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Influence of dose and route of administration on the kinetics of fluoxetine and its metabolite norfluoxetine in the rat

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Abstract. Fluoxetine (FL) is being used in neuropharmacology as a tool for studying various functional roles of serotoninergic neurons. Its kinetics was studied in rats, a species widely used in neurochemical studies, after IV (2.5–10 mg/kg) and oral (5–20 mg/kg) administration. When injected IV the drug followed apparent first-order kinetics up the 10 mg/kg dose. Its volume of distribution was large and total body clearance was relatively high compared to liver blood flow. The mean elimination half-lives ($t_{1/2}$) of FL and its active metabolite norfluoxetine (NFL) were about 5 and 15 h, respectively. The mean blood:plasma concentration ratios of FL and NFL approached unity and plasma protein binding was 85–90% for both compounds. After oral doses the kinetics of FL were complex. At the lowest dose tested (5 mg/kg) the drug was efficiently extracted by the liver (extraction ratio about 60%), resulting in bioavailability of only about 38%. Plasma areas under the curve (AUC) of the metabolite were approximately the same as after IV injection of the same dose; consequently the metabolite-to-parent drug ratio after oral administration (about 5) was approximately twice that after IV injection of FL (about 2.5). At higher doses, however, the oral bioavailability (e.g. C_{max} and AUC) appeared greater than expected, possibly because of transient saturation of FL first-pass metabolism in the case of the 10 mg/kg dose and concomitant saturation of elimination kinetics at the higher dose (20 mg/kg). The apparent elimination $t_1/_2$ of FL markedly increased and the metabolite-to-parent drug ratio declined with the higher dose, this also being consistent with saturable elimination. Brain concentrations reflected the plasma kinetics of FL and NFL and the metabolite-to-parent drug ratio varied with dose and time of administration and was modified at the highest dose tested. FL and its metabolite NFL distributed almost evenly in discrete brain areas and subcellular distribution was similar for both compounds. Neurochemical studies of FL should consider the formation of the active metabolite NFL and extrapolation of data across animal species requires consideration of dose dependence in the rat.

Key words: Fluoxetine – Norfluoxetine – Kinetics – Rat

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Fluoxetine (FL) is a new antidepressant which facilitates serotonergic transmission through inhibition of neuronal reuptake of serotonin (5HT). Its biochemical and pharmacological profile has been studied extensively in animals (see reviews Benfield et al. 1986; Schmidt et al. 1988). The selectivity and potency of this drug as an inhibitor of the 5HT reuptake pump has aroused considerable interest and it is being widely used as a tool for studying the functional role of the serotoninergic system.

In spite of its widespread pharmacological use, the physiological disposition and metabolism of FL in animals have still not been thoroughly investigated, apart from an initial tissue distribution study in rats and dogs (Parli and Hicks 1974) which showed that the drug concentrated in tissues and was extensively biotransformed by N-deethylation to norfluoxetine (NFL), an active metabolite which may contribute significantly to the parent drug's pharmacological and clinical effects (Horng and Wong 1976; Fuller et al. 1978; Schmidt et al. 1988). In comparison, there are many studies of the kinetics of FL and NFL in man under different conditions, in healthy subjects after single and repeated administration, in elderly and renally and hepatically impaired subjects (Benfield et al. 1986; Blackwell and Simon 1986; Robinson 1988).

Because disposition studies of FL in animals may help in understanding its mechanism of action and facilitate extrapolation of biochemical and pharmacological findings to man, we determined the single-dose profile and dose-proportion after intravenous and oral administration to rats, a species widely used in neurochemical studies of this drug. In addition, we examined the effect

of dose and route of administration on the presence of FL in relation to its metabolite NFL in brain, brain areas and subcellular components.

Materials and methods

Drugs and sources. FL (racemic mixture) and NFL hydrochloride were kindly supplied by E. Lilly, Florence, Italy.

Drug administration and plasma and blood sampling. Male CD-COBS rats (Charles River, Italy) weighing about 300 g were acclimatized to the research facility for 1 week before the study, receiving standard laboratory chow and water ad libitum. A chronic jugular cannula (PE-50 tubing) was implanted in each rat under chloral hydrate anesthesia 15 h before the study started. Rats received FL hydrochloride dissolved in isotonic saline in doses of 2.5, 5 and 10 mg/kg (7.2, 14.5, and 29 μmol/kg) as a bolus injection through a tail vein or dissolved in water at doses of 5, 10 and 20 mg/kg by direct esophageal intubation. Serial 0.1–0.3 ml blood samples were drawn at various times after dosing, centrifuged and the plasma was stored at −20° C until analysis.

The blood-plasma concentration ratios were determined from whole blood and plasma concentrations of FL and NFL 180 min after IV and oral administration of FL hydrochloride to rats.

Plasma protein binding was assessed in vitro by equilibrium dialysis. Rat fresh plasma was incubated at 37° C with FL and NFL (0.3–3 nmol/ml) for 30 min with shaking; 1 ml portions were dialyzed overnight at 37° C against isotonic Krebs-bicarbonate buffer (pH 7.4) in a Dianorm apparatus. Each side of the dialytic cells was then analyzed, with four to five replications for each concentration.

Brain distribution and subcellular localization. The brain distribution and subcellular localization of FL and its metabolite NFL was investigated 3 and/or 30 h after IV and oral administration of the parent drug. Brain regions were dissected according to Glowinsky and Iversen (1966).

Subcellular primary fractions were obtained by differential centrifugation following the procedure developed by Whittaker and Barker (1972). The nuclear (P_1), microsomal (P_3) and P_2 fractions (mitochondria and synaptosomes), resuspended in up to 2 ml 0.32 M sucrose, and the supernatant (S) from the last centrifugation (100000 *g*) was extracted and analyzed for FL and NFL as described below. Protein content was determined on samples of various fractions and supernatant by the method of Lowry et al. (1951).

Chemical analysis. Concentrations of FL and NFL in biological samples were determined by electron capture gas-liquid chromatography (GC-ECD) after the addition of nomifensine as internal standard. Plasma samples (0.05–0.15 ml) were deproteinized with 20% trichloroacetic acid and centrifuged. The clean supernatant was alkalinized using 1 N NaOH, then extracted twice with 1 ml benzene. The combined extracts were concentrated to about 0.5 ml. Then 100 μl of an ethyl acetate solution of heptafluorobutyric anhydride (25% v/v) were added and the samples were heated at 70° C for 30 min. After the reaction the samples were washed with water (1 ml) and 5% aqueous ammonia (0.5 ml) and 1–2 μl of the benzene phase were injected into the chromatographic column.

Blood (0.1 ml) was diluted in 0.5 ml water and extracted with 2.5 ml benzene. After centrifugation the supernatant was processed as described above. Brain tissue was homogenized (10 ml/g) in cold acetone-1 N formic acid (85:15 v/v) and centrifuged. The supernatant was shaken twice with n-heptane-chloroform (90:10 v/v), the organic phase was discarded and samples of the aqueous phase (0.05–0.15 ml) were used for parent drug and metabolite extraction as described for plasma.

The compounds were analysed on a Carlo Erba Fractovap equipped with a ^{63}Ni-ECD. The chromatographic column was a glass tube (2 m × 3 mm I.D) packed with 80–100 mesh Supelcoport with 3% OV-17 as the stationary phase. Column, injector port, and detector temperatures were 230, 250 and 275° C, respectively. Nitrogen flow rate was approximately 30 ml/min. Retention times were 4 min for NFL, 5 min for FL and 8 min for internal standard.

Standard curves were prepared daily spiking blank plasma, blood or brain tissue with known concentrations of FL and NFL (calculated as free bases). Because of the wide concentration range found for FL and NFL in the various biological matrices and in the different experimental conditions, different calibration graphs in the concentration range of approximately 0.02–1 nmol/ml (5–250 ng/ml) in body fluids and 0.1–3 nmol/g (25–1000 ng/g) in brain tissue were generally used. The calibration graphs were all linear with acceptable correlation coefficients ($r > 0.99$).

Intra-assay precision was assessed on control plasma and brain homogenates with different amounts of FL and NFL (see above). These samples were processed on the same day and the concentrations (mean ± S.D.) and coefficients of variation (CV) were calculated. The precision of the analysis was good at all the concentrations and the CV was less than 10% in all cases. The inter-assay precision was evaluated preparing plasma and brain samples containing approximately 0.02 and 0.3 nmol/ml (5 and 100 ng/ml) and 0.1 and 1 nmol/g (25 and 250 ng/g) FL and NFL. These were used as quality control samples throughout the disposition study to check the calibration data. The results indicated that the method has good reproducibility, the CV at the limit of detection being 10–15% and higher concentrations giving values below 10%. The efficiency of extraction from plasma, blood and brain tissue was, respectively, 75–80%, 70–75% and 55–66% for FL and 72–77%, 70–78% and 50–60% for its metabolite; the limits of sensitivity was approximately 0.02 nmol/ml plasma or blood and 0.03 nmol/g brain tissue.

Data analysis. Over the sampling interval the area under the concentration-time curve (AUC) was determined by the trapezoidal rule. The first moment of the plasma concentration-time profile (AUMC) was determined similarly after multiplying each concentration by its time. Then both values were extrapolated to infinity. The terminal slope (β), the last blood concentration (Ct) and the time of Ct were used in the calculation. The β values were calculated by linear least squares regression of the log blood concentrations versus time utilizing the data points in the log linear region of the blood concentration-time curves. The elimination half-life ($t_{1/2}$) was determined from β using the relationship $t_{1/2} = 0.693/\beta$. Total body clearance (Cl) was calculated from dose/AUC; the steady-state volume of distribution (V_{ss}) was calculated according to Benet and Galeazzi (1979). The maximum concentration (C_{max}) and the time (T_{max}) of its occurrence were read directly from the blood concentration-time data for both FL and NFL. Bioavailability (F) was calcualted as the ratio of the AUC for the oral doses to the mean AUC for intravenous doses.

The effect of dose on kinetic variables was assessed by analysis of variance and probabilities (P) less than 0.05 were considered statistically significant.

Results

Intravenous administration

Plasma concentration-time curves of FL and NFL after IV bolus injection of 2.5, 5 and 10 mg/kg FL hydrochloride to rats are shown in Fig. 1. Some kinetic parameters derived from these figures are summarized in Table 1. Plasma AUC of FL was linearly related to the dose

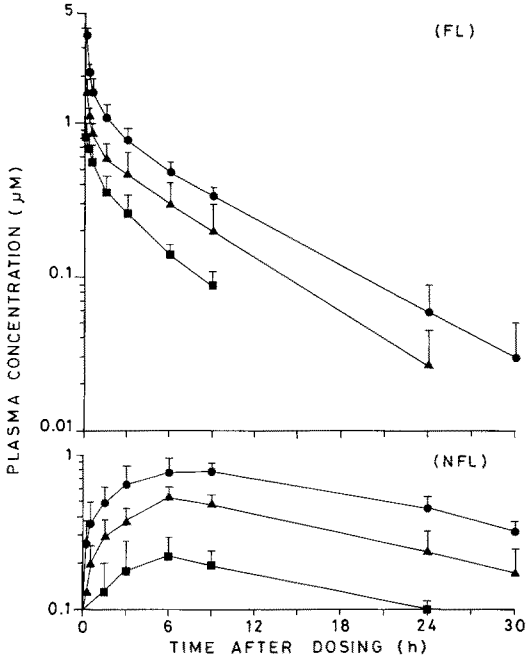

Fig. 1. Mean plasma concentration-time curves of fluoxetine (*FL*) and norfluoxetine (*NFL*) after IV injection of 2.5 (■), 5 (▲) and 10 (●) mg/kg FL hydrochloride. Each point is the mean ±SD of five rats

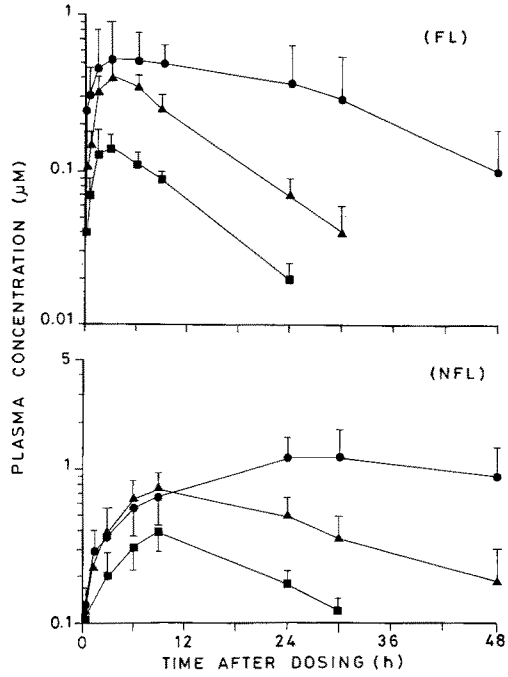

Fig. 2. Mean plasma concentration-time curves of fluoxetine (*FL*) and norfluoxetine (*NFL*) after oral doses of 5 (■), 10 (▲) and 20 (●) of FL hydrochloride. Each point is the mean ±SD of five rats

Table 1. Kinetic parameters of fluoxetine (FL) and norfluoxetine (NFL) after intravenous doses of fluoxetine hydrochloride to rats

Parameters	Dose (mg/kg)		
	2.5	5	10
FL			
Vss (l/kg)	15.9±6.1	19.1±8.6	20.2±4.5
Cl (ml/min/kg)	46.9±11.7	45.4±17.8	47.7±5.7
$t_{1/2}$ (h)	4.2±1.5	5.5±1.3	5.9±1.3
NFL			
AUC (nmol/ml·h)	5.7±1.0	14.3±4.5	24.9±2.7
AUC NFL/AUC FL	2.2±0.5	2.5±1.0	2.5±0.2
$t_{1/2}$ (h)	15.5±5.4	14.6±4.6	16.5±1.6

Each value is the mean ±SD of five rats

Table 2. Kinetic parameters of fluoxetine (FL) and norfluoxetine (NFL) after oral doses of fluoxetine hydrochloride to rats

Parameters	Dose (mg/kg)		
	5	10	20
FL			
T_{max} (h)	3.3±1.6	3.3±1.6	10.2±8.1*, +
C_{max} (nmol/ml)[a]	0.1±0.05	0.2±0.03*	0.2±0.1
AUC (nmol/ml·h)[a]	2.0±0.2	3.0±0.5	4.5±2.2**
$t_{1/2}$ (h)	7.0±0.9	7.7±0.9	13.2±2.7**
NFL			
T_{max} (h)	6.0±2.0	8.4±1.3	26.4±3.3**
C_{max} (nmol/ml)[a]	0.4±0.04	0.4±0.1	0.3±0.1
AUC NFL/AUC FL	5.3±1.1	4.1±0.8	3.0±0.9**
$t_{1/2}$ (h)	14.4±3.2	15.8±4.5	nd

Each value is the mean ±SD of 5 rats

[a] Normalized for a 5 mg/kg dose

nd = Not determinable

** $P<0.01$; * $P<0.05$ vs the 5 mg/kg dose

+ $P<0.05$ vs the 10 mg/kg dose

and analysis of variance revealed no difference between V_{ss}, Cl and $t_{1/2}$ for the doses used.

The metabolite was generally detected in plasma within 15–30 min of dosing and reached maximum concentrations between 6 and 9 h (Fig. 1). As in the case of FL, plasma AUC of the metabolite increased linearly with the dose and analysis of variance indicated no difference in T_{max}, C_{max} and $t_{1/2}$ for the doses of parent drug used. The mean metabolite-to-parent drug AUC ratio ranged from about 2 to 2.5 over the range investigated (Table 1). At higher doses (20 mg/kg) the animals suffered and most of them died within minutes of parenteral injection. Thus IV doses of FL exceeding 10 mg/kg were not utilized.

Oral administration

Mean plasma concentration-time data of FL and NFL after oral doses of 5, 10 and 20 mg/kg FL hydrochloride to rats appear in Fig. 2 and kinetic parameters are summarized in Table 2. FL was absorbed relatively slowly from the solution as indicated by the T_{max} which ranged from 1 to 6 h at the 5 and 10 mg/kg doses (mean 3.3 h) and more than 24 h at the highest dose tested ($P<0.01$

Table 3. Brain concentrations of fluoxetine (Fl) and norfluoxetine (NFL) after intravenous and oral administration of Fl hydrochloride to rats

Dose (mg/kg)	Brain concentrations at					
	3 h			30 h		
	FL	NFL	NFL/FL	FL	NFL	NFL/FL
2.5 IV	2.1±0.2	2.9±0.4	1.4±0.3	(n.d.)	0.8±0.2	(n.d.)
5 IV	2.5±0.4	2.7±0.4	0.9±0.2	0.02±0.00	0.8±0.4	47±17
10 IV	3.1±0.6	2.9±0.5	1.0±0.2	0.02±0.00	1.0±0.2	48±10
5 PO	0.9±0.2	3.2±0.4	3.8±0.4	0.02±0.00	0.8±0.3	46±16
10 PO	2.4±0.5**	2.5±0.5*	1.0±0.1**	0.02±0.01	1.1±0.5	54±12
20 PO	2.3±0.6**	1.5±0.3**,+	0.7±0.1**	0.17±0.04**,++	2.4±0.3**,++	14±4**,++

Results are expressed as nmol/g (normalized for dose of FL)

Each value is the mean ±SD of 5 rats

nd = Below level of quantitation

** $P<0.01$; * $P<0.05$ vs the 5 mg/kg oral dose
++ $P<0.01$; + $P<0.05$ vs the 10 mg/kg oral dose

versus the 5 and 10 mg/kg doses). Compared to the lowest dose the increase in C_{max} (normalized for the dose administered) was more than proportional for 10 and 20 mg/kg, although at the highest dose the difference did not reach significance probably because of broad data variability. The mean (±SD) AUC values, normalized to the 5 mg/kg dose, were 2.0±0.2, 3.0±0.5 and 4.5±2.2 after 5, 10 and 20 mg/kg, respectively, and the corresponding values for F, calculated as the ratio of the AUC for the oral doses to the mean AUC for IV doses, were 0.38±0.04, 0.56±0.08 and 0.84±0.40. The differences were statistically significant between the 5 and 20 mg/kg doses ($P<0.05$ to $P<0.01$). In all cases the apparent elimination $t_{1/2}$ of FL was significantly longer ($P<0.05$ to $P<0.01$) than after IV dosing but tended to rise with the oral dose (Table 2).

The T_{max} of the metabolite tended to rise with parent drug doses. Again the differences were statistically significant between the 5 and 20 mg/kg doses (Table 2). The metabolite-to-parent drug AUC ratio was also significantly lower for 20 mg/kg. At this dose the ratio had to be estimated by computation of the partial $AUC_{(0-48\ h)}$, because the AUC of the metabolite could not be extrapolated to infinity. At lower doses plasma $t_{1/2}$ (Table 2) and AUC values for NFL (10.3±1.4 and 24.7±6.4 nmol/ml·h, at the 5 and 10 mg/kg dose, respectively) were comparable to those in the IV experiments (Table 1).

Distribution in rat blood

No significant difference in plasma protein binding was observed when FL and NFL were incubated in vitro over the range of 0.3–3 nmol/ml with fresh plasma. The percentage of FL unbound ranged from a minimum of 10±1% to a maximum of 15±5%, mean 13±4% (±SD, $n=16$). Similarly, the percentage of NFL unbound, 11±3% (mean±SD, $n=20$) was not dependent on the concentration in plasma. Blood-to-plasma ratios averaged 0.83±0.17 and 0.85±0.15 ($n=16$) for FL and NFL, respectively, over the investigated dose range.

Brain distribution

In separate experiments other groups of rats were killed 3 and 30 h after intravenous (2.5–10 mg/kg) and oral (5–20 mg/kg) doses and the unchanged drug and its active metabolite were assayed in the brain, where in accordance with previous results (Parli and Hicks 1974), FL and FLN both concentrated, achieving levels 20–40 times those in plasma. At these times brain levels parallelled the plasma kinetics of FL and NFL and the metabolite-to-parent drug ratio changed with route and

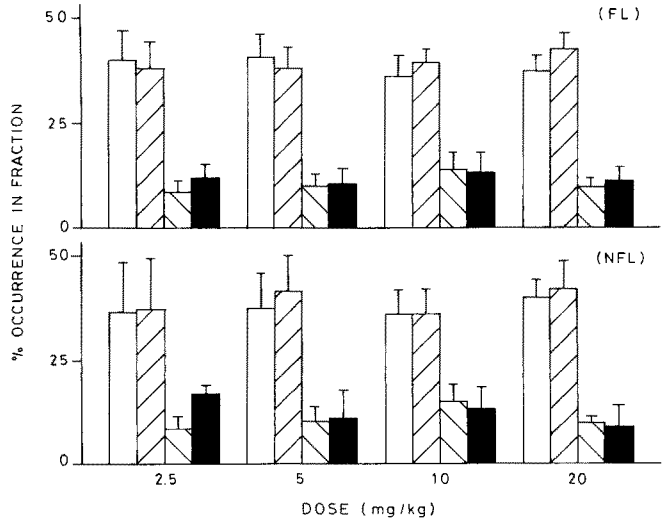

Fig. 3. Distribution of fluoxetine (*FL*) and norfluoxetine (*NFL*) in rat brain subcellular fractions after IV (2.5–5 mg/kg) and oral (10–20 mg/kg) administration of FL hydrochloride. The columns indicate mean value ±SD ($n=4$). □ Nuclei (P_1); ▨ mitochondria and synaptosomes (P_2); ▧ microsomes (P_3); ■ supernatant (S)

time of administration and even with dose, in the case of oral dosing (Table 3).

The pattern of drug and metabolite distribution in various brain regions and spinal cord was similar to that described for the whole brain (data not shown) and there was no evidence of preferential concentration of FL or NFL in any of the brain areas studied (cortex, hippocampus, striatum, brainstem and cerebellum) when assessed 3 h after parent drug administration.

The subcellular distribution pattern was also similar for FL and NFL but both compounds distributed unevenly in the rat brain subcellular fractions: about 40% was recovered in the P_1 (nuclei) and P_2 (mitochondria and synaptosomes) fractions and 10% in microsomes ($P3$) and soluble (S) fractions, regardless of the dose and route of administration (Fig. 3).

Discussion

Clinical pharmacokinetic studies in healthy volunteers and patients after single and repeated doses of FL have clarified most aspects of drug absorption, distribution and clearance. Although this drug is widely used in animal models to assess the functional role of the central serotoninergic system, little is known about pharmacologically relevant parameters such as bioavailability, rate of elimination and brain distribution of the parent drug and its active metabolite in animals. The present study evaluated the single dose and dose-proportionality kinetics of racemic FL and its metabolite NFL in the rat, using a specific chromatographic analytical method. The doses tested (2.5–20 mg/kg) are well within the range used for neurochemical studies with racemic FL.

Comparing the kinetic parameters in the rat of FL at low doses (2.5–10 mg/kg) with those known for man, elimination of FL was much faster in the rat, the apparent elimination $t_{1/2}$ of 4–7 h, depending on the route of administration, contrasting with 1–4 days in man after a single dose (Benfield et al. 1986; Blackwell and Simon 1986; Robinson 1988). However, if this is related to the different metabolic rates in the two species, i.e. the "physiological time", then clearance may not be so markedly different (Boxenbaum 1980). The volume of distribution for the rat was similar to that for man (20–40 l/kg) (Benfield et al. 1986) and greatly exceeded the body weight, indicating extensive distribution of the drug into tissues, a conclusion supported by previous tissue distribution studies (Parli and Hicks 1974) in which intraperitoneally administered FL rapidly attained higher concentrations in most organs of the rat (particularly liver, lung and brain) than in blood.

Plasma protein binding was extensive, as in man (Benfield et al. 1986), and did not change appreciably over a relatively broad range of concentrations. Plasma protein binding is not a limiting factor in the metabolic clearance of FL in the rat since, despite the fact that the free fraction in plasma was less than 15%, the estimated average value for hepatic extraction ($E = 1 - F$) was more than 50%.

FL is almost entirely eliminated from the body by biotransformation, only a small percentage being excreted unchanged in urine (Benfield et al. 1986; Robinson 1988). Among the metabolites so far identified, NFL is of pharmacological interest in both man and animals since it inhibits 5HT uptake in vivo and in vitro (Horng and Wong 1976; Fuller et al. 1978; Schmidt et al. 1988). Moreover, it is eliminated much more slowly than its parent drug by man and rats although as in the case of the parent drug its elimination $t_{1/2}$ is much shorter in the rat (about 15 h) than in man (about 7 days). However, while in rats the plasma concentrations of NFL (in terms of AUC) greatly exceeded those of FL, even after intravenous injection, concentrations of parent drug and metabolite are almost comparable in plasma after oral administration of FL to humans (Aronoff et al. 1984; Benfield et al. 1986; Schmidt et al. 1988). Thus in the rat NF should play a decisive role in the inhibition of serotonin uptake after FL administration while in man it would only contribute to the overall clinical outcome.

Mean bioavailability (F) of FL in the rat was about 38% at the lowest oral dose tested. The AUC of NFL after either IV or oral dosing showed, however, that the values were similar for each route. This could indicate that the drug is efficiently absorbed from the rat gastrointestinal tract but that it is also efficiently extracted by the liver (first-pass effect), resulting in relatively low availability. In man too FL is reported to be absorbed efficiently; in other species such as the dog the absolute oral bioavailability of the drug is about 72% (Benfield et al. 1986; Robinson 1988).

At larger oral doses the kinetics of FL was not linear. At 10 mg/kg the systemic availability (e.g. C_{max} and AUC) of the drug was greater than that anticipated from the lower dose, and at 20 mg/kg the metabolite-to-parent drug ratio also significantly changed and the apparent elimination $t_{1/2}$ of FL was longer than with lower doses.

Transient saturation of the first-pass metabolism appears the most likely explanation for the increase in FL C_{max} observed at the 10 mg/kg dose, since the drug is apparently well absorbed after oral administration. This effect is shared by a number of drugs undergoing a large first-pass metabolism on oral administration. Since saturation of hepatic metabolism will be a transient and concentration-dependent effect (i.e. FL concentration entering the liver with the portal blood), it is not surprising that there is a more pronounced effect on the C_{max} than the AUC of the drug, as observed at the 10 mg/kg dose. Together with concomitant saturation of hepatic clearance, this effect might explain the disproportionate rise in the FL AUC and the lower metabolite-to-parent drug ratios at the largest dose. Evidence of capacity-limited elimination of the drug and/or its active metabolite emerged from previous studies with FL in other animal species (Parli and Hicks 1974; Farid et al. 1986). In man chronic administration of FL apparently raises the drug elimination $t_{1/2}$ compared with a single dose (Benfield et al. 1986), further suggesting self-inhibition of metabolism and/or saturation of microsomal enzymes. Besides

this, FL may also inhibit oxidative drug metabolism in rodents within the dose range used in neurochemical studies (Fuller et al. 1976).

These findings were reflected in the brain where both compounds concentrated achieving concentrations several times those in plasma. They distributed almost evenly in discrete brain areas and subcellular distribution was also similar for both compounds. As previously observed after FL IP, the metabolite-to-parent drug brain concentration ratio increased with time (Schmidt et al. 1988) and, as in plasma, varied with route of FL administration and was different at the highest dose tested. Thus the relative contribution of the two compounds to the overall pharmacological effect of FL in vivo in the rat should also vary depending on the route, dose and possibly treatment schedule.

Overall, these results indicate that the kinetics of FL in the rat are complex and suggest the need for care in using this species for neurochemical studies with this drug. FL has relatively high clearance and is efficiently extracted by the liver, resulting in low bioavailability. The oral bioavailability is dose – dependent, thus complicating studies of the effect – disposition relationship; these may be further complicated by the presence of the active metabolite NFL in plasma and brain. Caution must in any case be exercised in extrapolating data from rat to man because of differences in FL and NFL kinetics between the two species.

FL is clinically available as the racemate and most published pharmacological and biochemical studies have been conducted with this form. However, increasing evidence show that the enantiomers of racemic drugs often differ pronouncedly in their pharmacodynamic and/or kinetic properties, hence the importance of studying the stereoselective pharmacology and disposition of the individual enantiomers (Williams and Lee 1985; Eichelbaum 1988). Although FL shows apparently little enantiospecificity regarding serotonin uptake inhibition in vitro and in vivo (Wong et al. 1985; Fuller and Snoddy 1986; Robertson et al. 1988) the kinetic and metabolic profile of the single enantiomers is not yet characterized in either man or animals. Accordingly, future studies should look into the disposition of the individual FL isomers after administration of the racemate and/or when administered as such. This will facilitate its utilization as a tool to study the mechanisms of serotonergic transmission and the central serotoninergic system's physiological role and should make for more reliable extrapolation of pharmacological and biochemical findings across species.

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References

Aronoff GR, Bergstrom RF, Pottratz ST, Sloan RS, Wolen RL, Lemberger L (1984) Fluoxetine kinetics and protein binding in normal and impaired renal function. Clin Pharmacol Ther 36:138–144

Benet LZ, Galeazzi RL (1979) Noncompartmental determination of the steady-state volume of distribution. J Pharm Sci 68:1071–1074

Benfield P, Hell RC, Lewis SP (1986) Fluoxetine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in depressive illness. Drugs 32:481–508

Blackwell B, Simon JS (1986) Second-generation antidepressants. Drugs of Today (Medicamentos de Actualidad) 22:611–633

Boxenbaum H (1980) Interspecies variation in liver weight, hepatic blood flow, and antipyrine intrinsic clearance: extrapolation of data to benzodiazepines and phenytoin. J Pharmacokinet Biopharm 8:165–176

Eichelbaum M (1988) Pharmacokinetic and pharmacodynamic consequences of stereoselective drug metabolism in man. Biochem Pharmacol 17:93–96

Farid NA, Bergstrom RF, Lemberger L, Ziege EA, Tenbarge J, Wolen RL, Dhahir PH (1986) Studies on the disposition of fluoxetine at steady state in man with the aud of stable and radioactive isotopes. In: 15th. CINP Congress, San Juan, Puerto Rico, Dec. 14–17, Book of Abstracts, P-234

Fuller RW, Snoddy HD (1986) Fluoxetine enantiomers as antagonists of *p*-chloroamphetamine effects in rats. Pharmacol Biochem Behav 24:281–284

Fuller RW, Rathbun RC, Parli CJ (1976) Inhibition of drug metabolism by fluoxetine. Res Commun Chem Pathol Pharmacol 13:353–356

Fuller RW, Snoddy HD, Perry KW, Bymaster FP, Wong DT (1978) Importance of duration of drug action in the antagonism of *p*-chloroamphetamine depletion of brain serotonin. Comparison of fluoxetine and chlorimpramine. Biochem Pharmacol 27:193–198

Glowinski J, Iversen LL (1966) Regional studies of catecholamines in the rat brain. I. The disposition of [^{3}H]norepinephrine, [^{3}H]dopamine and [^{3}H]DOPA in various regions of the brain. J Neurochem 13:655–669

Horng JS, Wong DT (1976) Effects of serotonin uptake inhibitor, Lilly 110140, on transport of serotonin in rats and human blood platelets. Biochem Pharmacol 25:865–867

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275

Parli CJ, Hicks J (1974) In vivo demethylation of Lilly 110140: 3-(*p*-trifluoromethylphenoxy)-N-methyl-3-phenylpropylamine to an active metabolite – Lilly 103947. Fed Proc 33:560

Robertson DW, Krushinski JH, Fuller RW, Leander JD (1988) Absolute configurations and pharmacological activities of the optical isomers of fluoxetine, a selective serotonin-uptake inhibitor. J Med Chem 31:1412–1417

Robinson CP (1988) Fluoxetine (Prozac®): An antidepressant which selectively inhibits serotonin reuptake. Drugs of Today (Medicamentos de Actualidad) 24:7–13

Schmidt MJ, Fuller RW, Wong DT (1988) Fluoxetine, a highly selective serotonin reuptake inhibitor: a review of preclinical studies. Br J Psychiatry [Suppl 3] 153:40–46

Whittaker VP, Barker LA (1972) The subcellular fractionation of brain tissue with special reference to the preparation of synaptosomes and their component organelles. In: Fried R (ed) Methods of neurochemistry, vol 2. Dekker, New York, pp 1–50

Williams K, Lee E (1985) Importance of drug enantiomers in clinical pharmacology. Drugs 30:333–354

Wong DT, Bymaster FP, Reid LR, Fuller RW, Perry KW (1985) Inhibition of serotonin uptake by optical isomers of fluoxetine. Drug Dev Res 6:397–403