DISPOSITION AND METABOLIC FATE OF ATOMOXETINE HYDROCHLORIDE: THE ROLE OF CYP2D6 IN HUMAN DISPOSITION AND METABOLISM

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ABSTRACT:

The role of the polymorphic cytochrome P450 2D6 (CYP2D6) in the pharmacokinetics of atomoxetine hydrochloride ([(−)-N-methyl(-γ-(2-methylphenoxy)benzenepropanamine hydrochloride; LY139603] has been documented following both single and multiple doses of the drug. In this study, the influence of the CYP2D6 polymorphism on the overall disposition and metabolism of a 20-mg dose of 14C-atomoxetine was evaluated in CYP2D6 extensive metabolizer (EM; n = 4) and poor metabolizer (PM; n = 3) subjects under steady-state conditions. Atomoxetine was well absorbed from the gastrointestinal tract and cleared primarily by metabolism with the preponderance of radioactivity being excreted into the urine. In EM subjects, the majority of the radioactive dose was excreted within 24 h, whereas in PM subjects the majority of the dose was excreted by 72 h. The biotransformation of atomoxetine was similar in all subjects undergoing aromatic ring hydroxylation, benzylc oxida- tion, and N-demethylation with no CYP2D6 phenotype-specific metabolites. The primary oxidative metabolite of atomoxetine was 4-hydroxyatomoxetine, which was subsequently conjugated forming 4-hydroxyatomoxetine-O-glucuronide. Due to the absence of CYP2D6 activity, the systemic exposure to radioactivity was prolonged in PM subjects (t1/2 = 62 h) compared with EM subjects (t1/2 = 18 h). In EM subjects, atomoxetine (t1/2 = 5 h) and 4-hydroxyatomoxetine-O-glucuronide (t1/2 = 7 h) were the principle circulating species, whereas atomoxetine (t1/2 = 20 h) and N-demethylatomoxetine (t1/2 = 33 h) were the principle circulating species in PM subjects. Although differences were observed in the excretion and relative amounts of metabolites formed, the primary difference observed between EM and PM subjects was the rate at which atomoxetine was biotransformed to 4-hydroxyatomoxetine.

Atomoxetine hydrochloride (LY139603; formerly known as atomoxetine hydrochloride) is known chemically as (−)-N-methyl-γ-(2-methylphenoxy)benzenepropanamine hydrochloride. Atomoxetine is a potent inhibitor of the presynaptic norepinephrine transporter with minimal affinity for other monoamine transporters or receptors (Wong et al., 1982; Gehlert et al., 1993). Atomoxetine is under development as a therapeutic agent for the treatment of attention deficit/hyperactivity disorder in children, adolescents, and adults.

Atomoxetine is predominantly metabolized by CYP2D6 (Ring et al., 2002); therefore, its single and multiple dose pharmacokinetics are influenced by the polymorphic expression of this enzyme (Farid et al., 1985). As a result, the pharmacokinetics of atomoxetine appear to have a bimodal distribution with two distinct populations. The enzymatic activity of CYP2D6 is determined by a genetic polymorphism (Evans et al., 1980; Steiner et al., 1988), and is an important source of intersubject variability in metabolism for a number of drugs, including debrisoquine, desipramine, and dextromethorphan (Wolf and Smith, 1999). Mutations or deletion of the CYP2D6 gene results in a minority of people (about 7% of the Caucasian population and <1% of the Asian population) who are poor metabolizers (PM1) of CYP2D6 substrates (Guengerich, 1995). The PM trait is inherited as an autosomal recessive characteristic that requires the presence of two defective CYP2D6 alleles. The majority of individuals are designated extensive metabolizers (EM) of CYP2D6 substrates and possess a range of activities considered to be normal CYP2D6 activity.

Although the role of CYP2D6 in the pharmacokinetics (Farid et al., 1985) and in vitro metabolism (Ring et al., 2002) of atomoxetine has been previously described, the influence of this polymorphic enzyme on the overall disposition and metabolism of atomoxetine has not been characterized. Therefore, the aim of the present study was to evaluate the primary routes of metabolism for atomoxetine and its metabolites, as well as the rate and extent to which these processes occur in individuals with normal and deficient CYP2D6 activity under steady-state conditions.

1 Abbreviations used are: PM, poor metabolizers; EM, extensive metabolizers; HPLC, high pressure liquid chromatography; F, absolute oral bioavailability; AUC, area under the time versus plasma concentration curve; CL, clearance; Cavg, average plasma concentration at steady state; LC/APCI/MS/MS, liquid chromatography-atmospheric pressure chemical ionization-tandem mass spectrometry; dpm, disintegrations per minute; LSC, liquid scintillation counting; MDA, minimum detectable activity; CI, clinical clearance; Cmax, maximal plasma concentration; Cavg, average plasma concentration; Cmin, minimal plasma concentration at steady state; Tmax, time to maximal plasma concentration; Vz, volume of distribution.
DISPOSITION OF ATOMOXETINE IN HUMANS

Materials and Methods

Reference Compounds and Other Materials. The following compounds were synthesized at Lilly Research Laboratories: atomoxetine hydrochloride, [L139603; (−)-N-methyl-γ-(2-methylphenoxyl)benzenepropanamine hydrochloride]; 3,14C-atomoxetine hydrochloride (radiochemical purity, 99%; specific activity, 22.34 μCi/mg), HCl-atomoxetine, N-desmethylandamoxetine hydrochloride [N-γ-(2-methylphenoxy)benzenepropanamine hydrochloride], 3,14C-N-desmethylandamoxetine oxalate (radiochemical purity, 99%; specific activity, 66.7 μCi/mg), 4-hydroxyadamoxetine oxalate [N-γ-(2-methyl-4-hydroxyphenoxy)benzenepropanamine oxalate], 3,14C-4-hydroxyadamoxetine oxalate (radiochemical purity, 99.4%; specific activity, 55.8 μCi/mg), 2-hydroxyethyladamoxetine hydrochloride [N-methyl-γ-(2-hydroxyphenoxy)benzenepropanamine hydrochloride], 2-carboxyamoxetine hydrochloride [(+)-N-methyl-γ-(2-carboxymethylphenoxy)benzenepropanamine hydrochloride], 4-hydroxyn,N-desmethylandamoxetine hydrochloride [N-methyl-γ-(2-methyl-4-hydroxy- phenoxy)benzenepropanamine hydrochloride], 2,4-dihydroxyadamoxetine hydrochloride [N-γ-(2-hydroxyethyl-4-hydroxyphenoxy)benzenepropanamine hydrochloride] and 4-hydroxy-2-carboxyamoxetine hydrochloride [N-methyl-γ-(2-carboxy-4-hydroxyphenoxy)benzenepropanamine hydrochloride].

HPLC grade ammonium acetate and acetonitrile were purchased from Fisher Scientific (Fair Lawn, NJ). Ultima-Gold XR, Permafluor V and Ultima-Fluor M were purchased from PerkinElmer Life Sciences (Boston, MA).

Subjects. The study was conducted at the Lilly Laboratory for Clinical Research (Indianapolis, IN). The protocol and informed consent documents were approved by the Institutional Review Board of Indiana University—Purdue University at Indianapolis. The study was conducted in accordance with the Declaration of Helsinki. All participants provided informed written consent before enrollment into the study. All volunteers were considered to be healthy on the basis of medical history, physical examination, electrocardiographic findings, and routine clinical laboratory tests. Volunteers with clinically abnormal results were excluded from the study.

CYP2D6 EM and PM subjects, as determined by genotyping and phenotyping analyses, were entered in this study. CYP2D6 genotype was performed by PPgx (Morrisville, NC). DNA from whole blood samples were isolated, purified, and analyzed for CYP2D6 genotype using a validated polynucleotide chain reaction method. CYP2D6 genotype was evaluated by testing for the non-wild type (PM) alleles *3, *4, *5, *6, *7, and *8. If patients were homozygous for any combination of these alleles, a PM genotype was assigned; otherwise, an EM genotype was assigned. CYP2D6 phenotype was performed using the urine ratio of dextromethorphan/dextrorphan following an oral dose of dextromethorphan (Harris Laboratories Inc., Lincoln, NE). Volunteers with a ratio greater than 0.3 were assigned a PM phenotype, and those with a ratio less than 0.3 were assigned an EM phenotype.

Study Design. This was an open-label study conducted in 7 healthy men whose CYP2D6 genotype was identified as EM or PM prior to study start. Multiple 20-mg doses of atomoxetine as the hydrochloride salt in a capsule were administered twice daily over 5 days following by a single radiolabeled atomoxetine (14C-atomoxetine as the hydrochloride salt in a capsule) 20-mg dose (actual dose 19.66 mg; 92.4 μCi) on the morning of the 6th day.

Sample Collection. Heparinized blood samples (approximately 12 mL) were collected from EM subjects 12 h prior to and immediately before administration of 14C-atomoxetine, and at approximately 1, 2, 3, 4, 6, 8, 12, 18, 24, 36, 48, and 72 h after dosing. For PM subjects, blood samples were obtained 12 h prior to and immediately before administration of 14C-atomoxetine, and at approximately 1, 2, 3, 4, 6, 8, 12, 18, 24, 48, 72, 96, 120, 144, 168, 192, and 216 h after dosing. Additional blood samples were collected at approximately 2 h after administration of 14C-atomoxetine for ex vivo plasma protein binding determination.

Urine was collected from −12 to 0, 0 to 2, 2 to 4, 4 to 8, 8 to 12, 12 to 24 h and at 24-h intervals thereafter through 168 h for the EM subjects and 246 h for the PM subjects after the administration of 14C-atomoxetine. Fecal samples were collected prior to the administration of 14C-atomoxetine and at 24-h intervals thereafter through 168 h for the EM patients and 246 h for the PM patients. A sample of expired air was collected for analysis of 14CO2 prior to administration of 14C-atomoxetine and at approximately 0.5, 1, 3, 6, 8, 12, and 24 h after dosing.

Determination of Radioequivalents. Radioactivity in whole blood and feces was determined by combustion followed by liquid scintillation counting (LSC). Radioactivity in plasma, urine, and breath was determined by LSC. For all samples, radioactivity levels were quantitated using a Packard Tri-Carb 2300 liquid scintillation counting system with external standard correction. Radioactivity calculations were based on the actual potency (19.66 mg) and radioactivity (92.4 μCi) measured for the dose prior to administration (specific activity of radiolabeled dose = 4.7 × 10−3 μCi/g).

The quantitation limit for radioactivity was calculated by determining the minimum detectable activity (MDA) of the assay (at the 99.7% confidence level). The MDA determines the count rate that is needed above the background count rate to be quantitated and is calculated by the equation: MDA = 3 × √cpm bg / h bg where cpm bg is the background counts per minute and h bg is the counting time. Units of cpm were converted to dpm using an estimated value of counting efficiency. For feces and whole blood samples values below the MDA value of 6 dpm, and 5 dpm for plasma and urine were reported as below quantitation limit.

Bioanalytical Methodology. Plasma concentrations of atomoxetine, N-desmethylationamoxetine, and 4-hydroxyatomoxetine, as well as the concentration of these analytes following treatment with a deconjugation reagent (β-glucuronidase, type B1; Sigma-Aldrich, St. Louis, MO), were determined using a validated liquid chromatography/atmospheric pressure chemical ionization/mass spectrometry/mass spectrometry (LC/APCI/MS/MS) method. The dynamic range for the assay was 1 to 800 ng/mL for N-desmethylationamoxetine and 4-hydroxyatomoxetine, and 2.5 to 2000 ng/mL for atomoxetine. The intra/interassay precision ranged from 1.77 to 14.81% for atomoxetine, 0.95 to 9.60% for 4-hydroxyatomoxetine, and 1.04 to 9.47% for N-desmethylationamoxetine. The intra/interassay accuracy ranged from −12.90% to 8.00% for atomoxetine, −11.50 to −1.69% for 4-hydroxyatomoxetine and −7.64 to 6.00% for N-desmethylationamoxetine. If required, additional analyses were conducted using a lower range method over the concentration ranges 1 to 100 ng/mL for N-desmethylationamoxetine and 4-hydroxyatomoxetine, and 0.25 to 25 ng/mL for atomoxetine. The intra/interassay precision ranged from 1.45 to 6.97% for atomoxetine, 0.88 to 6.87% for 4-hydroxyatomoxetine and 2.26 to 11.58% for N-desmethylationamoxetine. The intra/interassay accuracy ranged from −6.40 to −0.24% for atomoxetine, −4.80 to 4.80% for 4-hydroxyatomoxetine and −10.50 to 9.08% for N-desmethylationamoxetine. Taylor Technologies Inc. (Princeton, NJ) performed measurement of atomoxetine and its metabolites.

Briefly, samples were buffered with dilute trifluoroacetic acid and extracted by solid-phase extraction on a styrene-divinylbenzene-phase cartridge. The analytes were eluted, dried under nitrogen, and reconstituted for analysis. Liquid chromatographic separation was con-
compounds were detected and quantified by tandem mass spectrometry using atmospheric pressure chemical ionization. Both $^3$H$_2$-atomoxetine and $^3$H$_2$-4-hydroxyatomoxetine were used as the internal standards. The LC/APCI/MS/MS analysis was conducted on either a Finnigan TSQ 700 or TSQ 7000.

**Metabolite Profiling.** Plasma samples were profiled using HPLC with radiochemical and LC/MS/MS analysis. Approximately 250-μl aliquots from 2, 3, 4, 6, 8, and 12-h plasma were combined (1.5 ml) for each subject. Acetonitrile (3 ml) was added to the plasma, centrifuged, and the supernatant was evaporated to dryness under a gentle nitrogen stream. The residue was reconstituted in 200 μl of water/acetonitrile (90:10, v/v) and injected onto a Shimadzu HPLC system, consisting of two model LC-10AD pumps, a SIL-10A autosampler, a DGU-3A degasser, a CTO-10A column heater, and a model SCL-10A controller with a Berthold model LB 507A radiodetector equipped with a 150 μl ytrrium solid cell. For profiling plasma metabolites, samples were analyzed on a Zorbax Eclipse XDB-C18 (5 μm particle size, 0.46 × 15 cm) column using a gradient of 0.050 M ammonium acetate and acetonitrile. Solvent composition was initially maintained at 90:10 (0.050 M ammonium acetate/acetonitrile) and programmed at 1.67%/min until a mobile phase composition of 60% acetonitrile was achieved (30 min). The column was maintained at 30°C, and the flow rate was 1.0 ml/min.

Pooled urine samples were prepared for each subject [0 to 168 h for EM subjects (entire collection period) and 0 to 120 h for PM subjects (abridged collection period)] by combining aliquots for each time point base on the percentage of total collected urine volume for that subject. In the PM subjects, the collection intervals evaluated were chosen to obtain the maximal amount of radioactivity in the minimal amount of urine. Urine samples were concentrated by lyophilization for HPLC profiling and LC/MS/MS analysis. Residue was reconstituted with water/acetonitrile (90:10, v/v) to a volume approximately one-tenth the original volume. Samples were vortexed and then centrifuged through a Millipore nylon 0.45 μm centrifugal filter. The mean recovery of radioactivity was 89.1 ± 0.8% with a range of 84.5 to 94.8%. For profiling of urine metabolites, samples were analyzed in the same manner as described above for plasma.

The fecal homogenate with the highest amount of radioactivity per weight from each subject was extracted into acidic methanol, filtered, and profiled. The mean recovery was 78.6 ± 3.3% with a range of 64.3 to 88.7%. The radioactivity in fecal homogenates was profiled using the same HPLC system as described above.

For metabolite identification, extracts of plasma, urine, and fecal homogenates were analyzed by LC/MS and LC/MS/MS on either a Finnigan TSQ 700 or TSQ 7000. Analytes were separated on a Zorbax Eclipse XDB-C18 column using chromatographic conditions as described for profiling except that 0.025 M ammonium acetate was used in place of 0.050 M to reduce ion current. Analysis was performed by injecting aliquots of extracts onto the analytical column coupled to the mass spectrometer via a splitting tee such that the flow rate to the ion source was approximately 200 μl/min, the remaining effluent being diverted to the radiochemical detector. For positive electrospray full-scan analysis, the mass spectrometer was programmed to scan from m/z 200 to 800 every second. For MS/MS experiments, collision gas (argon) pressure was maintained at 2.0 millitorr, and the collision offset voltage was −20 to −30 eV.

**Plasma Protein Binding Analysis.** Plasma samples collected at 2 h after administration of $^{14}$C-atomoxetine, and blank plasma spiked with $^{14}$C-atomoxetine, $^{14}$C-N-desmethylatomoxetine (3-14C-N-desmethylatomoxetine oxalate) or $^{14}$C-4-hydroxyatomoxetine (3-14C-4-hydroxyatomoxetine oxalate) were evaluated for protein binding. Following ultracentrifugation [100,000 rpm (approximately 430,000g), 37°C, 4 h], the amount of radioactivity in the supernatant was determined by LSC. The fraction of radioactivity bound to protein was calculated from the radioactivity concentrations in the spiked sample and supernatant. The protein binding was calculated as follows: % plasma protein binding = (1 − Cf/Cp) × 100 where Cf is the amount of radioactivity in protein-free fraction and Cp is the amount of radioactivity in plasma.

The ability of $^{14}$C-atomoxetine to bind to fatty acid and globulin-free human albumin (41.6 mg/ml), human α1-acid glycoprotein (0.9 mg/ml), and IgG (11.6 mg/ml) dissolved in Krebs-Ringer buffer (pH 7.4) was also evaluated.

**Pharmacokinetic Analysis.** Single dose pharmacokinetic parameter estimates for $^{14}$C-radioequivalents were calculated with noncompartmental analysis by using WinNonlin professional version 2.1 (Pharsight Corp, Mountain View, CA). The maximum plasma concentration ($C_{\text{max}}$), and the corresponding time of the maximum concentration ($T_{\text{max}}$) were observed values. The elimination rate constant ($\lambda_e$) was determined as the slope of the linear regression for the cumulative elimination of radioactivity after oral administration of a 20-mg dose of $^{14}$C-atomoxetine to CYP2D6 extensive and poor metabolizer subjects.

**Fig. 1.** Cumulative elimination of radioactivity after oral administration of a 20-mg dose of $^{14}$C-atomoxetine to CYP2D6 extensive and poor metabolizer subjects. Data are expressed as mean ± S.E.M.
terminal log-linear portion of the concentration-time curve. Terminal half-life \( (t_{1/2}) \) was calculated as \( \ln(2)/\lambda_d \). The area under the plasma concentration-time curve \( (\text{AUC}_{0-\infty}) \) were estimated by the linear trapezoidal method and extrapolated to infinite time. Apparent clearance \( (\text{CL}_{ss}/F) \) and apparent volume of distribution \( (V_z/F) \) were calculated as \( \text{dose/\text{AUC}_{0-\infty}} \) and as \( (\text{CL}_{ss}/F)/\lambda_d \), respectively.

The pharmacokinetic evaluation of the steady-state atomoxetine, \( N \)-desmethylatomoxetine, and 4-hydroxyatomoxetine plasma concentration data employed noncompartmental pharmacokinetic methods of analysis. The minimum plasma concentration \( (C_{ss,\text{min}}) \), the maximum plasma concentration \( (C_{ss,\text{max}}) \), and the corresponding times of these maximal concentrations \( (T_{\text{max}}) \) were identified from the measured samples and recorded. The elimination rate constant \( (\lambda_d) \) and terminal half-life \( (t_{1/2}) \) were calculated as described above when sufficient data were available. The area under the plasma concentration time curve \( (\text{AUC}_{0-\infty}) \) over the 12-h dosing interval \( (\tau) \) was estimated by the linear trapezoidal method. Average steady-state plasma concentration \( (C_{ss,\text{avg}}) \), apparent plasma clearance \( (\text{CL}_{ss}/F) \), and apparent volume of distribution \( (V_z/F) \) were also calculated.

The reported plasma concentrations of the conjugated metabolites of atomoxetine are derived values and were not determined directly. The plasma concentrations of the conjugated metabolite data employed noncompartmental pharmacokinetic methods of analysis. The minimum plasma concentration \( (C_{ss,\text{min}}) \), the maximum plasma concentration \( (C_{ss,\text{max}}) \), and the corresponding times of these maximal concentrations \( (T_{\text{max}}) \) were identified from the measured samples and recorded. The elimination rate constant \( (\lambda_d) \) and terminal half-life \( (t_{1/2}) \) were calculated as described above when sufficient data were available. The area under the plasma concentration time curve \( (\text{AUC}_{0-\infty}) \) over the 12-h dosing interval \( (\tau) \) was estimated by the linear trapezoidal method. Average steady-state plasma concentration \( (C_{ss,\text{avg}}) \), apparent plasma clearance \( (\text{CL}_{ss}/F) \), and apparent volume of distribution \( (V_z/F) \) were also calculated.

The reported plasma concentrations of the conjugated metabolites of atomoxetine are derived values and were not determined directly. The plasma concentrations of the conjugated metabolite concentrations were determined by subtracting the analyte concentration in nonhydrolyzed plasma from hydrolyzed plasma when concentrations of hydrolyzed plasma were greater than concentrations of the nonhydrolyzed plasma. The only conjugated metabolite observed for 4-hydroxyatomoxetine during the metabolic characterization portion of the study was an \( O \)-glucuronide; therefore, all 4-hydroxyatomoxetine-derived conjugated metabolites were assumed to be 4-hydroxyatomoxetine-\( O \)-glucuronide. No conjugates of atomoxetine or \( N \)-desmethylatomoxetine were observed.

**Fig. 2.** HPLC radiochromatograms of pooled urine samples following a single oral 20-mg dose of \(^{14}C\)-atomoxetine in extensive metabolizer (0 to 168 h pooled urine samples) and poor metabolizer (0 to 120 h pooled urine samples) subjects.
Results

Subject Characteristics. Seven male subjects were entered into and completed the study. The four EM subjects ranged from 38 to 54 years of age with a mean age of 45 years. The mean EM subject body mass index was 22.8 kg/m². The three PM subjects ranged from 19 to 49 years of age with a mean age of 35 years. The mean PM subject body mass index was 24.5 kg/m².

Excretion of Atomoxetine and Its Metabolites. Urine and feces were collected until excreted radioactivity levels were less than 5 times that of environmental background. The recovery of radioactivity in urine and feces 168 h after the administration of [14C]-atomoxetine for the EM subjects and at 264 h for the PM subjects was measured. The total recovery was similar in all subjects, independent of their CYP2D6 metabolic status. The combined recovery for EM and PM subjects was 97.2 ± 1.1% with 89.0 ± 3.5% excreted in urine and 8.2 ± 3.2% in feces. The overall mean cumulative excretion of radioactivity in urine and feces is shown in Fig. 1 for EM and PM subjects. Although the elimination of radioactivity into the urine was the primary route of excretion regardless of CYP2D6 metabolic status, the amount of fecal excretion was different between EM and PM subjects. Although each of the individual urine samples were evaluated for each subject, the relative amount of each metabolite excreted in urine was determined by preparing a composite pooled urine sample. For each subject, pooled urine samples were based on the total urine volume each individually collected time point represented [0 to 168 h for EM subjects (entire collection period) and 0 to 264 h in the majority of PM subjects. Radioactivity was not associated with expired air.

Biotransformation of Atomoxetine. Pooled plasma samples (2- to 12-h samples) were profiled for each subject. The profiles obtained for all EM subjects were dominated by a single radioactive peak, 4-hydroxyatomoxetine-O-glucuronide, which accounted for approximately 67% of the total radioactivity in the plasma. 4-Hydroxyatomoxetine-O-glucuronide was identified by selective reaction monitoring-MS in which the transitions of m/z 448 (M + H⁺) to m/z 272 and 148 were monitored. In the plasma radioprofiles from PM subjects, unchanged parent drug and 4-hydroxyatomoxetine-O-glucuronide were the only detectable radioactive components. However, due to the low amount of radioactivity associated with the chromatographic peaks, the relative contribution of these analytes could not be defined.

Although each of the individual urine samples were evaluated for each subject, the relative amount of each metabolite excreted in urine was determined by preparing a composite pooled urine sample. For each subject, pooled urine samples were based on the total urine volume each individually collected time point represented [0 to 168 h for EM subjects (entire collection period) and 0 to 120 h for PM subjects (abridged collection period)] (Fig. 2). In the PM subjects, the collection intervals evaluated were chosen to obtain the maximal amount of radioactivity in the minimal amount of urine. Although

Fig. 3. HPLC radiochromatograms of urine samples (2–4, 8–12, and 24–48 h) following a single oral 20-mg dose of [14C]-atomoxetine in extensive metabolizer and poor metabolizer subjects.
of the 6th day.

to the prolonged excretion of radioactivity into the feces. Represen-
ted its LC/MS/MS properties with those obtained from an

carboxyatomoxetine (1)\textsuperscript{b} 12 258 1213, 144, 105, 30

In each of the fecal samples, the largest radioactive peak observed

\begin{table}
\centering
\caption{Retention time, molecular ions, and characteristic product ions of atomoxetine and its metabolites}
\begin{tabular}{|c|c|c|c|}
\hline
\textbf{Compound} & \textbf{HPLC Retention Time (min)} & \textbf{Molecular Ion ($[M+H]^{+}$, m/z)} & \textbf{Characteristic Product Ions} \\
\hline
4-Hydroxy-N\textsuperscript{-}desmethylatomoxetine-O-glucuronide & 6 & 434 & 330, 258, 206, 134, 30 \\
4-Hydroxyatomoxetine-O-glucuronide & 6.5 & 448 & 272, 148, 44 \\
4-Hydroxy-2-carboxyatomoxetine-O-glucuronide & 7 & 478 & 302, 148, 44 \\
Dihydroxyatomoxetine-O-glucuronide (1')\textsuperscript{b} & 8 & 464 & 322, 288, 148, 44 \\
Hydroxy-2-carboxyatomoxetine-O-glucuronide\textsuperscript{a} & 9 & 478 & 302, 148, 44 \\
Hydroxyatomoxetine (1')\textsuperscript{b} & 11 & 272 & 254, 226, 155, 148, 117, 44 \\
2-Hydroxyethylatomoxetine-O-glucuronide & 11 & 448 & 272, 150 \\
Dihydroxyatomoxetine-O-glucuronide (2)'\textsuperscript{b} & 11 & 464 & 288, 166, 44 \\
4-Hydroxy-N\textsuperscript{-}desmethylatomoxetine\textsuperscript{b} & 12 & 258 & 213, 134, 117, 105, 30 \\
4-Hydroxyatomoxetine\textsuperscript{b} & 13 & 272 & 213, 144, 44 \\
2-Hydroxyethylatomoxetine\textsuperscript{b} & 15 & 272 & 150, 107, 44 \\
Hydroxyatomoxetine (2)' & 16 & 272 & 150, 123, 115, 44 \\
N\textsuperscript{-}Desmethylatomoxetine\textsuperscript{b} & 19 & 242 & 197, 145, 105, 30 \\
Atomoxetine\textsuperscript{b} & 20 & 256 & 197, 148, 133, 117, 44 \\
\hline
\end{tabular}
\end{table}

\textsuperscript{a} Specific hydroxylation sites for these metabolites have not been defined.

\textsuperscript{b} HPLC retention time and fragmentation characteristics were identical to authentic standard.

\begin{table}
\centering
\caption{Metabolites of atomoxetine identified in urine from CYP2D6 extensive and poor metabolizer subjects following oral administration of $^{14}$C-atomoxetine}
\begin{tabular}{|c|c|c|c|c|}
\hline
\textbf{Compound} & \textbf{Poor Metabolizer Subjects} & \textbf{Extensive Metabolizer Subjects} \\
\hline
\textbf{Subject 1} & \textbf{Subject 2} & \textbf{Subject 3} & \textbf{Subject 4} & \textbf{Subject 5} & \textbf{Subject 6} \\
\hline
4-Hydroxy-N\textsuperscript{-}desmethylatomoxetine & +\textsuperscript{a} & + & N.D. & + & N.D. & N.D. \\
4-Hydroxyatomoxetine & + & + & + & + & + & + \\
2-Hydroxyethylatomoxetine & + & + & + & + & + & + \\
Hydroxyatomoxetine (1')\textsuperscript{b} & N.D. & + & + & + & + & + \\
Hydroxyatomoxetine (2)' & + & + & N.D. & + & N.D. & N.D. \\
N\textsuperscript{-}Desmethylatomoxetine & + & + & N.D. & N.D. & N.D. & N.D. \\
4-Hydroxy-N\textsuperscript{-}desmethylatomoxetine-O-glucuronide & + & + & + & + & + & + \\
4-Hydroxyatomoxetine-O-glucuronide & + & + & + & + & + & + \\
2-Hydroxyethylatomoxetine-O-glucuronide & + & + & + & + & + & + \\
Dihydroxyatomoxetine-O-glucuronide (1)'\textsuperscript{b} & + & + & + & + & + & + \\
Dihydroxyatomoxetine-O-glucuronide (2)'\textsuperscript{b} & N.D. & + & + & N.D. & N.D. & N.D. \\
4-Hydroxy-2-carboxyatomoxetine-O-glucuronide\textsuperscript{a} & + & + & + & + & + & + \\
Hydroxy carboxyatomoxetine-O-glucuronide\textsuperscript{a} & + & + & + & + & + & + \\
Atomoxetine\textsuperscript{b} & + & + & + & + & + & + \\
\hline
\end{tabular}
\end{table}

\textsuperscript{a} Multiple 20-mg doses of atomoxetine were administered twice daily over 5 days followed by a single radiolabeled atomoxetine 20-mg dose (actual dose 19.66 mg; 92.4 equivalents) in plasma was similar for EM and PM subjects with each peak occurring at a median T\textsubscript{max} of 2 h (Tables 4 and 5; Fig. 4). The mean area under the concentration curve (AUC\textsubscript{0-\infty}) was 4-fold higher in PM subjects compared with EM subjects and mean half-life was longer (18 h versus 62 h) as shown in Tables 4 and 5. The mean apparent clearance (CL\textsubscript{app}/F) for PM subjects was about 25% of EM subjects’ clearance with apparent volume of distribution (V\textsubscript{d}/F) the same in both groups.

The mean maximal concentration at steady state (C\textsubscript{ss,max}) for atomoxetine was almost 6-fold higher in PM subjects with peak concentration occurring at a median T\textsubscript{max} of 2 h. Mean AUC\textsubscript{0-\infty}, was 8-fold higher in PM subjects. Only EM subjects had measurable 4-hydroxyatomoxetine concentrations sufficient to calculate pharmacokinetic parameters. The individual 4-hydroxyatomoxetine C\textsubscript{ss,max} values were only about 1 to 3% of the atomoxetine C\textsubscript{ss,max} values. Mean C\textsubscript{ss,max} plasma concentrations for N-desmethylatomoxetine were almost 40-fold higher in PM subjects compared with EM subjects rising
from 6% of atomoxetine concentrations to 34% (based on AUC₀₋₉h). The mean half-life of N-desmethylatomoxetine was approximately 9 h in EM subjects and 33 h in PM subjects.

In EM subjects, 4-hydroxyatomoxetine-O-glucuronide concentrations were higher than atomoxetine or other metabolites with mean \( C_{ss,\text{max}} \) of 414 ng/ml occurring at a median \( T_{\text{max}} \) of 2 h and a mean \( \text{AUC}_{0-\infty} \) of 2.74 \( \mu \)g·h/ml. In PM subjects, these values were considerably lower than either atomoxetine or N-desmethylatomoxetine. The mean \( C_{ss,\text{max}} \) was 88 ng/ml occurring at a median \( T_{\text{max}} \) of 4 h and a mean \( \text{AUC}_{0-\infty} \) of 0.935 \( \mu \)g·h/ml. Although there is a lower amount of 4-hydroxyatomoxetine-O-glucuronide formed in PM subjects compared with EM subjects, plasma concentrations are higher than most other metabolites. The mean half-life of 4-hydroxyatomoxetine-O-glucuronide was approximately 7 h in EM subjects and 19 h in PM subjects.

**Plasma Protein Binding of Atomoxetine and Its Metabolites.**

The in vitro plasma protein binding demonstrated that atomoxetine was highly bound to plasma protein (98.7 ± 0.3% bound). Plasma protein binding of N-desmethylatomoxetine (99.1 ± 0.1% bound) was similar to atomoxetine, whereas the binding of 4-hydroxyatomoxetine to plasma protein (66.6 ± 0.3% bound) was substantially less than atomoxetine. Following incubation with albumin, a\(_{1}\)-acid glycoprotein or immunoglobulin G, atomoxetine was extensively bound to albumin (97.5 ± 0.1%), whereas binding to a\(_{1}\)-acid glycoprotein (77.3 ± 2.2%) and IgG (14.5 ± 0.8%) was much lower.

The percentage of plasma protein binding of total radioactivity at 2 h after administration of \(^{14}\)C-atomoxetine in EM subjects and PM subjects was 53.8 ± 4.9% and 96.7 ± 0.5%, respectively. Radioactivity of the 2-h plasma and ultracentrifuged protein-free plasma samples from each of the EM subjects showed a single radiolabeled
component that was identified by selective reaction monitoring-MS as 4-hydroxyatomoxetine-O-glucuronide. Radioprofiles of the 2-h plasma samples from PM subjects showed only a single radioactive peak having the same HPLC retention time as atomoxetine. No detectable peaks were present in radioprofiles of protein-free plasma from the PM subjects.

Discussion

Atomoxetine is a potent and selective inhibitor of the presynaptic norepinephrine transporter that has been developed for the treatment of attention deficit/hyperactivity disorder in children, adolescents, and adults. In clinical studies the safety and tolerability of atomoxetine in

### TABLE 5

<table>
<thead>
<tr>
<th>Parameter</th>
<th>14C-Equivalents</th>
<th>Atomoxetine</th>
<th>4-Hydroxyatomoxetine</th>
<th>N-Desmethylatomoxetine</th>
<th>4-Hydroxyatomoxetine-O-glucuronide</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{max} (ng/ml)</td>
<td>427.75 (30.5)</td>
<td>914.72 (30.5)</td>
<td>259.22 (39.6)</td>
<td>88.00 (16.9)</td>
<td></td>
</tr>
<tr>
<td>C_{min} (ng/ml)</td>
<td>502.84 (29.2)</td>
<td>193.09 (40.6)</td>
<td>234.89 (41.2)</td>
<td>69.27 (16.4)</td>
<td></td>
</tr>
<tr>
<td>C_{avg} (ng/ml)</td>
<td>703.63 (26.9)</td>
<td>6.00 (3.00–6.00)</td>
<td>77.88 (17.0)</td>
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<td></td>
</tr>
<tr>
<td>T_{max} (h)</td>
<td>2.00 (2.00–6.00)</td>
<td>6.00 (3.00–6.00)</td>
<td>4.00 (2.00–6.00)</td>
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<td></td>
</tr>
<tr>
<td>AUC_{0–inf} (μg · h/ml)</td>
<td>18.4 (13.5)</td>
<td>20.0 (16.8–25.2)</td>
<td>33.3 (27.7–42.7)</td>
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<td></td>
</tr>
<tr>
<td>CL/F (l/h/kg)</td>
<td>0.0157 (22.6)</td>
<td>0.0357 (26.2)</td>
<td>2.82 (41.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V/F (l/kg)</td>
<td>1.41 (22.6)</td>
<td>1.06 (42.9)</td>
<td>0.935 (17.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Multiple 20-mg doses of atomoxetine were administered twice daily over 5 days followed by a single radiolabeled atomoxetine 20-mg dose (actual dose 19.66 mg; 92.4 μCi) on the morning of the 6th day.
* Single dose parameters are reported for 14C-equivalents: C_{max}, T_{max}, t_{1/2}, AUC_{0–inf}, CL/F, V/F.
* Median (range).
* Mean (range).

**FIG. 4.** Mean plasma concentration-time profiles (cartesian and semilog) for CYP2D6 extensive and poor metabolizer subjects.

Multiple 20-mg doses of atomoxetine were administered twice daily over 5 days followed by a single radiolabeled atomoxetine 20-mg dose (actual dose 19.66 mg; 92.4 μCi) on the morning of the 6th day.
PM patients has not been different from that of EM patients (Michelson et al., 2001), despite the greater exposure at comparable doses. To gain insight into the similarities and differences in the disposition of atomoxetine and its metabolites among EM and PM patients, 14C-atomoxetine hydrochloride was administered to individuals with normal and deficient CYP2D6 activity. The results of this study demonstrate that although there are differences in the rate at which atomoxetine is cleared from the systemic circulation between EM and PM subjects; the absorption, metabolism, and excretion of atomoxetine and its metabolites are similar and independent of CYP2D6 activity.

In both EM and PM subjects, atomoxetine is well absorbed from the gastrointestinal tract and cleared from the body primarily by metabolism with the majority of its metabolites being eliminated by excretion into the urine. In EM subjects, nearly all of the radioactive dose was excreted within 24 h with total radioactivity being almost exclusively excreted in urine. Excretion was much slower in PM subjects with the majority of the radioactive dose being excreted within 72 h after 14C-atomoxetine administration. Although the primary route of excretion was via the urine, 13.1 to 21.6% of total radioactivity was excreted in feces of PM subjects. The greater amount of the radioactivity excreted in feces of PM subjects is likely related to the slower rate of metabolic elimination of atomoxetine, compared with EM subjects, rather than differences in the overall routes of biotransformation.

In both EM and PM subjects, atomoxetine was primarily cleared from the body by oxidative metabolism. The primary oxidative (phase I) metabolite of atomoxetine produced by both EM and PM subjects was 4-hydroxyatomoxetine, which was subsequently conjugated forming the primary ultimate metabolite of atomoxetine, 4-hydroxyatomoxetine-O-glucuronide. However, the relative amount of metabolites derived from secondary routes of biotransformation,
N-desmethylatomoxetine- and 2-hydroxymethylatomoxetine-derived metabolites, was greater in the PM subjects (22% of the dose) compared with EM subjects (3% of the dose). Very little atomoxetine was excreted into the urine unchanged, indicating the relatively minor role for direct renal elimination of atomoxetine. The difference in the relative distribution of metabolites between EM and PM subjects is directly related to the differences in longer residence time of atomoxetine. The lack of CYP2D6 in PM subjects allows for secondary or lower affinity metabolic processes to participate in the systemic clearance of atomoxetine.

The essential role of CYP2D6 in this metabolic conversion for the clearance of atomoxetine was recently demonstrated in vitro by Ring et al. (2002). Utilizing human liver microsomes containing a full complement of cytochrome P450 enzymes, a mean intrinsic clearance (CL_{int}) value of 103 μl/min/mg was obtained for the formation of 4-hydroxyatomoxetine; however, microsomal samples deficient in CYP2D6 exhibited a mean CL_{int} value of 0.2 μl/min/mg. Clearly, CYP2D6 is the primary enzyme responsible for the formation of 4-hydroxyatomoxetine, but when CYP2D6 is not present a number of other isozymes of cytochrome P450 (including CYP2C19, CYP3A, CYP1A2, CYP2A6, and CYP2E1) are capable of forming 4-hydroxyatomoxetine at a substantially slower rate. The role of CYP2D6 in the in vivo metabolic elimination of atomoxetine is demonstrated by the slower plasma clearance of atomoxetine in PM subjects (mean CL_{p} = 0.0357 l/h/kg) compared with EM subjects (mean CL_{p} = 0.373 l/h/kg). Thus, the difference in atomoxetine exposures in EM and PM subjects is attributed to a decrease in the rate of formation of 4-hydroxyatomoxetine, resulting in a reduction in the overall rate of elimination of atomoxetine in PM subjects.

Based on the identified metabolites, three major phase I metabolic pathways are involved in the biotransformation of atomoxetine in humans: aromatic ring hydroxylation, benzylic oxidation, and N-demethylation (Fig. 5). Although each of these metabolic pathways is distinct, aromatic ring hydroxylation appears to be the penultimate step for the further biotransformation of each of these metabolites. Subsequent O-glucuronidation of the ring-hydroxylated metabolites is the only phase II metabolic pathway to participate in the conjugation of the hydroxylated metabolites. From the metabolic profiles of atomoxetine in EM and PM subjects, it can be concluded that the same major metabolites of atomoxetine were produced regardless of CYP2D6 metabolic status. Furthermore, no CYP2D6 phenotype-specific metabolites were observed. The pharmacological activity of the primary oxidative metabolites of atomoxetine has been evaluated across a battery of receptors, channels, and transporters (data not shown). Similar to atomoxetine, 4-hydroxyatomoxetine has demonstrated a selective blockade of the presynaptic norepinephrine transporter. However, the other oxidative metabolites, including N-desmethylatomoxetine, appear to be pharmacologically inactive relative to atomoxetine.

As previously reported by Farid et al. (1985), individuals lacking CYP2D6 activity have a slower systemic clearance that results in higher steady-state plasma concentrations of atomoxetine and N-desmethylatomoxetine compared with EM subjects. As expected, due to the accumulation of atomoxetine and N-desmethylatomoxetine following repeated administration, the combined amount of unlabeled atomoxetine and its metabolites exceeded the amount of radiolabeled analytes in the plasma. Although N-desmethylatomoxetine was a major circulating metabolite in PM subjects, the contribution of this metabolic pathway to the overall metabolism of atomoxetine was relatively minor (approximately 6% of the total dose). Furthermore, in EM subjects, who have relatively low plasma concentrations of N-desmethylatomoxetine, the ultimate amount of this metabolite produced was only slightly lower (approximately 3% of the total dose) than that observed in PM subjects. Thus, the high plasma concentrations of N-desmethylatomoxetine in PM subjects are not due to enhanced production of N-desmethylatomoxetine, but rather its slow systemic clearance. Following its formation, N-desmethylatomoxetine must undergo hydroxylation and subsequent O-glucuronidation prior to its excretion. This hydroxylation appears to be mediated by CYP2D6 and, therefore, is slower in PM subjects resulting in accumulation of N-desmethylatomoxetine in the plasma.

Atomoxetine has been developed to treat children, adolescents, and adults; however, this study was conducted only in adults and did not evaluate the metabolism and disposition of atomoxetine in either adolescents or children. Previously, Long et al. (1999) demonstrated that the pharmacokinetics observed in pediatric patients, after adjustment for body weight, were consistent with adult pharmacokinetic data. For example, the half-life averaged approximately 4 h in adult EM subjects (Farid et al., 1985), which is similar to that in pediatric patients (approximately 3 h). Minimal accumulation in plasma at steady state was observed in these EM pediatric patients, which is similar to that seen in EM adults in previous pharmacokinetic studies (Farid et al., 1985). Furthermore, the apparent plasma clearance and apparent volume of distribution, after normalizing for body weight, were similar in pediatric patients and adult subjects. Thus, it is reasonable to conclude that the disposition of atomoxetine in EM and PM pediatric patients would follow the same pattern as was observed in adult subjects.

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References


