions

Participated in research design: Yoshida, Yamazaki

Conducted experiments: Shimizu, Uehara, Nishimura, Suemizu

Contributed new reagents or analytic tools: Uehara, Tanaka, Saito, Suemizu, Yoshida

Performed data analysis: Shimizu, Uehara, Ohyama, Nishimura, Yamazaki

Wrote or contributed to the writing of the manuscript: Yamazaki
References


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Footnotes

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Declaration of Interest

The authors declare that they have no conflicts of interest.
Figure legends

Fig. 1. The case selection process (A and B) and time to onset of selected events (C and D) in patients receiving atomoxetine or paroxetine alone: data were taken from the Japanese Adverse Drug Event Report database. (A, B) The number of patients (n) is shown. (C, D) The number of adverse events (n) is indicated.

Fig. 2. Atomoxetine oxidation activities of (A) human liver microsomes inactivated by mechanism-based inhibitors and untreated human liver microsomes as controls and (B) recombinant human P450 enzymes. Atomoxetine (10 μM) was incubated with sets of control and inactivated human liver microsomes (0.20 mg/mL) or recombinant human P450 enzymes (10 pmol/mL) at 37°C for 10 min. The mean ± standard deviation values of triplicate measurements are shown. Numbers in parentheses indicate percentages with respect to the control. **p < 0.01.

Fig. 3. Plasma concentrations (A, B), urinary concentrations (C, D), and pharmacokinetic parameters obtained using non-compartment models (E-G) for atomoxetine, 4-hydroxyatomoxetine, and N-desmethylatomoxetine after intravenous administration of atomoxetine at 3.0 mg/kg body weight in untreated humanized-liver mice (open symbols, n = 6) and in those pretreated for 3 days with paroxetine (8.0 mg/kg, solid symbols, n = 6). In panels A and B, the mean values and SD bars are shown. In panels C-G, plots and mean bars
for individual mice are shown for atomoxetine (A, circles), unconjugated 4-hydroxyatomoxetine (4OH, inverted triangles), conjugated 4-hydroxyatomoxetine [4OH(G), triangles], and N-desmethylatomoxetine (N-DesCH₃, squares). **Significantly different from untreated mice, p < 0.01.

**Fig. 4.** Plasma concentrations of paroxetine after oral administration of 8.0 mg/kg body weight in untreated humanized-liver mice (open symbols, n = 5) and in those pretreated for 3 days with paroxetine (8.0 mg/kg, closed symbols, n = 6). The mean values and SD bars are shown.

**Fig. 5.** Estimated and measured plasma (A, B) and urinary (C, D) concentrations of atomoxetine (A), total 4-hydroxyatomoxetine [i.e., the sum of unconjugated and glucuronide forms, (4OH(T)], and N-desmethylatomoxetine (N-DesCH₃) after intravenous administration of atomoxetine (3.0 mg/kg body weight) using the current PBPK models. Lines represent the results of the PBPK models. The measured mean values and SD bars in untreated humanized-liver mice (open symbols, n = 6) and mice pretreated for 3 days with paroxetine (8.0 mg/kg, solid symbols, n = 6) are also shown.

**Fig. 6.** Measured and estimated human plasma (A, B), hepatic (C, D), and urinary (E, F) concentrations of atomoxetine (in blue), total 4-hydroxyatomoxetine (in red), and N-desmethylatomoxetine (in black) in pediatric patients. Dose-normalized individual plasma
and urinary concentrations after oral atomoxetine administration in CYP2D6*1 carriers [normal allele groups (2D6*1 or *2) (Gaedigk et al., 2008), n = 8, A, C, and E] and non-CYP2D6*1 [impaired allele group (2D6*10, *36-*10 or *36-*36), n = 5, B, D, and F] are shown. Four (two and two) urinary samples taken from extensive and intermediate-metabolizer patients, respectively, were currently available. The solid (and broken) lines represent the PBPK model results with the hepatic intrinsic clearance values (with 80-120% ranges) on the 15th day after repeated administration twice daily for 14 days.
Table 1. Pharmacokinetic parameters of atomoxetine and its metabolites after an intravenous dose of 3.0 mg/kg body weight in both untreated humanized-liver mice and those pretreated for 3 days with paroxetine (8.0 mg/kg)

<table>
<thead>
<tr>
<th>Group</th>
<th>Atomoxetine C₀, µg/mL</th>
<th>AUC, nmol•h/mL</th>
<th>Total 4-hydroxyatomoxetine Cₘₐₓ, µg/mL</th>
<th>AUC, nmol•h/mL</th>
<th>N-Desmethyl-atomoxetine Cₘₐₓ, µg/mL</th>
<th>AUC, nmol•h/mL</th>
<th>Sum of AUC, nmol•h/mL</th>
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<tr>
<td>Untreated, n = 6</td>
<td>0.47 ± 0.09 (1.0)</td>
<td>2.0 ± 0.15 (1.0)</td>
<td>2.5 ± 0.4 (1.0)</td>
<td>23 ± 2 (1.0)</td>
<td>0.003 ± 0.001 (1.0)</td>
<td>0.038 ± 0.006 (1.0)</td>
<td>25 ± 2 (1.0)</td>
</tr>
<tr>
<td>Treated, n = 6</td>
<td>0.61 ± 0.15 (1.3)</td>
<td>8.7 ± 0.32 (4.4*)</td>
<td>0.59 ± 0.38 (0.52*)</td>
<td>12 ± 6 (0.84)</td>
<td>0.032 ± 0.017 (11*)</td>
<td>0.61 ± 0.32 (16*)</td>
<td>21 ± 5 (0.84)</td>
</tr>
</tbody>
</table>

The numbers in parentheses are the fold values compared with the untreated group.

*Significantly different compared with untreated mice, p < 0.05.
Table 2. Chemical properties of atomoxetine, 4-hydroxyatomoxetine, and N-desmethylatomoxetine used for PBPK modeling

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Atomoxetine</th>
<th>4-Hydroxy-atomoxetine</th>
<th>N-Desmethyl-atomoxetine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight</td>
<td>MW</td>
<td>255</td>
<td>271</td>
<td>241</td>
</tr>
<tr>
<td>Octanol–water partition coefficient</td>
<td>logP</td>
<td>3.94</td>
<td>3.45</td>
<td>3.67</td>
</tr>
<tr>
<td>Acid dissociation constant</td>
<td>pKₐ</td>
<td>9.6 (base)</td>
<td>9.4 (base)</td>
<td>9.2 (base)</td>
</tr>
<tr>
<td>Plasma unbound fraction</td>
<td>fᵤ,p</td>
<td>0.157</td>
<td>0.750</td>
<td>0.194</td>
</tr>
<tr>
<td>Blood–plasma concentration ratio</td>
<td>R_b</td>
<td>0.872</td>
<td>0.827</td>
<td>0.889</td>
</tr>
<tr>
<td>Liver–plasma concentration ratio</td>
<td>Kₚ,h</td>
<td>7.27</td>
<td>9.83</td>
<td>7.15</td>
</tr>
</tbody>
</table>

The acid dissociation constant (pKₐ), plasma unbound fraction (fᵤ,p), and octanol–water partition coefficient (logP) values for atomoxetine, 4-hydroxyatomoxetine, and N-desmethylatomoxetine were obtained by in silico estimation using ACD/Percepta, Simcyp, and ChemDraw software, respectively. Parameters such as fᵤ,p, R_b, and Kₚ,h are assumed to be the same in mice and humans.

The liver (kidney)-to-plasma concentration ratios (Kₚ,h/Kₚ,r) and the blood-to-plasma concentration ratio (R_b) were calculated from the fᵤ,p and logP values as follows (Adachi et al., 2023c):

\[
K_{p,h} = \frac{0.02289 \cdot P + 0.72621}{0.001719 \cdot P + 0.960581} \times \frac{1 + f_{u,p}}{2}
\]

\[
R_b = 0.45 \cdot (K_b \cdot f_{u,p} - 1) + 1 \quad \text{where} \quad \log K_b = 0.617 \cdot \log \left(\frac{1 - f_{u,p}}{f_{u,p}}\right) + 0.208
\]
Table 3. Physiological, experimental, and final calculated parameters for humanized-liver mouse and human PBPK models for atomoxetine, 4-hydroxyatomoxetine, and N-desmethylatomoxetine

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Abbreviation (unit)</th>
<th>Humanized-liver mouse</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction absorbed × intestinal availability</td>
<td>$F_a \cdot F_g$</td>
<td>(1)</td>
<td>1</td>
</tr>
<tr>
<td>Absorption rate constant</td>
<td>$k_a$ (1/h)</td>
<td>(5.93 ± 0.02)</td>
<td>4.41</td>
</tr>
<tr>
<td>Volume of systemic circulation for atomoxetine</td>
<td>$V_{1_{,substrate}}$ (L)</td>
<td>0.0961 ± 0.0030$^a$</td>
<td>278</td>
</tr>
<tr>
<td>Hepatic intrinsic clearance for atomoxetine</td>
<td>$CL_{h,\text{int} }$ (L/h)</td>
<td>0.671 ± 0.015$^a,b$</td>
<td>671$^c$</td>
</tr>
<tr>
<td>Hepatic clearance for atomoxetine</td>
<td>$CL_{h_{,substrate}}$ (L/h)</td>
<td>0.0635</td>
<td>50.4</td>
</tr>
<tr>
<td>Renal clearance for atomoxetine</td>
<td>$CL_{r_{,substrate}}$ (L/h)</td>
<td>0.0001</td>
<td>0.0252</td>
</tr>
<tr>
<td>Volume of systemic circulation for 4-hydroxyatomoxetine</td>
<td>$V_{1_{,4-\text{hydroxyatomoxetine}}}$ (L)</td>
<td>0.0233 ± 0.0025$^a$</td>
<td>75.6</td>
</tr>
<tr>
<td>Volume of systemic circulation for N-desmethylatomoxetine</td>
<td>$V_{1_{,N-\text{desmethylatomoxetine}}}$ (L)</td>
<td>0.756 ± 0.014$^a$</td>
<td>2130</td>
</tr>
<tr>
<td>Hepatic intrinsic clearance for 4-hydroxyatomoxetine</td>
<td>$CL_{h_{,4-\text{hydroxyatomoxetine}}}$ (L/h)</td>
<td>0.00923 ± 0.00091$^a$</td>
<td>9.23</td>
</tr>
<tr>
<td>Hepatic intrinsic clearance for N-desmethylatomoxetine</td>
<td>$CL_{h_{,N-\text{desmethylatomoxetine}}}$ (L/h)</td>
<td>0.527 ± 0.014$^a$</td>
<td>527</td>
</tr>
<tr>
<td>Hepatic clearance for 4-hydroxyatomoxetine</td>
<td>$CL_{h_{,4-\text{hydroxyatomoxetine}}}$ (L/h)</td>
<td>0.00663</td>
<td>6.46</td>
</tr>
<tr>
<td>Hepatic clearance for N-desmethylatomoxetine</td>
<td>$CL_{h_{,N-\text{desmethylatomoxetine}}}$ (L/h)</td>
<td>0.0623</td>
<td>49.6</td>
</tr>
<tr>
<td>Renal clearance for 4-hydroxyatomoxetine</td>
<td>$CL_{r_{,4-\text{hydroxyatomoxetine}}}$ (L/h)</td>
<td>0.00155</td>
<td>0.307</td>
</tr>
<tr>
<td>Renal clearance for N-desmethylatomoxetine</td>
<td>$CL_{r_{,N-\text{desmethylatomoxetine}}}$ (L/h)</td>
<td>0.0001</td>
<td>0.0248</td>
</tr>
</tbody>
</table>

$^a$ Data are presented as mean ± standard deviation.

$^b$ A decreased hepatic intrinsic clearance for atomoxetine (0.309 L/h) was used to model the presence of co-administered P450 2D6 inactivator paroxetine, resulting in suppression of plasma $C_{\text{max}}$ (24%) and AUC (52%) values of atomoxetine and fraction metabolized by P450 2D6 (0.9) and the unchanged part (0.671 × 0.9 × 0.4 + 0.671 × 0.1).

$^c$ A decreased hepatic intrinsic clearance for atomoxetine (309 L/h) was used to model the presence of paroxetine.

The original metabolic ratio of 4-hydroxyatomoxetine and N-desmethylatomoxetine (0.95 and 0.05) from atomoxetine by hepatic clearance was also modified to 0.70 and 0.30 in the presence of paroxetine.

A set of differential equations was solved for the concentrations in mice:

$$V_h \frac{dC_h}{dt} = -\frac{Q_h \cdot C_h \cdot R_b}{K_{p,h}} - CL_{h,\text{int}} \cdot \frac{C_h}{K_{p,h}} \cdot f_{u,p} + Q_h \cdot C_b$$
\[
V_1 \frac{dC_b}{dt} = R_{i.v.} - (Q_h + Q_r) \cdot C_b + \frac{Q_h \cdot C_h \cdot R_b}{K_{p_h}} + \frac{Q_r \cdot C_r \cdot R_b}{K_{p_r}}
\]

\[
V_r \frac{dC_r}{dt} = Q_r \cdot C_b - \frac{Q_r \cdot C_r \cdot R_b}{K_{p_r}} - C_L_r \cdot \frac{C_r}{K_{p_r}} \cdot f_{u,p}
\]

where \(C_h, C_r, C_b, \) and \(R_{i.v.}\) are the hepatic, renal, and blood substrate concentrations and the rate of administration, respectively (Kamiya et al., 2022). \(V_h, V_r,\) and \(Q_h/Q_r\) are the liver (0.85 mL) and kidney (0.34 mL) volumes and the blood flow rates of the systemic circulation to the hepatic/renal compartments (0.16 L/h) in mice (25 g body weight), respectively.

A set of differential equations was also solved for concentrations in humans:

\[
\frac{dx_g}{dt} = -k_a \cdot X_g \quad \text{when at } t = 0, X_g(0) = F_a \cdot F_g \cdot \text{dose}
\]

\[
V_h \frac{dC_h}{dt} = k_a \cdot X_g - \frac{Q_h \cdot C_h \cdot R_b}{K_{p,h}} - C_L_{h,\text{int}} \cdot \frac{C_h}{K_{p,h}} \cdot f_{u,p} + Q_h \cdot C_b
\]

\[
V_1 \frac{dC_b}{dt} = -(Q_h + Q_r) \cdot C_b + \frac{Q_h \cdot C_h \cdot R_b}{K_{p,h}} + \frac{Q_r \cdot C_r \cdot R_b}{K_{p_r}}
\]

\[
V_r \frac{dC_r}{dt} = Q_r \cdot C_b - \frac{Q_r \cdot C_r \cdot R_b}{K_{p,r}} - C_L_r \cdot \frac{C_r}{K_{p,r}} \cdot f_{u,p}
\]

where \(X_g\) is the amount of drug in the gut. \(V_h, V_r,\) and \(Q_h/Q_r\) are the liver (1.5 L) and kidney (0.28 L) volumes and the blood flow rates of the systemic circulation to the hepatic/renal compartments (96.6 L/h) in adult humans (70 kg body weights) (Kamiya et al., 2022). A multiplicative factor of 0.744 was applied to the virtual absorption rate constant (the number in parenthesis) in humanized-liver mice to generate the human absorption rate constant (Kamiya et al., 2022).
Table 4. Output values for atomoxetine, 4-hydroxyatomoxetine, and N-desmethylandatomoxetine by humanized-liver mouse PBPK modeling

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Atomoxetine</th>
<th>4-Hydroxyatomoxetine</th>
<th>N-Desmethylatomoxetine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_{\text{max}}$, ng/mL (fold to experimental)</td>
<td>758 (2.9)</td>
<td>1250 (0.52)</td>
<td>2.39 (1.1)</td>
</tr>
<tr>
<td>AUC, ng h/mL (fold)</td>
<td>1250 (2.5)</td>
<td>8760 (1.2)</td>
<td>31.6 (1.9)</td>
</tr>
<tr>
<td>Urine, 0–24 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean concentration (fold, range), µg/mL</td>
<td>1.16 (6.7, 4.4–15)</td>
<td>132 (2.7, 2.2–4.2)</td>
<td>0.028 (14, 8.9–34)</td>
</tr>
<tr>
<td>Treated with paroxetine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_{\text{max}}$, ng/mL</td>
<td>783 (1.4)</td>
<td>750 (1.4)</td>
<td>12.7 (0.43)</td>
</tr>
<tr>
<td>AUC, ng h/mL</td>
<td>2090 (0.58)</td>
<td>6440 (0.80)</td>
<td>186 (0.35)</td>
</tr>
<tr>
<td>Urine, 0–24 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean concentration (fold, range), µg/mL</td>
<td>1.93 (2.0, 1.5–3.0)</td>
<td>97.2 (3.5, 2.8–6.4)</td>
<td>0.166 (1.1, 0.60–2.6)</td>
</tr>
</tbody>
</table>

Numbers in parentheses indicate fold values relative to the experimental results shown in Figs. 3 and 4.
Table 5. Patients administered with prescribed atomoxetine.

<table>
<thead>
<tr>
<th>No.</th>
<th>Patient age (years), gender (weight)</th>
<th>Time after last administration (morning/evening dose, mg)</th>
<th>CYP2D6 genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12, M (30 kg)</td>
<td>1.3 h (25/25 mg)</td>
<td>*10-*36/*10-*36</td>
</tr>
<tr>
<td>2</td>
<td>10, M (28 kg)</td>
<td>2.0 h (25/25 mg)(^a)</td>
<td>*1/*1</td>
</tr>
<tr>
<td>3</td>
<td>14, F (50 kg)</td>
<td>1.5 h (30/30 mg)</td>
<td>*1/*10</td>
</tr>
<tr>
<td>4</td>
<td>9, F (36 kg)</td>
<td>4.0 h (25/35 mg)</td>
<td>*1/*2-*2</td>
</tr>
<tr>
<td>5</td>
<td>11, M (41 kg)</td>
<td>2.3 h (35/35 mg)</td>
<td>*2-*2/*10</td>
</tr>
<tr>
<td>6</td>
<td>13, F (40 kg)</td>
<td>4.0 h (40/40 mg)(^b)</td>
<td>*2/*10-*36</td>
</tr>
<tr>
<td>7</td>
<td>13, M (40 kg)</td>
<td>5.5 h (35/35 mg)</td>
<td>*10-*36/*36-*36</td>
</tr>
<tr>
<td>8</td>
<td>10 M (25 kg)</td>
<td>1.0 h (15/15 mg)</td>
<td>*10-*36/*10-*36</td>
</tr>
<tr>
<td>9</td>
<td>10, M (33 kg)</td>
<td>4.0 h (20/20 mg)</td>
<td>*1/*10</td>
</tr>
<tr>
<td>10</td>
<td>10, M (33 kg)</td>
<td>1.8 h (20/20 mg)</td>
<td>*2/*10</td>
</tr>
<tr>
<td>11</td>
<td>8, M (29 kg)</td>
<td>1.5 h (25/25 mg)</td>
<td>*1/*5</td>
</tr>
<tr>
<td>12</td>
<td>13, M (50 kg)</td>
<td>2.5 h (40/50 mg)(^c)</td>
<td>*10-*36/*36-*36</td>
</tr>
<tr>
<td>13</td>
<td>13, M (36 kg)</td>
<td>6.5 h (20/25 mg)(^d)</td>
<td>*10-*36/*10-*36</td>
</tr>
</tbody>
</table>

Urine samples were collected 4\(^a\), 11\(^b\), 2.5,\(^c\) and 6.5\(^d\) h after last administrations in the morning.
Patients treated with atomoxetine
\( n = 425 \) out of 775,555 (in 2004-22)

Patients treated **solely** with atomoxetine
\( n = 58 \)

Patients for whom data were available for the calculation of the time to onset of adverse events resulting from doses of 10-100 mg/day atomoxetine or unknown dosage
\( n = 16 \) (including 3 cases of >20 years old)

Patients for whom data were available for the calculation of the time to onset of adverse events dependent on increased drug exposure
\( n = 14 \) (including 2 cases of >20 years old)

Patients treated with paroxetine
\( n = 5832 \) out of 775,555 (in 2004-22)

Patients treated **solely** with paroxetine
\( n = 651 \)

Patients for whom data were available for the calculation of the time to onset of adverse events resulting from doses of 10 and 20 mg/day paroxetine dosage
\( n = 81 \)

Patients for whom data were available for the calculation of the time to onset of adverse events dependent on increased drug exposure
\( n = 70 \)

---

**Fig. 1**

(A) Patient

(B) Patient

(C) Event

(D) Event

Cumulative incidence rate

Median (interquartile range) day
10 (2–319), numbers of events = 19
10 mg - 100 mg and unknown doses combined

Median (interquartile range) day
10 mg (broken line); 5 (1–21), numbers of events = 54
20 mg (solid line); 3 (0–15), \( n = 62 \)
log rank test for two low doses, \( p = 0.15 \)
Fig. 2

(A) Liver microsomes

Atomoxetine oxidation, pmol/min/mg protein

- 4-Hydroxy-atomoxetine
- N-Desmethyl-atomoxetine

Untreated

P450 2D6 inactivated

P450 2D6

(B) Recombinant P450

Atomoxetine oxidation, nmol/min/nmol P450

- 4-Hydroxy-atomoxetine
- N-Desmethyl-atomoxetine

P450 2D6.1

P450 2D6.10

P450 2C19

(22**)

(20**)
Fig. 3

(A) Untreated [Atomoxetine] and [metabolites] in plasma, ng/mL

(B) Treated with paroxetine [Atomoxetine] and [metabolites] in plasma, ng/mL

(C) Untreated [Atomoxetine] and [metabolites] in urine, ng/mL

(D) Treated with paroxetine [Atomoxetine] and [metabolites] in urine, ng/mL

(E) Atomoxetine

(F) Total 4-hydroxy-atomoxetine

(G) N-Desmethyl-atomoxetine

Time after intravenous administration, h

Urine, 0-24 h

[Cmax, AUC]

Prereated

Untreated

**
Fig. 4

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Fig. 5

(A) Untreated

(B) Treated with paroxetine

(C) Untreated

(D) Treated with paroxetine
Fig. 6

(A) Harboring CYP2D6*1

(B) Harboring no CYP2D6*1

(C)

(D)

(E)

(F)

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