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Pharmacokinetics and pharmacodynamics of norfluoxetine in rats: Increasing extracellular serotonin level in the frontal cortex

Ying Qu *, Leah Aluisio, Brian Lord, Jamin Boggs, Kenway Hoey, Curt Mazur, Timothy Lovenberg

Johnson and Johnson Pharmaceutical Research and Development, L. L. C., 3210 Merryfield Row, San Diego, CA 92121, USA

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ABSTRACT

Norfluoxetine is the most important active metabolite of the widely used antidepressant fluoxetine. Although the pharmacokinetics/pharmacodynamics (PK/PD) relationship and neurochemical profile of fluoxetine is well characterized in human and in animals, little is known about the effect of its metabolite. The aim of this study was to characterize extracellular level of serotonin (5-hydroxytryptamine, 5-HT)-time profile of norfluoxetine after acute administration over 18 h post dose and to establish the relationship between this pharmacodynamic (PD) profile and its pharmacokinetic (PK) properties. Following subcutaneous administration of fluoxetine in rats, plasma and brain PK of fluoxetine and norfluoxetine were monitored respectively by liquid chromatography/tandem mass spectrometry (LC/MS/MS). The extracellular level of 5-HT in the frontal cortex was measured by microdialysis as a PD endpoint. Norfluoxetine when directly administrated to rats caused a significant increase in extracellular level of 5-HT in the frontal cortex and maintained for 18 h. This result is correlated well with higher plasma and brain concentration and longer plasma and brain retention time of norfluoxetine. Our results showed that norfluoxetine contributes to 5-HT transporter inhibition and extends fluoxetine efficacy.

1. Introduction

Fluoxetine (Prozac) is widely used for the treatment of depression (Fuller, 1995; Masand and Gupta, 1999). Its biochemical and pharmacological profiles have been studied extensively in animals and human (Fuller, 1995; Qu et al., 2006, 2003; Stahl, 1998; Wong et al., 1995). However the mechanism of drug action related to its clinical efficacy has not been fully understood. It is generally believed that a significant part of the therapeutic activity of fluoxetine is attributable to its most important active metabolite norfluoxetine (Fuller et al., 1992), since clinical pharmacokinetic (PK) study has shown that plasma norfluoxetine level were 100–130% those of fluoxetine (Tulloch and Johnson, 1992). Fluoxetine is a selective serotonin (5-hydroxytryptamine, 5-HT) transporter inhibitor, which exerts its behavioral and clinical therapeutic effect by blocking the transport of 5-HT at the 5-HT transporter, thereby increasing extracellular level of 5-HT in serotonergic synaptic cleft of many brain regions, which mediate a variety of behaviors. *In vivo* microdialysis has been extensively used to document the changes of extracellular level of 5-HT in rat brain after administration of fluoxetine (Beyer et al., 2002; Boothman et al., 2006; Bymaster et al., 2002; Fuller, 1994; Kobayashi et al., 2008; Koch et al., 2002;). It has been reported that norfluoxetine is as potent as the parent drug itself as an inhibitor of 5-HT uptake *in vitro* (Fuller, 1995; Wong et al., 1995) and *in vivo* (Fuller et al., 1992). However, the effect of norfluoxetine on the extracellular level of 5-HT in rat brain has never been reported.

In clinical study, fluoxetine has a mean half-life of 1–3 days and is subject to hepatic metabolism by cytochrome P450 enzymes (Mandrioli et al., 2006). Norfluoxetine is its N-demethylated metabolite with a longer half-life of 4–16 days (Mandrioli et al., 2006). We hypothesize that the time course of extracellular 5-HT level in following the acute norfluoxetine treatment may also relate to longer plasma half-life of norfluoxetine.

Therefore we designed the following experiments to evaluate both PK and pharmacodynamic (PD) features of norfluoxetine. 1). The effect of acute systemic administration of norfluoxetine (3 and 10 mg/kg s.c.) on extracellular level of 5-HT in the frontal cortex of freely moving rats was examined by performing a 21-hour *in vivo* microdialysis experiment. 2). A comparison of plasma and brain PK of fluoxetine and norfluoextine in rat after acute administration (3 and 10 mg/kg, s.c.) of fluoxetine. Based on the findings of these experiments, we established the PK/PD relationship of norfluoxetine, which is indicated by extracellular level of 5-HT and the plasma and brain concentration of the norfluoxetine.

2. Methods

2.1. Drugs

Fluoxetine hydrochloride, norfluoxetine hydrochloride and 5-hydroxytryptamine were purchased from Research Biochemicals

* Corresponding author. Present address: Department of Psychiatry, University of California, San Diego, USA. Tel.: +1 858 692 0779.
E-mail address: yingqu68@gmail.com (Y. Qu).

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International of Sigma (Natick, MA, USA). Other chemicals and reagents were of HPLC or analytical grade of purity. HPLC water was purchased from J. T. Baker (Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA) and purified by passing through a C18 solid phase extraction column (Sep-Pak C18, Waters, USA).

2.2. Microdialysis experiments

Male Sprague–Dawley rats (Charles River Laboratories, MA, USA) weighing 300–350 g were used. Each rat was given a 0.05 ml s.c. injection of Buprenex 0.06 mg/kg (buprenorphine hydrochloride) 5 min prior to anesthesia. Animals were anesthetized with an isoflurane/air mixture and stereotaxically implanted with a guide cannula (Eicom, Japan) in the prefrontal cortex (incisor bar −3.5 mm, +3.2 mm anterior, 0.8 mm lateral and 1 mm ventral to Bregma) (Paxinos and Watson, 1997). The guide cannula was secured in place with skull screws and dental cement. Animals were allowed at least 3 days to recover from surgery prior to experimentation.

Dialysis experiments were conducted between 8:00 am and 4:00 am the following day, in a controlled environment. The animals remained in their home cage throughout experimentation. Dialysis probes (Eicom, molecular weight cut-off 5,5000 Da, 0.22 mm out diameter, 4 mm active membrane length) were perfused with aCSF (147 mM NaCl, 4 mM KCl, 0.85 mM $MgCl_2$, 2.3 mM $CaCl_2$, pH 7.4) at a flow rate of 1 µl/min and implanted the afternoon prior to sample collection. The probe was connected via FEP tubing to a liquid swivel (QM, Instech, USA) mounted on a counter-balance arm. The following morning 3 h of baseline samples were collected into a 96-well plate (Sarstedt, 96 well multiply PCR, USA) via a four-channel fraction collector (Eicom). Norfluoxetine (3 or 10 mg/kg s.c.) was subcutaneously administrated to the animal. Samples were collected every 60 min for 18 h into the 96-well plate maintained at 4 °C containing 15 µl of the antioxidant (1 mM oxalic acid and 3 mM l-cysteine in 0.1 M acetic acid). The 60 µl microdialysis sample plus 15 µl antioxidant was aliquot to 30 µl for 5-HT analysis. Vehicle animals received 5% N-methyl-2-pyrrolidone solution (v/v, 1 ml/kg, s.c.) injection. New probes were used every time without determining *in vitro* recovery.

2.3. Analysis of extracellular serotonin levels

Quantification of extracellular levels of 5-HT in microdialysis samples of the frontal cortex was achieved by high-performance liquid chromatography (ESA Model 582) coupled to electrochemical detector (CoulArray Coulometric, ESA) with dual channel coulometric microdialysis cell (ESA 5014B) (Barbier et al., 2007). Separation was performed on a C18 column (Hypersil, 150×3.2 mm I.D.) at room temperature. The mobile phase consisted of 75 mM NaH_2PO_4, 0.5 mM Disodium-EDTA, 350 mg/L 1-octanesulfonic acid, pH 3.1, 1.0% THF, and 9.0% ACN. Flow rate was 0.4 ml/min. 22 µl microdialysate was injected by an autosampler (ESA Model 540). The first electrode of the detector was set at −90 mV (reduction) and the second at +280 mV (oxidation). All values for microdialysis studies were calculated as percentage change at each time point compared with the average of three baseline values. The overall effect of norfluoxetine treatments on extracellular levels of 5-HT was determined by a two way ANOVA analysis with treatment as the independent variable and time as the repeated measurement. If significant, the ANOVA was followed by post-hoc Duncan's multiple range test (SigmaStat, SPSS Inc., www.spss.com). Student paired *t*-test was used to compare the extracellular 5-HT level in light and dark phases.

2.4. Plasma sample preparation

Following administration of 3 or 10 mg/kg fluoxetine (s.c.), 250 µl blood samples via venipuncture of the lateral tail vein were collected into heparinized tubes over a time course as follow: 0.5, 1, 2, 4, 6, 8, 9, 10, 12 and 24 h. Then centrifuged at 14,000 rpm for 5 min. The plasma was retained and kept frozen in a −20° C freezer waiting for liquid chromatography/tandem mass spectrometry (LC/MS/MS) analysis.

The thawed plasma samples (50 µl) were mixed with 50 µl dimethyl sulfoxide (DMSO) and 150 µl acetonitrile, at room temperature for 10 min and centrifuged for 10 min at 14,000 rpm. 150 µl of supernatant was diluted with 450 µl of 10 mM ammonium acetate (pH = 7) for LC/MS/MS injection.

2.5. Brain sample preparation

Following administration of 3 or 10 mg/kg (s.c.) fluoxetine, brains were taken out at 1, 6, 24, or 48 h immediately after scarified by CO_2 asphyxiation and homogenized in 6 ml of phosphate buffered saline (Dulbecco HyQ DPBS/MODIFIED, HyClone, Utah, USA) and stored at −20° C. For LC/MS/MS analysis, the thawed homogenized brain samples (50 µl) were mixed with 50 µl DMSO, and 150 µl methanol. After incubation at room temperature for 10 min, 250 µl acetonitrile was added and waiting at room temperature for another 10 min, the samples were centrifuged for 10 min at 14,000 rpm. The supernatants were diluted 1:1 with 10 mM ammonium acetate (pH = 7) for LC/MS/MS injection.

2.6. Drug analysis by using LC/MS/MS

LC/MS/MS system consisted of Agilent 1100 series LC (Agilent Technologies, Wilmington, DE, USA) including a vacuum degasser, a binary pump, and an autosampler coupled to an Applied Biosystems/MDS SCIEX API-4000 triple–quadrupole mass spectrometer equipped with a TurboIonSpray source (Foster City, CA, USA). LC separations were achieved using 5-µm C18 column (50×2.1 mm i.d.; MAC-MOD http://www.mac-mod.com) at 40 °C with gradient elution. Mobile phase A was prepared by mixing 5% acetonitrile with 95% 10 mM ammonium acetate buffer (pH = 7) (v/v) and mobile phase B was prepared by mixing 95% acetonitrile with 5% 10 mM ammonium acetate buffer (pH 7) (v/v). A gradient elution over 7 min at a flow rate of 0.4 ml/min was used. Fluoxetine and norfluoxetine were monitored using the single-reaction monitoring (SRM) mode. The SRM transition m/z 310.1 → 44.1 and 296.1 → 134.0 were sequentially monitored for the detection of fluoxetine and norfluoxetine, respectively.

2.7. PK data analysis

WinNonlin software was used to analyze the data. A non-compartmental pharmacokinetic model was used to determine the following pharmacokinetic parameters: C_{max} (µM), peak plasma or brain concentration; T_{max} (h), time to maximum plasma or brain concentration; $AUC_{0-\infty}$ (µM h), area under the plasma or brain-time curve extrapolated to infinity; MRT (h), mean retention time; $t_{1/2}$ (h), half life.

3. Results

3.1. Effects of norfluoxetine on extracellular level of 5-HT

The effects of vehicle or norfluoxetine (3, 10 mg/kg s.c.) administration on time course of extracellular level of 5-HT in the frontal cortex of the freely moving rat are shown in Fig. 1. The acute administration of norfluoxetine (3.0 mg/kg s.c., $n = 5$) evoked a significant increase of 5-HT from basal levels in the frontal cortex of rats, to a maximum of 346 ± 50% at 10 h post dose and the average over the 18-hour treatment period was sustained at 277 ± 15% of basal values. Acute administration of a higher dose of norfluoxetine (10 mg/kg s.c., $n = 6$) evoked a larger increase of extracellular 5-HT level, to a maximum of 432 ± 56% at 9 h and the average over the 18-hour treatment period was

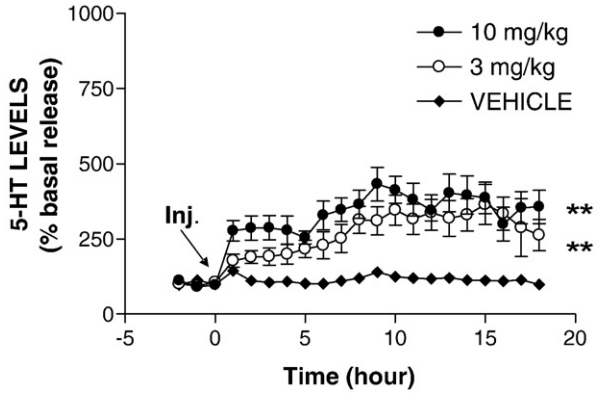

Fig. 1. Effect of norfluoxetine on extracellular level of 5-HT in the frontal cortex of free moving rat. Values are mean ± S.E.M. of extracelllular 5-HT levels and expressed as a percentage of the average of three baseline samples (defined as 100%). Two way ANOVA–post-hoc Duncan's multiple range test were used for comparison. Control ($n=5$), 3 mg/kg ($n=6$) and 10 mg/kg ($n=6$) norfluoxetine were subcutaneously administrated. Asterisks indicate significance of overall effect of drug treatment versus vehicle, $^{**}P<0.01$.

sustained at 344 ± 13% of basal values. The two-way ANOVA analysis indicated a significant dose effect of norfluoxetine treatment ($F_{2,\ 216}=10.7,\ p=0.002$) and time ($F_{17,\ 216}=8.8, p<0.001$), as well as dose across time interaction ($F_{34,\ 216}=2.7,\ p<0.001$), compared to vehicle treatment.

In addition, after administration of norfluoxetine, the average increases of extracellular level of 5-HT during the dark phase are significantly larger (paired *t*-test was used) than in the light phase for two different doses. For 3 mg/kg ($n=5,\ p=0.001$), the average extracellular 5-HT concentration is 88 ± 2.1 fg/μl in the light phase and 124 ± 2.5 fg/μl in the dark phase. For 10 mg/kg ($n=6,\ p=0.0028$), they are 153 ± 9.9 fg/μl and 208 ± 9.6 fg/μl respectively.

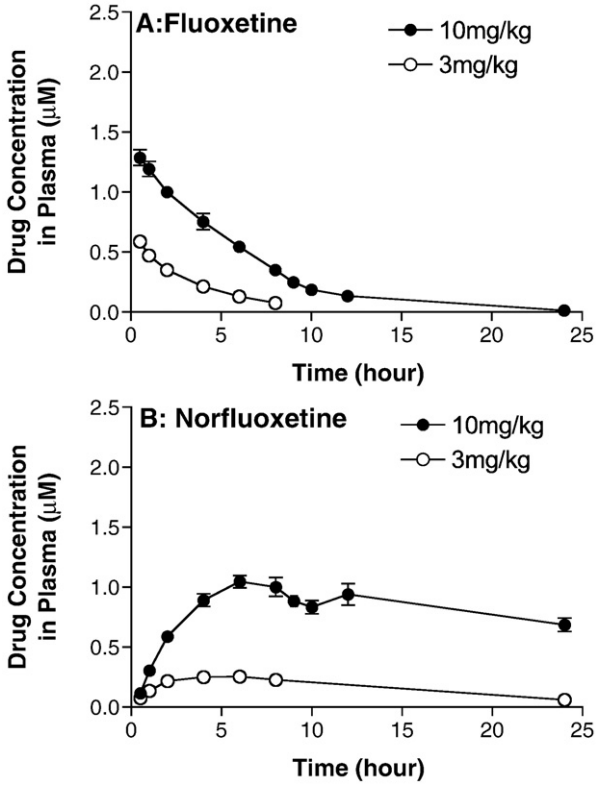

Fig. 2. Time course of plasma concentrations of fluoxetine and norfluoxetine. Plasma concentrations (mean ± S.E., $n=10$) of fluoxetine (panel A) and norfluoxetine (panel B) were measured following subcutaneous administration of 3 or 10 mg/kg fluoxetine.

Table 1
Pharmacokinetic parameters for FLX and NFLX following acute subcutaneous administration.

Drug		C_{max} (μM)	T_{max} (h)	AUC $_{0-\infty}$ (μM h)	MRT (h)	$t_{1/2}$ (h)
3 mg/kg FLX	In plasma	0.46	1	1.95	3.00	2.08
	In brain	11.0	1	80.3	4.09	2.83
10 mg/kg FLX	In plasma	1.55	1	9.71	3.91	2.71
	In brain	24.2	1	276	5.36	3.71
3 mg/kg NFLX	In plasma	0.24	6	3.75	9.40	6.51
	In brain	7.21	6	117	12.1	8.37
10 mg/kg NFLX	In plasma	1.07	6	31.4	17.0	11.8
	In brain	31.7	6	977	18.5	12.9

Mean ± SD, $n=5$.
Abbreviations: C_{max} (μM), peak plasma or brain concentration; T_{max} (h), time to maximum plasma or brain concentration; AUC $_{0-\infty}$ (μM h), area under the plasma or brain-time curve extrapolated to infinity; MRT (h), mean retention time; $t_{1/2}$(h), half life.

3.2. Plasma concentrations of fluoxetine and norfluoxetine

PK profiles of fluoxetine and its metabolite, norfluoxetine in rats were evaluated after subcutaneous administration of 3 or 10 mg/kg of fluoxetine. Plasma concentration–time curves of fluoxetine and norfluoxetine are shown in Fig. 2, Panels A and B. A non-compartmental model was used to determine the PK parameters, which are summarized in Table 1.

Both 3 and 10 mg/kg of fluoxetine showed rapid elimination from the plasma. The elimination is from 0.5 h through 9 h at dose of 3 mg/kg and from 0.5 h through 24 h at dose of 10 mg/kg (Fig. 2A). Plasma AUC (area under the plasma concentration–time curve extrapolated to infinity) of fluoxetine was linearly related to the dose and no significant difference between time to maximum plasma concentration (T_{max} (h)), mean retention time (MRT (h)) and half life ($t_{1/2}$(h)).

The metabolite, norfluoxetine, was generally detected in plasma 0.5 h after dosing. At the dose of 10 mg/kg, norfluoxetine concentration is slowly increased by N-demethylation of fluoxetine and maintained a steady state-like profile from T_{max} of 6 h through 24 h. 3 mg/kg norfluoxetine maintained a steady state-like profile as well (Fig. 2B). As in the case of fluoxetine, plasma AUC of norfluoxetine increased linearly with dose, but has different MRT and $t_{1/2}$.

3.3. Brain concentrations of fluoxetine and norfluoxetine

In separate experiments, rats were scarified at 1, 6, 24 and 48 h after subcutaneous administration of 3 or 10 mg/kg of fluoxetine. The unchanged drug and its active metabolite were analyzed in the brain tissue. Brain concentration–time curves of fluoxetine and norfluoxetine are shown in Fig. 3, Panels A and B respectively. Both brain AUC of fluoxetine and norfluoxetine are 28 and 40 times higher than those in plasma respectively. The time course of brain concentration of fluoxetine and norfluoxetine paralleled to the plasma kinetics of these drugs. Non-compartmental model was used to determine the PK parameters in the brain, which are summarized in Table 1 as well.

Both 3 and 10 mg/kg of fluoxetine rapidly passed blood-brain barrier and reached to their T_{max} at 1 h after dosing, then started to eliminate within 24 h (Fig. 3A). Brain AUC of fluoxetine was linearly related to the dose and no difference between T_{max}, MRT and $t_{1/2}$.

Norfluoxetine concentration in the brain was slowly increased by N-demethylation of fluoxetine and maintained a steady state-like profile. T_{max} are 6 h at both 3 and 10 mg/kg dose (Fig. 3B). At the dose of 10 mg/kg, norfluoxetine eliminated in 48 h, had MRT at 18.5 h and $t_{1/2}$ at 12.9 h. 3 mg/kg norfluoxetine eliminated in 24 h, had MRT at

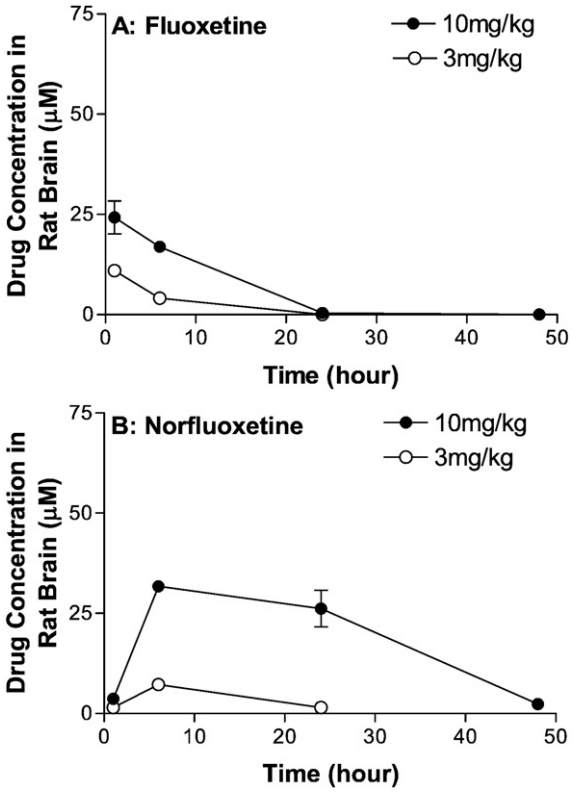

Fig. 3. Time course of brain concentrations of fluoxetine and norfluoxetine. Brain concentrations (mean ± S.E., $n = 5$) of fluoxetine (panel A) and norfluoxetine (panel B) were measured following subcutaneous administration of 3 or 10 mg/kg fluoxetine.

12.1 h and $t_{1/2}$ at 8.37 h. Brain AUC of norfluoxetine increased linearly with dose, but had a different MRT and $t_{1/2}$ compared to plasma.

4. Discussion

4.1. Effect norfluoxetine on extracellular level of 5-HT in the frontal cortex of rats

To measure the extracellular level of 5-HT in the frontal cortex of freely moving rats, an *in vivo* microdialysis sampling technique was designed in our lab to collect microdialysis samples from four freely moving rats over 21 h in the same time frame as a pharmacokinetic study. The basal dialysate levels for 5-HT in the frontal cortex of rats in this study are consistent with previous report showing the same brain region and using the same size microdialysis probe (Rocher et al., 1996).

Acute fluoxetine administration to rats has been shown to increase extracellular 5-HT level in many brain regions including striatum, thalamus frontal cortex, caudate-putamen and raphe nuclei (Wong et al., 1995) and well documented by others (Beyer et al., 2002; Boothman et al., 2006; Bymaster et al., 2002; Fuller, 1994; Kobayashi et al., 2008; Koch et al., 2002). In earlier studies, it has been reported that norfluoxetine is as potent as the parent drug itself as an inhibitor of 5-HT transporter *in vitro* (Fuller, 1995; Wong et al., 1995) and *in vivo* (Fuller et al., 1992). In *in vitro* study, it has been showed that both fluoxetine and norfluoxetine are racemic mixtures containing equal amounts of R and S enantiomers. S-norfluoxetine inhibits 5-HT transporter with an inhibition constant (Ki) of 20 nM which is as potent as both R-fluoxetine (Ki = 21 nM) and S-fluoxetine (Ki = 16 nM), is 14 time more potent than R-norfluoxetine (Ki = 268 nM) (Wong et al., 1995). In the *in vivo* studies, norfluoxetine only have been compared as 5-HT uptake inhibitors based on its antagonism of p-chloroamphetamine-induced depletion of serotonin in brain and lowering of tissue concentrations of 5-HT metabolite, 5-hydroxyindoleacetic acid (5-HIAA) in brain (Fuller et al., 1992). It has been found (Bourdeaux et al., 1998; Wong et al., 1995) that both fluoxetine and norfluoxetine lead to reduce levels of 5-HT in platelets. However, the *in vivo* effect of norfluoxetine on extracellular level of 5-HT has never been reported. In present study, a 21 h *in vivo* microdialysis experiment was first time used to examine the change of extracellular concentration of 5-HT in the frontal cortex of rats by direct administration of norfluoxetine. Acute norfluoxetine treatment at both 3 and 10 mg/kg dose showed significant and, similar, increases in the extracellular 5-HT of 2 to 3 fold of basal levels over 18 h. These results show that norfluoxetine is as potent as the fluoxetine with respect to extracellular level of 5-HT.

In addition, we also observed that the extracellular levels of 5-HT in the frontal cortex are significantly higher in waking (dark phase) compared to sleeping (light phase) in norfluoxetine treated animal. The extracellular 5-HT change across the light/dark cycle corroborates previous findings in the dorsal raphe nucleus (DRN) and frontal cortex (Rueter et al., 1997; Portas et al., 1998). The extracellular level of 5-HT in the frontal cortex has been shown to parallel DRN neuronal activity (Wright et al., 1990) and is related to general behavioral states/activity (Rueter and Jacobs, 1996). In the literature, 5-HT presents something of a paradox in respect of its role in sleep and waking behavior, although its importance to both is undoubted. Early experiments suggest that an increase in 5-HT transmission actually helps to induce sleep (Jouvet and Pujol, 1974). In contrast to the evidence that 5-HT activity decreases arousal, direct stimulation of raphe neurons, or system administration of a 5-HT precursor, actually increases waking (Jacobs and Azmitia, 1992). A link between extracellular 5-HT level and increased waking is supported by evidence from *in vivo* microdialysis of cats and rats (Portas et al., 1998). This suggests that 5-HT has either an excitatory influence on behavior and/or an inhibitory effect on sleep (Rusak and Bina, 1990). Norfluoxetine has long brain retention time of 18 h and maintains a steady high plasma and brain concentration for 18 h. Thus it may continually inhibit serotonin transporter and increase the 5-HT neurotransmission across the light/dark cycle. Therefore, the enhanced 5-HT neurotransmission by norfluoxetine may increase the excitatory influence on the motor activity associated with waking, as has been suggested.

4.2. PK and PD relationship of fluoxetine and norfluoxetine

Clinical PK studies in healthy volunteers and patients after single and repeated doses of fluoxetine have clarified most aspects of drug absorption, distribution and clearance (Altamura et al., 1994; Goodnick, 1994; Hiemke and Hartter, 2000). Although this drug is widely used in animal models to assess the functional role of central serotoninergic system, little is known about PK relevant parameters such as time to maximum brain concentration, brain distribution of the parent drug and its active metabolite in animals. The present study evaluated the PK profiles of fluoxetine and norfluoxetine by acute administration of 3 and 10 mg/kg fluoxetine in rat.

Comparing the kinetic parameters of fluoxetine in the rats at 3 and 10 mg/kg with those known for human, elimination of fluoxetine in this study was much faster in rat (elimination $t_{1/2}$ of 2–3 h) while $t_{1/2}$ was 1–4 days for man after a single oral dose (Benfield et al., 1986; Gram, 1994). This result was similar to a previous rat studies which showed a $t_{1/2}$ of 4–7 h by single intravenous or oral administration (Caccia et al., 1990). Norfluoxetine was eliminated much more slowly than its parent drug in both man and rats. In this study, the $t_{1/2}$ of norfluoxetine is 6.5 h for 3 mg/kg and 12 h for 10 mg/kg in rats. In a previous rat study, $t_{1/2}$ was 15 h for both intravenous or oral administration at 2.5–10 mg/kg (Caccia et al., 1990) and $t_{1/2}$ was 7 days for human (Benfield et al., 1986; Gram, 1994). Therefore the PK finding in this study is consistent with previous studies in human and animals.

In present study, we observed that norfluoxetine in the plasma not only has a longer retention time than that of fluoxetine, but also has higher plasma concentration. Similar findings were made by others, under

steady-state conditions, the plasma concentration of racemic norfluoxetine normally exceeds the concentration of racemic fluoxetine (Baumann and Rochat, 1995). Thus in rat, norfluoxetine may play a decisive role in the inhibition of serotonin uptake after fluoxetine administration.

In present PK study, the drug concentrations are much higher in the brain than in the plasma. At 3 and 10 mg/kg fluoxetine, brain peak concentration (C_{max}) is 24 and 16 times higher than plasma C_{max} respectively; brain AUC is 41 and 28 times higher than plasma AUC respectively. For both dose of norfluoxetine, brain C_{max} is 30 times higher than plasma C_{max}. Similarly brain AUC is 31 times higher than plasma. These results suggest that fluoxetine and norfluoxetine quickly and efficiently penetrate the blood-brain barrier to reach and act on 5-HT transporters in the brain.

After fluoxetine administration, norfluoxetine is slowly produced by fluoxetine metabolism and reached to the peak concentration at 6 h, then maintains a steady state-like profile through 24 h in both brain and plasma. The mean retention time of norfluoxetine in plasma is 17 h. This result is consistent with the microdialysis finding, after 10 mg/kg direct norfluoxetine treatment, the extracellular concentration of 5-HT in the frontal cortex is increased and maintained a steady for 18 h. At the dose 3 mg/kg, the mean retention time of norfluoxetine in plasma is 12 h. The effect of 5-HT level in the frontal cortex is increased and kept until the end of the experiment. These results provide a clear relationship between the norfluoxetine concentration in both plasma and brain and the norfluoxetine effect on extracellular 5-HT concentration in the frontal cortex of rats. After norfluoxetine injection, the extracellular concentration of 5-HT was immediately increased at the first hour which is due to its quick blood-brain barrier penetration. This result is consistent with PK results.

In additional, fluoxetine often takes several weeks to months to achieve its antidepressant effects in clinical setting and its therapeutic action when chronically administered has been ascribed to desensitization of pre-synaptic 5-HT_{1A} and 5-HT_{1B} autoreceptors, further augmenting extracellular 5-HT (Blier and de Montigny, 1994). However, the effect of chronic nonfluoxetine treatment on serotonin level and other molecular system change has not been reported. Gardier et al. (1994) examined the effects of repeated administration of fluoxetine (5, 10, or 15 mg/kg., twice daily for 21 days) on brain and plasma concentrations of fluoxetine and norfluoxetine in rats during the 21 days regimen as well as after cessation of drug treatment. In their studies, norfluoxetine concentration in plasma and brain was ten times higher than those of fluoxetine during the period of drug treatment. Although fluoxetine accumulated more markedly in the rat brain than norfluoxetine, it disappeared completely from plasma and brain after treatment stopped, while norfluoxetine persisted up to 7 days after treatment stopped. Therefore, the therapeutic action when chronically administered fluoxetine might directly relate to norfluoxetine as well.

5. Conclusion

In this study, 21 h *in vivo* microdialysis experiments were performed to measure the changes of extracellular level of 5-HT, which is used as the PD ending point for selective serotonin transporter inhibitor. It was the first time to observe that norfluoxetine, when directly administrated to rats, caused the significant increases in the extracellular level of 5-HT in the frontal cortex by norfluoxetine administration correlates positively with the plasma and brain PK of norfluoxetine. Due to higher plasma and brain concentrations and longer plasma and brain retention time of norfluoxetine, norfluoxetine contributes to 5-HT transporter inhibition and extends fluoxetine's efficacy.

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