

A Comparison of Brain and Serum Pharmacokinetics of R-Fluoxetine and Racemic Fluoxetine: A ¹⁹F MRS Study

Michael E Henry^{*1}, Mark E Schmidt², John Hennen¹, Rosemond A Villafuerte¹, Michelle L Butman¹, Pierre Tran², Lynn T Kerner¹, Bruce Cohen¹ and Perry F Renshaw¹

¹McLean Hospital, Belmont, MA, USA; ²Eli Lilly and Company, Indianapolis, IN, USA

Racemic fluoxetine consists of *R*- and *S*-fluoxetine, which are metabolized to *R*- and *S*-norfluoxetine, respectively. This study was designed to compare brain levels achieved with *R*-fluoxetine to those achieved with racemic fluoxetine in healthy subjects using fluorine-19 (¹⁹F) magnetic resonance spectroscopy (MRS). In all, 13 healthy volunteers received study drug for 5 weeks using a dosing schedule designed to achieve steady state for 20 mg/day racemic fluoxetine, 80 mg/day *R*-fluoxetine, or 120 mg/day *R*-fluoxetine. The resulting brain drug levels were measured using ¹⁹F MRS. At 5 weeks, the racemate, 80 and 120 mg/day *R*-fluoxetine groups had mean brain levels of 25.5, 34.9, and 41.4 μM, respectively. In the serum, *R*-norfluoxetine, which is thought to be an inactive metabolite, accounted for 17, 71, and 63% of the fluoxetine/norfluoxetine concentration, respectively. When the relative proportion of active to total species in serum are taken into account, the data suggest that doses of *R*-fluoxetine greater than 120 mg/day would be needed to achieve brain levels of active drug comparable to 20 mg/day of racemate. The 120 mg/day *R*-fluoxetine group experienced a mean increase in QTc interval of 44 ms, with one individual having an increase of 89 ms, which suggests that higher doses may not be tolerable. While these data support the use of MRS to aid in defining the therapeutic dose range for drug development, they also highlight the need for additional studies with concurrent animal models to establish the validity of using serum drug/metabolite ratios to interpret MRS determined brain drug levels. *Neuropsychopharmacology* (2005) **30**, 1576–1583, advance online publication, 11 May 2005; doi:10.1038/sj.npp.1300749

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INTRODUCTION

Racemic fluoxetine is an antidepressant with well-documented efficacy (Emslie *et al*, 2002; Cheer and Goa, 2001), pharmacokinetics (Bergstrom *et al*, 1992, 1993), and therapeutic dose range (Fava *et al*, 2002; Schmidt *et al*, 2002). Despite its widespread use, fluoxetine's inhibition of the cytochrome P450 IID6 isoenzyme (CYP2D6), implicated in drug interactions with certain other medications, and its side effects (eg sexual dysfunction, apathy) have demonstrated the need for antidepressant agents with shorter elimination half-lives, fewer drug–drug interactions, and a more benign side effect profile (Vandel, 2003).

Recently, it has been recognized that component stereoisomers of medications can have markedly different pharmacodynamics and toxic side effect profiles from the racemate (Wade *et al*, 2002; Tonini *et al*, 2001). Racemic fluoxetine consists of two stereoisomers, *R*- and *S*-fluoxetine (Robertson *et al*, 1988), which are metabolized to *R*- and

S-norfluoxetine, respectively (Jannuzzi *et al*, 2002). Both enantiomers of the parent compound are potent inhibitors of the serotonin reuptake pump, although *R*-fluoxetine also has modest affinity at the 5-HT_{2A} and 5-HT_{2C} receptors compared to *S*-fluoxetine (Koch *et al*, 2002; Owens *et al*, 2001). In contrast, the enantiomers of the metabolite norfluoxetine have more pronounced differences. *S*-norfluoxetine binds to the transporter with an affinity similar to *R*- and *S*-fluoxetine and is therefore regarded as an active metabolite (Koch *et al*, 2002). *R*-norfluoxetine has no affinity for the serotonin reuptake pump or other monoaminergic receptors and is therefore regarded as an inactive metabolite (Koch *et al*, 2002).

Racemic fluoxetine inhibits its own metabolism largely through the action of its metabolite norfluoxetine at the P450 IID6 isoenzyme. The two enantiomers of norfluoxetine also have important differences in their inhibition of this enzyme. *S*-norfluoxetine is a potent inhibitor and contributes significantly to the half-life of the racemate (Stevens and Wrighton, 1993). *R*-norfluoxetine appears to be much less active at the IID6 enzyme (Stevens and Wrighton, 1993), which is reflected in *R*-fluoxetine's half-life of 3.3 vs 6.3 days for *S*-fluoxetine; and in *R*-norfluoxetine's half-life of 9.5 vs 23.9 days for *S*-norfluoxetine (Eli Lilly and Company, December, 1999). In light of these differences between *R*-fluoxetine and the racemate, development of the

*Correspondence: Dr ME Henry, McLean Hospital, Brain Imaging Center, 115 Mill Street, Belmont, MA 02478, USA, Tel: +1 617 855 2986; Fax: +1 617 855 3810; E-mail: mehenny@mclean.harvard.edu
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R isomer as an antidepressant was begun. Owing to the differences in pharmacokinetics between the two compounds, the therapeutic dose of *R*-fluoxetine needed to be determined (as per the usual drug development paradigm) before pivotal efficacy trials could begin.

As the chemical structure of fluoxetine contains three chemically identical fluorine atoms and clinically efficacious doses of the racemate accumulate in the central nervous system in micromolar concentrations, brain drug levels can be measured using fluorine-19 (¹⁹F) magnetic resonance spectroscopy (MRS) (Bolo *et al*, 2000; Henry *et al*, 2000). Data from previous clinical trials of fluoxetine suggest that 20 mg/day is a well-tolerated, efficacious dose of racemic fluoxetine (Beasley *et al*, 2000; Schmidt *et al*, 2002). Although the activity of *R*-fluoxetine at the 5-HT_{2A} and 2C receptors has been postulated to contribute to its antidepressant effects, the magnitude of that contribution cannot currently be quantified. Therefore, we conservatively hypothesized that in order to demonstrate clinical efficacy, doses of *R*-fluoxetine would have to produce brain levels of the parent drug comparable to those achieved with 20 mg/day of the racemate. ¹⁹F MRS offers a noninvasive, nonradioactive technique for comparing the brain drug levels achieved by the two compounds.

In light of the differences in elimination half-life and the need to compensate for the absence of an active metabolite, we also hypothesized that significantly higher oral doses of *R*-fluoxetine would be required to produce brain levels comparable to those achieved with the racemate. To test this hypothesis, healthy volunteers were given either *R*-fluoxetine or the racemate, as described in the Methods and Materials section. The resulting brain drug levels were measured after 3 and 5 weeks on drug, and again after 1 week off-drug, using ¹⁹F MRS methods. Lastly, since *R*-fluoxetine has shown some propensity towards prolongation of the QTc interval (Eli Lilly and Company, December,

1999), electrocardiograms (ECGs) were recorded at baseline and throughout the study.

The objectives of the study were (1) to describe the accumulation and initial elimination kinetics of *R*-fluoxetine compared to the racemate in the CNS; (2) to test the hypothesis that significantly higher oral doses of *R*-fluoxetine would be required to produce brain levels comparable to those achieved by 20 mg/day of the racemate; and (3) to obtain additional safety data in terms of risk of prolongation of QTc intervals for the 120 mg/day dose of *R*-fluoxetine. We summarize the results of these analyses in this report.

MATERIALS AND METHODS

The study was reviewed and approved by the McLean Hospital Institutional Review Board. In all, 15 healthy subjects were recruited via advertisement. Prior to enrollment informed consent was obtained and subjects underwent a semistructured clinical interview to confirm the absence of significant psychiatric and medical illness. A blood sample was collected at the screening visit to determine each subject's genotype for CYP2D6 metabolism and ensure that none of the enrolled subjects were poor metabolizers.

Medication Dosing

Subjects were blindly randomized to receive either (i) 80 mg/day of *R*-fluoxetine, (ii) 80 mg/day for 1 week followed by 4 weeks of 120 mg/day, or (iii) 60 mg/day of racemic fluoxetine for 1 week followed by 20 mg/day for 4 weeks (Figure 1). This dosing schedule for racemic fluoxetine has previously been shown to approximate steady concentrations of the 20 mg/day dose by 5 weeks (Bergstrom *et al*, 1993). Subjects were asked to take study drug orally each morning and come into the clinic weekly

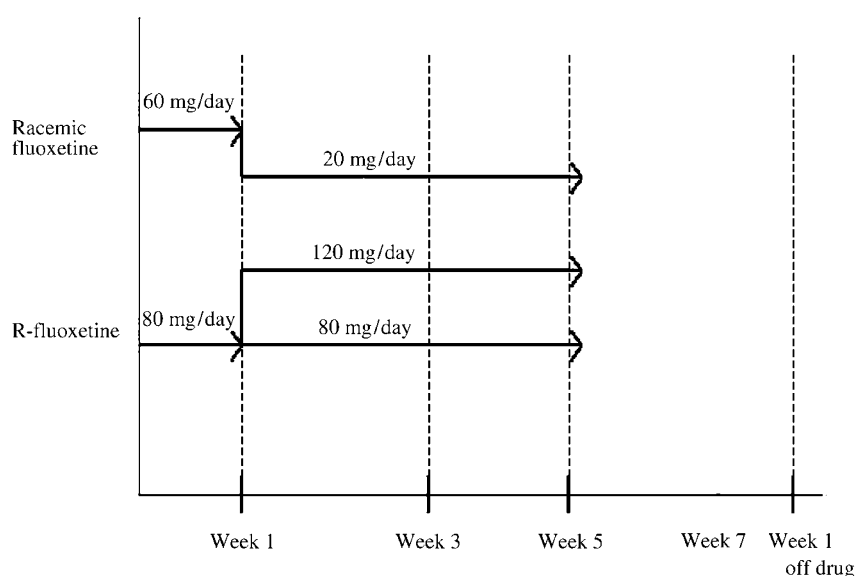


Figure 1 Dosing schedule. Subjects were blindly randomized to receive either (i) 80 mg/day of *R*-fluoxetine, (ii) 80 mg/day for 1 week followed by 4 weeks of 120 mg/day, or (iii) 60 mg/day of racemic fluoxetine for 1 week followed by 20 mg/day for 4 weeks. Each of the dashed lines indicates visits where an ECG, scan, and blood draw were performed.

for the duration of the 6-week study. For dose 35, scheduled for the last day of the fluoxetine regimen, subjects took the medication during the visit; serum samples were obtained both before the dose was taken and 2 h after dosage.

Scanning

Subjects underwent 19-F MRS scans prior to initiating drug (baseline), after 3 weeks on drug, after 5 weeks on drug, and 1 week after drug discontinuation. The magnetic resonance imaging and spectroscopy methods have been described in detail elsewhere (Christensen *et al*, 1998, 1999). Sample 19-F MRS spectra are shown in Figure 2 for each treatment group. Spectroscopy data were acquired using a 1.5 T GE Signa Scanner (General Electric, Milwaukee, WI) and a volume head coil (US Asia Instruments, Highland Heights, OH) capable of being tuned to either the proton or fluorine resonance frequency. Quadrature detection, which increases the sensitivity of measurement compared to detection using linear coils, was employed at the 19-F frequency (Claassen-Vujcic *et al*, 1996). Spectra were acquired using a simple, nonlocalized pulse acquisition method (TR = 1 s; number of averages \approx 1200; total scan time = 20 min). An external reference standard was placed beside the head to optimize data acquisition and normalize the brain signal. The signal intensity was calibrated using a 10 μ M solution of racemic fluoxetine, which was scanned immediately after each subject's scan. Preliminary studies of standardized solutions demonstrated that the 19-F MRS resonance frequencies and relaxation times for R- and S-fluoxetine were nearly identical. Brain volume was estimated from structural scans obtained at one of the scanning sessions during the study for each subject. Drug concentrations were determined by dividing the total amount of drug in the brain by the calculated brain volume.

Brain Volume Measurements

Structural scans were obtained for each patient at one of the scanning sessions during the study. The volume of each subject's brain was estimated from analysis of the two-dimensional axial slices of the structural scans using Cine software (Kikinis *et al*, 1992) running on a Sparc2 workstation (Sun Microsystems, Mountainview, CA). The area of brain calculated from each slice was then multiplied by the slice thickness and the thickness of the skipped region between slices. The measurements for all of the slices were summed to estimate brain volume (Christensen *et al*, 1998).

Serum Samples

Serum drug level samples were obtained coincident with the spectroscopy scans. Blood was centrifuged using a Beckman (Beckman Coulter Inc., Fullerton, CA) tabletop centrifuge for 20 min. Serum was pipetted into freezer tubes and stored at 0°C. Samples were shipped under dry ice to CoVance Central Laboratory Services (Indianapolis, IN). Samples were analyzed for the R and S enantiomers of fluoxetine and norfluoxetine using gas chromatography and mass spectrometry.

Electrocardiograms

ECGs were obtained at the screening visit, the day of the baseline scan (day 0), day 7, day 14, day 21, day 28, day 35, and 7 days after the last dose. To measure the diurnal variation in QTc, three ECGs, each separated by a minimum of 2 h, were obtained on day 0 and day 35. The ECG tracings were electronically transmitted to an outside reference laboratory and interpreted by a board-certified cardiologist using Bazet's correction (Biomedical Services, St Louis, MO) (Al-Khatib *et al*, 2003).

Statistical Analyses

We obtained brain and serum drug levels within each of the three medication treatments (R-fluoxetine, 80 mg/day; R-fluoxetine, 120 mg/day; and racemic fluoxetine, 20 mg/day), summarizing these measurements at each assessment time as mean \pm standard deviation (mean \pm s.d.) or mean with 95% confidence interval (95% CI). The QTc measurements also were summarized as mean \pm s.d. or mean with 95% CI. Differences among the three treatments in change-from-baseline in the brain drug levels (for each stereoisomer and their respective metabolites), serum drug levels, and QTc measurements were assessed using random effects regression modeling methods, adjusting for clustering within subjects. For some of these modeling analyses, positive skewness was apparent, and these skewed drug-level or QTc measurements were logarithmically transformed prior to analysis. We estimated the slope of the QTc change over the time period week baseline (day 0) to day 35 for each subject and compared these slope estimates among the three treatment groups using ordinary linear regression (*t*-test) methods. We examined the interaction between treatment group and time at the weeks 3–5 time interval to determine if the QTc peak values occurring then differed significantly between the 120 mg/day subgroup and the other two (80 and 20 mg/day) subgroups considered together. Lastly, random effects regression modeling methods were used to compare the values obtained at baseline to those obtained at day 42 (1 week off study medication).

Statistical significance required two-tailed $p < 0.05$. Analyses employed commercial microcomputer programs (Stata[®], Stata Corporation, College Station, TX; and Statview-5[®], SAS Institute, Cary, NC).

RESULTS

Of the 15 subjects enrolled, six male and seven female subjects completed the protocol. One subject in the 80 mg/day group was withdrawn from the study for noncompliance with the study protocol, and excluded from data analysis. Of the remaining 14 subjects, one subject who completed the protocol was excluded from the analysis when pretreatment serum and brain drug levels revealed that the subject, who randomized to the racemic fluoxetine group, was taking fluoxetine prior to entry into the study. One subject in the 120 mg/day R-fluoxetine group was withdrawn from the study after week 4 due to termination of the study by the sponsor. This subject was included in data analysis until withdrawal. The age ranges for the three groups were 23–51 (mean = 34 ± 12 , $n = 4$), 21–45

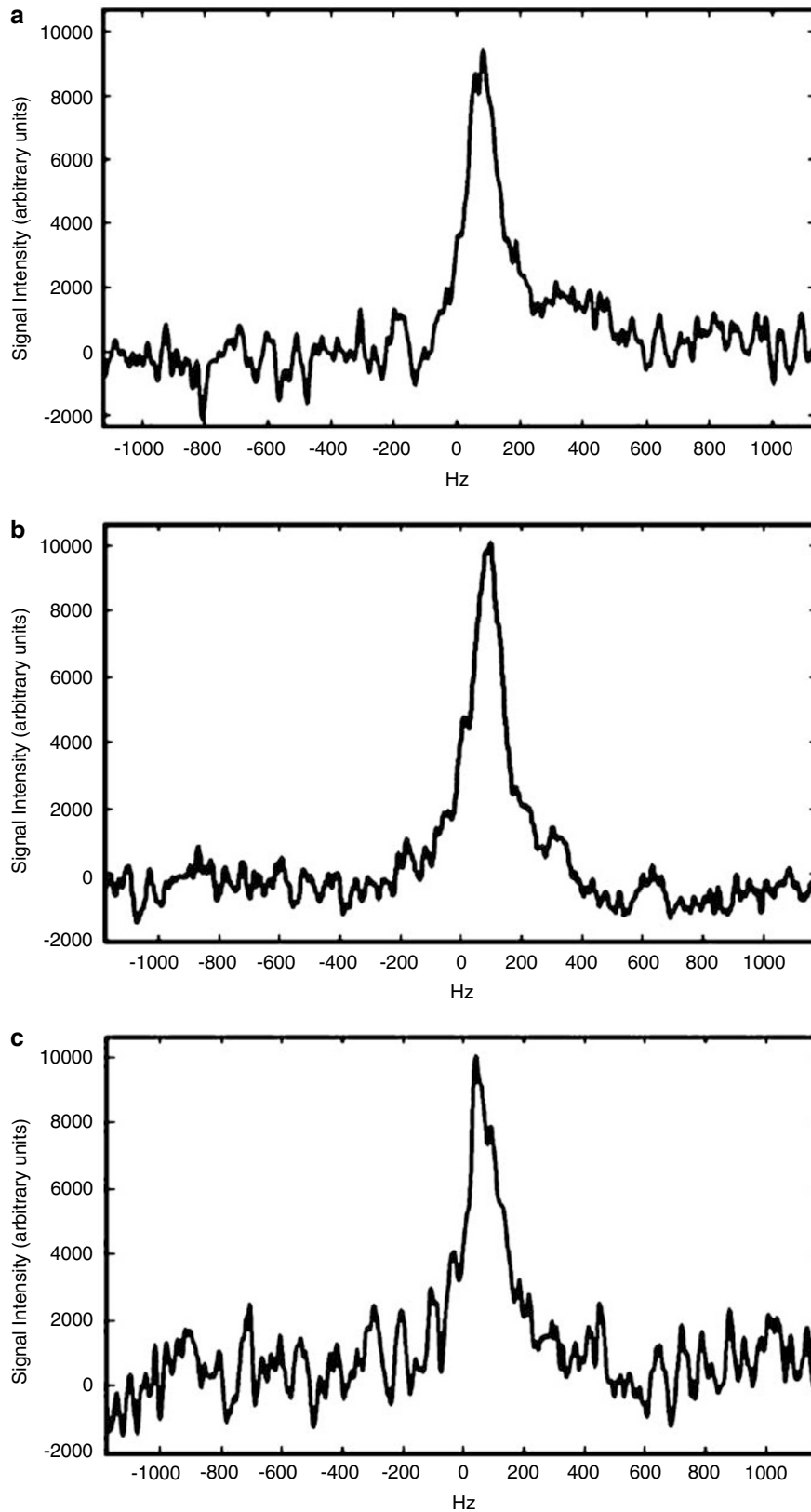


Figure 2 Representative fluorine MR spectra for each treatment group: (a) 80 mg/day *R*-fluoxetine, (b) 120 mg/day *R*-fluoxetine, and (c) 20 mg/day racemic fluoxetine.

(mean = 32 ± 10 , $n = 4$), and 19–47 (mean = 32 ± 10 , $n = 4$) years for the racemate, 80 mg/day, and 120 mg/day groups, respectively.

The serum accumulation and elimination kinetics of the racemate and *R*-fluoxetine subgroups for both the individual stereoisomers and their respective metabolites are

shown in Table 1 and Figure 3a. Unlike 19-F MRS, the serum samples obtained permitted measurement of the individual stereoisomers and their respective metabolites. At week 5, the compounds active at the reuptake pump (*R*- and *S*- fluoxetine and *S*-norfluoxetine) (Koch *et al*, 2002) accounted for 83, 29, and 37% of the serum drug/metabolite concentration for the racemate, 80 mg/day, and 120 mg/day of *R*-fluoxetine groups, respectively. As noted in Table 1, there were no medication group differences at baseline, but as expected, there were impressive racemate *vs* 80 mg/day and racemate *vs* 120 mg/day differences at week 5.

The brain accumulations of fluorinated compounds are shown in Table 2 and Figure 3b. At week 5, the brain concentration of fluoxetine plus fluorinated metabolites was $25.5 \pm 6.6 \mu\text{M}$ for the racemate, $34.9 \pm 13.8 \mu\text{M}$ for the 80 mg/day of *R*-fluoxetine group, and $41.4 \pm 12.9 \mu\text{M}$ for the 120 mg/day of *R*-fluoxetine group. These week 5 racemate *vs* 80 mg/day and racemate *vs* 120 mg/day contrasts, although large, were not statistically significant because of the small sample sizes involved. When corrected by the percent of inactive *R*-norfluoxetine in the serum (see Discussion below for rationale), the brain concentration of active compounds was $21.2 \mu\text{M}$ for the racemate ($25.5 \mu\text{M} \times 83\%$), $10.1 \mu\text{M}$ for the 80 mg/day group ($34.9 \mu\text{M} \times 29\%$), and $15.3 \mu\text{M}$ for the 120 mg/day *R*-fluoxetine group ($41.4 \mu\text{M} \times 37\%$).

After 1 week off-drug, the serum concentration of *R*-fluoxetine had decreased by 38% from the week 5 concentration for the racemic fluoxetine group, *vs* 53% for the 80 mg/day group and 48% for the 120 mg/day group. The concentration of *R*-fluoxetine in the brain had decreased by 52% for the racemic fluoxetine group, while the concentration decreased by 54 and 47% from the week 5 concentration for the 80 mg/day and 120 mg/day groups, respectively.

As noted in Methods and Materials, QTc interval data were obtained at each of the scanning dates (baseline, week 3, week 5, and 1 week after drug discontinuation). Table 3 and Figure 4 summarize QTc values for the three groups at each of the scanning dates. As noted in Table 3, the QTc intervals were substantially longer at weeks 3 and 5 compared to baseline, and Figure 4 suggests that most of this difference was due to elevated QTc intervals within the 120 mg/day subgroup. This interaction was statistically significant, when examined by random effects modeling methods ($z = 2.42$, $p = 0.015$).

Table 1 Serum Accumulation and Elimination Data at Baseline (Week 0), Week 3, Week 5, and 1 Week Off-Drug for Three Fluoxetine Treatment Groups: 20 mg/day Racemate, 80 mg/day *R*-Fluoxetine, and 120 mg/day *R*-Fluoxetine

Characteristics	Racemate	80 mg/day	120 mg/day
Serum level (mM)			
Baseline (week 0)	0	0	0
Week 3	0.935 ± 0.08	1.095 ± 0.30	1.798 ± 0.32
Week 5	0.939 ± 0.08	1.048 ± 0.41	2.020 ± 0.63
Week 6 (1 week off-drug)	0.575 ± 0.07	0.514 ± 0.25	1.010 ± 0.46

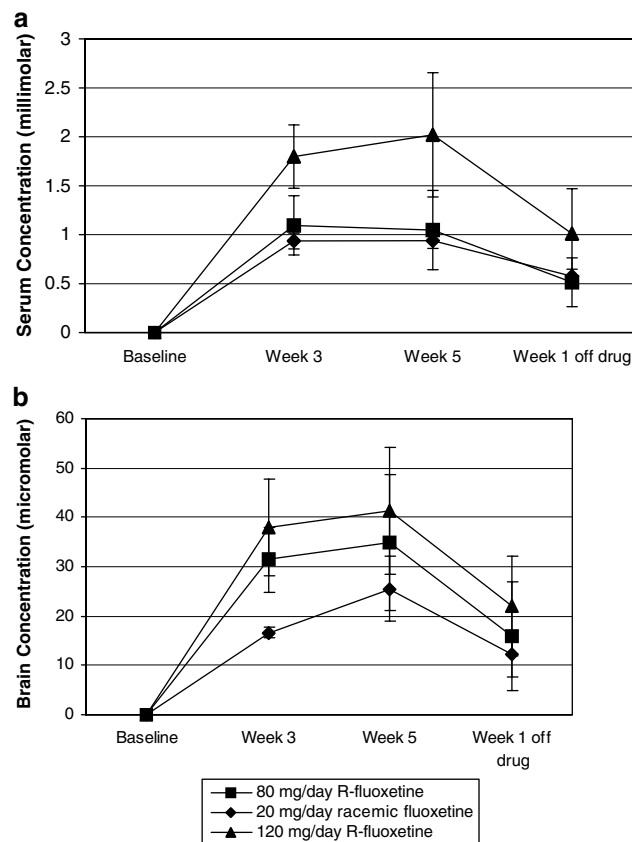


Figure 3 Concentration of fluoxetine in (a) serum and (b) brain at baseline, 3 weeks on drug, 5 weeks on drug, and 1 week after drug discontinuation. Bars indicate standard error at each time point. Concentrations in serum were measured using gas chromatography and mass spectrometry and concentrations in brain were measured using 19-F MRS.

Table 2 Brain Accumulation and Elimination Data at Baseline (Week 0), Week 3, Week 5, and 1 Week Off-Drug for Three Fluoxetine Treatment Groups: 20 mg/day Racemate, 80 mg/day *R*-Fluoxetine, and 120 mg/day *R*-Fluoxetine

Characteristics	Racemate	80 mg/day	120 mg/day
Brain level (μM)			
Baseline (week 0)	0	0	0
Week 3	16.63 ± 1.07	31.42 ± 6.68	37.98 ± 9.83
Week 5	25.48 ± 6.60	34.87 ± 13.8	41.39 ± 12.9
Week 6 (1 week off-drug)	12.23 ± 4.43	15.90 ± 11.1	22.04 ± 10.2

We also examined the individual subject changes over time in QTc interval over the time period baseline through week 5, by estimating the slope of the QTc *vs* scan-week regression separately for each subject. These slope data are summarized in Table 3. The average slope within the 120 mg/day subgroup was significantly higher than the mean slope within the racemic fluoxetine group ($z = 3.36$, $p = 0.001$), and also higher than the mean slope within the

80 mg/day subgroup, although the latter difference did not reach statistical significance ($z = 0.96$, $p = 0.340$).

We also examined the elevated QTc interval value for the 120 mg/day subgroup at week 5, to see if this elevation differed significantly from the remaining QTc interval measurements among all three treatment groups. This interaction effect was found to be statistically significant ($z = 2.51$, $p = 0.012$).

Lastly, all three medication groups showed statistically comparable values at 1 week off-drug compared with their baseline values ($z = 1.03$, $p = 0.300$), even the 120 mg/day R-fluoxetine group when examined independent of the other two groups ($z = 1.36$, $p = 0.170$).

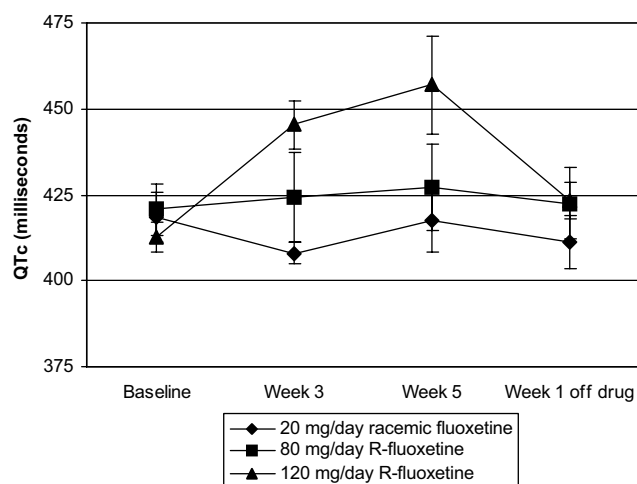


Figure 4 QTc values for each medication group at baseline, 3 weeks on drug, 5 weeks on drug, and 1 week after drug discontinuation. Bars indicate standard error at each time point.

DISCUSSION

The differences in serum and brain pharmacokinetics between R-fluoxetine and racemic fluoxetine found in this study were consistent with previously described differences in metabolism of the enantiomers (Stevens and Wrighton, 1993). It is of note that the concentrations achieved in the brain of both racemic fluoxetine (25.5 μ M) and 80 and 120 mg/day of R-fluoxetine (34.9 and 41.4 μ M, respectively (parent drug plus metabolites)) were approximately 20 times those of the parent compound and metabolites in the serum (0.94, 1.10, and 2.02 μ M, respectively) for both compounds. This is consistent with earlier studies of racemic fluoxetine, and most likely reflects the fact that the drugs are relatively lipophilic and also accumulate in vesicles on the basis of pH gradients (Bolo *et al*, 2000; Strauss *et al*, 2002). It also highlights the fact that these compounds have a large volume of distribution and their pharmacokinetics are dependent on equilibration across multiple compartments.

The MRS signal observed is also dependent on the kinetics of the fluorinated metabolites that are able to enter and accumulate in the brain (Bolo *et al*, 2000). The primary metabolites of concern are R- and S-norfluoxetine. As described above, R-norfluoxetine is thought to be inactive at both the reuptake pump and the IID6 isoenzyme, while S-norfluoxetine is active at the reuptake pump and is a more potent inhibitor of the IID6 isoenzyme than its parent compound (Koch *et al*, 2002). Therefore, knowing the relative contribution of each of these chemical species to the MRS signal would permit a determination of the amount of active drug in the brain.

Direct sampling of brain tissue is not a practical method for determining the relative amount of each chemical species present and the animal data addressing this issue are limited. Wikell and colleagues (1999), however, have measured the concentrations of the S and R enantiomers of

Table 3 QTc Interval Data at Baseline (Week 0), Week 3, Week 5, and 1 Week Off-Drug for Three Fluoxetine Treatment Groups: 20 mg/day Racemate, 80 mg/day R-Fluoxetine, and 120 mg/day R-Fluoxetine

Characteristics	Racemate	80 mg/day	120 mg/day	R vs 80 mg ^a		R vs 120 mg ^b	
				z	p	z	p
Subjects	4	4	4	—	—	—	—
<i>QTc interval</i>							
Baseline (week 0)	418.4 \pm 14.7	420.7 \pm 15.4	412.6 \pm 9.5	0.29	0.77	-0.96	0.34
Week 3	408.0 \pm 6.2	424.3 \pm 26.1	445.4 \pm 15.8	—	—	—	—
Week 5	417.3 \pm 18.4	427.3 \pm 25.0	456.9 \pm 31.9	0.70	0.48	2.42	0.015
Weeks 0-5	414.6 \pm 15.3	425.4 \pm 21.6	435.3 \pm 26.3	0.99	0.33	2.68	0.007
Week 6 (1 week off-drug)	411.3 \pm 15.3	422.5 \pm 20.6	423.3 \pm 12.2	0.97	0.33	1.03	0.30
QTc last \leq QTc baseline	3/4 (75%)	2/2 (50%)	1/4 (25%)	2.00	0.37 ^c	—	—
Slope, weeks 0-5 ^d	-0.25 \pm 1.97	1.22 \pm 2.73	8.81 \pm 5.62	0.96	0.34	3.36	0.001

Data are N (%) (and χ^2 (df=2)-statistic) for nominal factors and mean \pm SD and (z-statistic) for continuous measures.

^aContrast R vs 80 mg compares racemate subgroup with R-fluoxetine 80 mg/day subgroup.

^bContrast R vs 120 mg compares racemate subgroup with R-fluoxetine 120 mg/day subgroup.

^cFisher's exact p-value = 0.77.

^dPostmodeling test of slopes for 80 vs 120 mg/day subgroups: and χ^2 (df = 1) = 7.21, $p = 0.007$.

citalopram and their metabolite demethylcitalopram in the serum and cortex of rats at steady state. In the serum, the ratio of S- to R-citalopram was 0.94 vs 1.00 for the brain. Similarly, the ratio of S- to R-demethylcitalopram was 0.36 for serum vs 0.28 for cortex. Thus, at least in this model of citalopram pharmacokinetics, the ratio of the stereoisomers in the brain mirrors their relative concentrations in the serum. If fluoxetine and norfluoxetine likewise have brain ratios for the R- and S- enantiomers that are similar to those in the serum, then based on the relative amount of inactive R-norfluoxetine in the serum of the subjects at week 5 (73% for 120 mg/day vs 17% for the racemate), these data suggest that R-fluoxetine will need to be dosed above 120 mg/day (15.3 μ M active species in the brain) to have antidepressant efficacy that is comparable to 20 mg/day of the racemate (21.2 μ M active species in the brain). It should be noted that these estimates assume that R-norfluoxetine has negligible antidepressant effects at sites other than the serotonin reuptake pump.

While the 19-F MRS data suggest that doses higher than 120 mg/day may be needed, the adverse event (QTc) profile suggests that higher doses may not be tolerated. In the 120 mg/day R-fluoxetine group, there was a statistically significant increase in mean QTc observed at week 5. As a group, the subjects receiving 120 mg/day of R-fluoxetine had a mean increase in QTc of 44 ms between days 0 and 35 on drug. In the most extreme case, one individual had an increase of 89 ms, which exceeds the 60 ms threshold thought to significantly increase risk of Torsades de Pointes syndrome (Noel *et al*, 2003). In light of these findings, it is possible that doses of R-fluoxetine above 120 mg/day would cause unacceptable prolongation of the QTc interval in vulnerable individuals (Curtis *et al*, 2003; McConathy and Owens, 2003).

The main limitation of this study is that it provides little information about the concentration of active drug, receptor occupancy, or the degree to which the drug is trapped in vesicles or otherwise bound. The small sample size, which is usually a limitation of such studies, highlights the potential of this method. In this study, despite a small number of subjects, we were able to demonstrate central nervous system accumulation of fluoxetine and its metabolites, with a weighting towards an inactive metabolite in the serum, and a concerning adverse event profile. While these data support the use of MRS to aid in defining the therapeutic dose range for drug development, they also highlight the need for additional studies using concurrent animal models to establish the validity of using serum drug/metabolite ratios to interpret brain drug levels. This type of information, when obtained early in the clinical discovery process, can significantly reduce the number of trials needed to define the therapeutic dose range and contribute to more efficient utilization of resources in early drug discovery.

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