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Fluoxetine: juvenile pharmacokinetics in a nonhuman primate model

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Abstract

Rationale—The selective serotonin reuptake inhibitor (SSRI) fluoxetine is the only psychopharmacological agent approved for use in children. While short-term studies of side effects have been performed, long-term consequences for brain development are not known. Such studies can be performed in appropriate animal models if doses modeling therapeutic use in children are known.

Objectives—To identify a daily dose of fluoxetine in juvenile monkeys which would result in serum fluoxetine and norfluoxetine concentrations in the therapeutic range for children.

Methods—Juvenile (2.5 year old rhesus monkeys, n=6) received single administration of doses of 1, 2 and 4 mg/kg-d fluoxetine on a background of chronic dosing at an intermediate level to provide steady state conditions to model therapeutic administration. Using nonlinear modeling, standard pharmacokinetic parameters were derived. Cerebrospinal fluid monoamine neurotransmitters were assayed to confirm the pharmacological effects.

Results—Dose-dependent AUC and Cmax values were seen while Tmax and absorption/elimination half-lives were minimally influenced by dose. A dosage of 2 mg/kg-d given over a 14 week period led to steady state serum concentrations of active agent (fluoxetine + norfluoxetine) similar to those recorded from drug monitoring studies in children. Cisternal cerebrospinal fluid concentrations of serotonin increased significantly over the 14 week period, while concentrations of the serotonin metabolite (5-HIAA) were lower but not significantly different.

Conclusions—2 mg/kg/d fluoxetine in juvenile rhesus monkeys provides an internal dose similar to therapeutic use in children and will help establish a valuable animal model for understanding fluoxetine therapeutic and potential adverse effects in children.

Keywords

fluoxetine; rhesus monkeys; pharmacokinetics; childhood therapy

Introduction

Fluoxetine, a selective serotonin reuptake inhibitor (SSRI), is the only psychopharmacological agent approved for use in children. Clinical studies in children report wide individual variability in therapeutic response to fluoxetine and drug monitoring shows a corresponding broad range of serum concentrations at therapeutic doses (Wilens et al. 2002; Koelch et al. 2012). Body weight as well as genetic and lifestyle factors may be involved (Blazquez et al. 2012).

Detailed single dose pharmacokinetic studies have not been performed to date in children. The rhesus monkey model offers an opportunity to identify basic pharmacokinetic parameters in the juvenile stage of life. Rhesus monkeys, like humans, undergo a prolonged postnatal period of behavioral and brain development prior to puberty making them a valuable model for childhood.

This study employed a 3-dose, intra-subject design in rhesus monkeys 2.5 years of age, a maturational stage equivalent to about 10 years in children. Doses were selected based on fluoxetine use in children, and pharmacokinetic studies and therapeutic use of fluoxetine in rhesus (Clarke et al. 1998; Clarke et al. 1999; Laudenslager and Clarke 2000; Anderson 2004; Fontenot et al. 2005; Fontenot et al. 2009; Sawyer and Howell 2011). Prior to and during the single-dose studies, monkeys were maintained on a daily dosing regimen at an intermediate dose. Fluoxetine PK is known to change with duration of dosing (Bergstrom et al. 1988). Conducting the single-dose studies on a background of chronic dosing enhances their relevance for clinical use and provides a more efficient approach than three separate experiments with chronic administration at each dose.

While age and weight were held constant, subjects of both sexes were used in the study. To further define the population, polymorphisms of monoamine oxidase A (MAOA) and the serotonin transporter (5HTLLPR), available from routine colony genotyping, were identified. Rhesus display polymorphisms of the MAOA (Newman et al. 2005) and 5HTLLPR (Lesch et al. 1997) genes similar to those in humans. Both MAOA (Yu et al. 2005), and 5HTLLPR (Kim et al. 2006; Silva et al. 2010) polymorphisms demonstrate associations with therapeutic response to fluoxetine in humans, but associations with PK have not been studied.

Pharmacological action of the chronic dosing regimen was evaluated by assay of cerebrospinal fluid (CSF) for monoamine neurotransmitters (serotonin, norepinephrine and dopamine) and their metabolites. Fluoxetine has been shown to increase all three monoamine neurotransmitters in brain tissue in animal studies and also in plasma in human studies (Bymaster et al. 2002; Koch et al. 2002; Blardi et al. 2005). While neurotransmitter concentrations in lumbar CSF obtained in human studies are usually too low to measure, the metabolites are commonly used as markers for SSRI action (De Bellis et al. 1993). Several studies in human clinical settings have shown reduced CSF concentrations of the serotonin metabolite 5-HIAA in response to SSRIs (De Bellis et al. 1993; Lundmark et al. 1994; Sheline et al. 1997).

Methods

Subjects

Six rhesus monkeys (*Macaca mulatta*) approximately 2.5 years of age were selected from the colony of over 5000 rhesus monkeys at the California National Primate Research Center (CNPRC) to form a group balanced for sex and MAOA genotype. Monkeys weighed 4.41 ± 1.13 kg at the beginning of the study and gained $.25 \pm .06$ kg during the study. Most 3–4 month-old rhesus in the colony are routinely screened for MAOA and 5HTLLPR genotype as an aid to researchers and for colony management. Three females (1 high-MAOA genotype, 2 low-MAOA genotype) and three males (2 high-MAOA, 1 low-MAOA) were included as subjects. A complete blood count (CBC) and physical exam were obtained prior to dosing. The juvenile rhesus were transferred from outdoor group housing to indoor housing 6 months prior to initiation of the study, with the exception of one subject with a 1 month adaptation. They were continuously paired with a cagemate of the same sex and age in indoor housing during the study. Background information on individual subjects is provided in Online Resource 1.

Dose selection

Fluoxetine mg/kg dosing was based on the Food and Drug Administration (FDA) recommended range of therapeutic doses in children (10–60 mg) (FDA 2003) and normative data on body weights of children 6–10 years of age giving a range of 0.5–4.5 mg/kg. This range also included doses shown to be pharmacologically active in adult and juvenile macaques. A dose of 2 mg/kg-d was chosen for the chronic oral dosing, and single-dose pharmacokinetic studies were conducted at 1, 2 and 4 mg/kg.

Chronic oral dosing at 2 mg/kg-d

This regimen allowed evaluation of steady state internal dose. Liquid fluoxetine (20 mg/5 mL) was purchased from Webster Veterinary. Monkeys were dosed daily in the morning between 8 am and 9 am throughout the ~4 month study period. They first learned to come to the front of the cage to sip palatable liquids (chocolate or strawberry Boost®, Kool-Aid®) from the tip of a 6 mL syringe which was gradually emptied into their mouths. A “chaser” syringe filled with plain Boost was given immediately following the dose along with a small treat (raisin, grape, marshmallow, other small fruit) to reinforce acceptance of the dose. Fluoxetine hydrochloride has a bitter taste that is masked by mint in the commercially available liquid formulation and requires further modification for individual palatability. Daily doses were administered either via the dosing syringe or in a fig cookie or piece of banana when necessary.

Gavage dosing for single-dose pharmacokinetics

The pharmacokinetic (PK) studies were initiated after 4 weeks of daily dosing. The young monkeys were trained for arm-pull blood sampling prior to initiation of the first PK study. In this technique the monkey moves to the front of the cage adjacent to a small opening in the cage door, and the arm is guided through the opening for venipuncture. They were fasted in the morning prior to each study and remained in their homecage throughout the PK study

with access to water throughout and food beginning 2 h after dosing. A baseline 0.5 mL blood sample was collected via armpull just prior to dosing via nasogastric intubation. Fluoxetine doses were mixed with Boost® in a 2:1 Boost®:fluoxetine volume. Further 0.5 mL samples were collected at 30 minutes intervals for 4 h, with a final sample at 10 h post-dosing.

CSF monoamine assays

To assess the pharmacological effect of the chronic treatment with 2 mg/kg fluoxetine, cerebrospinal fluid (CSF) samples were obtained prior to the initiation of dosing and at the end of the study 4 weeks after the last single-dose study, 14 weeks after the onset of dosing. Animals were fasted, sedated with ketamine (10 mg/kg i.m.) and dexmedetomidine (0.0075–0.015 mg/kg), and reversed with equal volume of atipamezole at the end of the procedure. A 0.5 mL sample was collected under sterile conditions via passive accumulation from the cisterna magna and frozen at –80° C for shipping to a collaborating laboratory for high pressure liquid chromatography (HPLC) analysis of a panel of monoamines as described previously (Golub et al. 2012). Serotonin, the serotonin metabolite 5HIAA, and the dopamine metabolites DOPAC and HVA were quantifiable in all samples, while epinephrine, norepinephrine and dopamine were not detected in any of the samples.

Serum fluoxetine/norfluoxetine analyses

The fluoxetine and norfluoxetine liquid chromatography mass spectrometry (LC/MS-MS) assays (Chu and Metcalfe 2007; Li et al. 2011) were adapted for monkeys. Extraction used Biotage Isolate SLE200 system. LC utilized a Waters acquity ultra high performance liquid chromatography (UPLC) system with a Phenomenex Synergy Polar-RP 1000 column. The MS-MS was done on an AB Sciex 4000 Q trap using a turbo spray ESI ion source. Duplicate spiked samples were included in each assay. Fluoxetine/norfluoxetine recoveries were 112%/108%; intra assay reliabilities (%coefficient of variation) were 10.7/6.6; inter assay reliabilities (%CV) were 2.8/3.3 and limits of quantitation (LOQs) (ng/mL) were 2/2.

Pharmacokinetic and statistical analysis

Noncompartmental and single compartmental pharmacokinetic parameters for fluoxetine and norfluoxetine were calculated using the WinNonLin program, Version 2.1 (Pharsight, Sunnyvale CA). AUC was computed with the trapezoidal rule. Akaike's Information Criterion was used to evaluate model fit. Concentrations of CSF monoamine neurotransmitters and metabolites were analyzed using repeated measures analysis of variance with time point (baseline, end of chronic dosing) as the repeated measure and sex as an additional independent variable.

Results

Fluoxetine serum levels after single-dose nasogastric administration are shown in Figure 1 along with pharmacokinetic model fit and derived parameters from group data. Achieved internal doses (AUC and Cmax) showed graded increase with administered dose. Absorption and elimination coefficients were stable over the 1 and 2 mg/kg dose increasing slightly at the highest dose. Peak doses were reached at 4 h for the two lower doses and

close to 5 h at the high dose. Terminal half-lives could not be determined due to limited post-peak data points and the background maintenance dosing. Monkeys of both sexes and MAOA genotypes produced comparable data (Online Resource 1). Information on 5HTTLPR genotype, derived from routine colony genotyping, showed one subject with the L/L polymorphism (Online Resource 1). This subject demonstrated higher serum fluoxetine concentrations in response to single doses, and also higher daily baseline fluoxetine + norfluoxetine concentrations (Figure 2 inset).

Serum concentrations of active agent (fluoxetine + norfluoxetine) from the single-dose study at 2 mg/kg-d are shown in Figure 2, along with an inset showing active agent at baseline (24 h after dosing) at 3 timepoints during the 14 weeks of chronic daily administration of this dose. Serum concentrations were consistent within and between individuals. One animal was an exception in having elevated fluoxetine and norfluoxetine values as mentioned above. The average 2–10 h post-dosing serum concentrations from the single dose study were considered representative of daily therapeutic levels for purposes of comparison with human drug monitoring studies in children. Combined fluoxetine and norfluoxetine concentrations at this time were 336 ± 40 ng/mL with a fluoxetine:norfluoxetine ratio of 0.51 ± 0.05 .

CSF monoamines and metabolites

Plots of changes in CSF serotonin and monoamine metabolites by sex are shown in Figure 3. Serotonin was the only monoamine neurotransmitter present in CSF at concentrations (8 to 14 ng/mL) that could be detected by the assay. Metabolites of the monoamine neurotransmitters dopamine (DOPAC, HVA) and serotonin (5-HIAA) were detected in all samples. There were no effects of MAOA polymorphism on CSF analytes. In general, serotonin and the monoamine metabolites were present at higher levels in females than males ($F(1,4)=2.63$, $p=.03$) across all analytes ($F(1,5)=29.49$, $p=.006$ for 5-HIAA, $F(1,5)=44.43$, $p=.003$ for DOPAC). From baseline to conclusion of fluoxetine dosing, serotonin increased significantly ($F(1,5)=12.07$, $p=.01$) while 5-HIAA showed a nonsignificant decrease ($F(1,5)=2.15$, $p=.25$). The 5-HIAA/5-HT ratio declined significantly ($F(1,5)=25.93$, $p=.004$). The dopamine metabolite HVA increased after dosing ($F(1,5)=14.98$, $p=.01$) with a similar trend for DOPAC ($F(1,5)=3.16$, $p=.11$).

Discussion

Our study attempted to compare single dose PK under steady state conditions maintained by chronic dosing at an intermediate dose. The pre-dosing values confirmed that plasma fluoxetine and norfluoxetine levels from chronic dosing were steady state during the single-dose studies. Because body weight is an important determinant of serum drug level in children (Wilens et al. 2002) we used mg/kg dosing and subjects within a narrow weight range.

Although sample size was small, under the controlled conditions of the study, individual variability was limited and dose-dependent AUC and C_{max} values were obtained across a clinically relevant dose range. T_{max} of 3–4 h in the young monkeys can be compared to values of 6–8 h reported in adult humans; no single-dose studies with oral dosing were

identified in adult monkeys or in children. Terminal half-life values could not be obtained due to the background from chronic maintenance dosing.

Our population represented some of the potential sources of genetic variability in human populations, although the study was not powered to examine the influence of genetic factors on PK. MAOA polymorphisms did not appear to influence PK in single dose or chronic dosing components; however, there was some indication, based on one of the subjects, that 5HTTLPR polymorphism could influence fluoxetine metabolism. Another gene of interest is CYP2D6 which metabolizes fluoxetine to norfluoxetine (Ereshefsky et al. 1996; Alfaro et al. 2000; Qu et al. 2009) and has high and low expression polymorphisms in humans. The CYP2D6 gene is recognized in rhesus monkeys, although genetic variability and regulatory control have not been studied (Yasukochi and Satta 2011). We do not have information on CYP2D6 polymorphism in the present study but fluoxetine/norfluoxetine ratios (2 mg/kg chronic dosing, 4 h timepoint, $.51 \pm .05$ mean $\pm$ sem) were not highly variable.

Sex of subjects also had no apparent effect. Clinical and pharmacodynamic gender differences have been reported for fluoxetine in adult humans but we did not find studies of PK gender differences. Extrapolation of fluoxetine PK across species and ages is complicated by the differences between acute and chronic dosing (Hiemke and Hartter 2000) and the limited and varied designs of available studies.

The goal of this study was to identify a dosing regimen that would parallel therapeutic serum concentrations in drug monitoring studies of children. A dose of 2 mg/kg-d was indicated by comparison of fluoxetine+norfluoxetine serum concentrations with human studies. Combined fluoxetine+norfluoxetine serum levels were 336 ng/mL 4 h after a daily dose of 2 mg/kg-d in the young monkeys. In drug monitoring studies, Wilens et al. (Wilens et al. 2002) reported steady state serum concentrations averaging 366 ng/mL fluoxetine +norfluoxetine 8–12 h after a daily dose of 20 mg/d in children 6–11 years old. Koelch et al. (Koelch et al. 2012) reported concentrations of 224 ng/mL when measured before a daily dose of 20 mg/d in children and adolescents 8–19 years of age. A recent study of male juvenile rhesus reported serum levels of fluoxetine of 41 ± 25 and norfluoxetine of 159 ± 78 ng/mL (mean $\pm$ SD) after chronic dosing at 3 mg/kg/d. With this same sampling (4 weeks of daily dosing, sample taken 24 h after last daily dose), we found values of fluoxetine 11 ± 13 and norfluoxetine 126 ± 54 ng/mL (mean $\pm$ SD) (Shrestha et al. 2014).

Simple allometric scaling would not appear useful for human to monkey extrapolation of fluoxetine doses. Based on an average body weight of 40 kg and dose of 20 mg for children in Wilens et al. (Wilens et al. 2002) the dosage is 0.5 mg/kg-d. Using allometric extrapolation ($bw^{3/4}$), the dose for 4.5 kg juvenile rhesus would be 0.86 mg/kg-d, much lower than the dose of 2 mg/kg identified in the present study. A similar conclusion concerning allometric extrapolation was reached in a study of adult rhesus (Sawyer and Howell 2011).

Human studies typically use lumbar CSF and do not detect 5-HT. However, studies in monkeys using cisternal CSF demonstrated reduced 5-HT in response to sertraline (Anderson et al. 2002; Anderson et al. 2005) and support the use of this measure for reflecting SSRI effects on serotonin receptor blockade (Anderson 2004). In monkey infants

9.5–10.5 months of age, 2 mg/kg fluoxetine for 2 weeks reduced levels of 5HIAA in CSF to about 60% of pretreatment levels (Clarke et al. 1999). Although the decline in 5HIAA was consistent it was small (3% of initial values). In contrast, a recent study of rhesus reported CSF concentrations of 5HIAA using three fluoxetine doses (0.5, 2.0 and 3.0 mg/kg/d) after 4 weeks of daily oral administration and found reductions to 60% of control in the 2.0 mg/kg/d group (Shrestha et al. 2014). Details of this study, such as the age and sex of the subjects, the number, order, and time between dosing periods, and the site of the CSF tap were not reported making comparisons difficult. The longer duration of treatment in our study (14 weeks) may be relevant. Epinephrine, norepinephrine and the metabolite MHPG were not detected in the present study, but were reported and were not found to be affected by fluoxetine in the study of Clarke et al. (1999). The dopamine metabolite HVA was significantly elevated in CSF at the end of chronic treatment in the present study. In few available studies of HVA response to fluoxetine in adult human CSF, concentrations were slightly reduced (Martensson et al. 1989; Sheline et al. 1997). It is possible that dopamine is more sensitive to fluoxetine in younger subjects. However, because our study had an intra-subject pre-post treatment design, it is possible that factors other than treatment such as age or season influenced CSF monoamines.

In conclusion, under steady state conditions, a dose of 2 mg/kg-d administered to juvenile rhesus monkeys produced therapeutic levels comparable to those recommended for children. Serum concentrations of fluoxetine after a single administration could be adequately modeled with a single compartment model yielding estimates of basic PK parameters across a dose range of 1–4 mg/kg. Sex and MAOA polymorphism did not appear to influence fluoxetine PK, but there was an indication that 5HTTLPR polymorphisms should be further examined in this regard.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

All procedures conformed to the Guide for the Care and Use of Laboratory Animals, 8th Edition, issued by the US National Academy of Sciences. Fluoxetine and norfluoxetine assays were performed at the West Coast Metabolomics Center, an NIH Regional Resource Core, under the supervision of Mine Palazoglu. Erica Unger, Pennsylvania State University, performed the CSF analysis. Andrew Campbell, PhD, MPH, Andrew Campbell Consulting, performed the PK analysis. The authors appreciate the contribution of CNPRC Research Services personnel in conducting dosing and blood sampling for the single-dose studies and Alicia Bulleri for additional technical assistance. Supported by NIH grants R01HD065826 (Golub) and OD011107 (Lewin).

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Fig 1.

Single dose studies of fluoxetine administered by gavage to juvenile (2.5 year old) rhesus monkeys (n=6). Graphs show serum concentrations from a one-compartment pharmacokinetic model (line) along with raw data points (group average). Pharmacokinetic parameters from the models are provided in the table below

Fig 2.

Serum fluoxetine + norfluoxetine (active agent) concentrations from administration of a single dose of 2 mg/kg fluoxetine after 4 weeks of daily administration of the same dose. Group mean and s.e.m. are shown. The inset shows the daily pre-dosing baseline concentrations across 14 weeks of dosing. The range of therapeutic levels in children is outlined in the black box

Fig 3.

Concentrations of serotonin (5-HT) and its major metabolite (5-HIAA) in CSF of juvenile monkeys after 14 weeks of fluoxetine administration at 2 mg/kg-d. Analysis for monoamine neurotransmitters and their metabolites in CSF additionally detected concentrations of the dopamine metabolites DOPAC and HVA, which are also shown. Statistical analysis indicated effects of sex for 5HIAA ($p=.006$) and DOPAC ($p=.003$) and effects of weeks for 5HT ($p=.01$) and HVA ($p=.01$)