# Pharmacokinetics of Fluphenazine, a Highly Lipophilic Drug, Estimated from a Pulse Dose of a Stable Isotopomer in Dogs at Steady State

JOHN W. HUBBARD,* SALIM HADAD, JIANG-PING LUO, GORDON MCKAY, AND KAMAL K. MIDHA

Contribution from *College of Pharmacy & Nutrition, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, S7N 5C9.*

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**Abstract** □ The potential utility of a pulse dose of a deuterium-labeled isotopomer (FLU-$D_4$) in elucidating the pharmacokinetics of fluphenazine (FLU) at steady state was investigated in dogs. The single-dose oral pharmacokinetics of FLU in dogs were established. After resting the dogs for 3 weeks, the animals were dosed to steady state with oral FLU administered at 12-h intervals. Following 15 doses, one dose of FLU was replaced by a pulse dose of FLU-$D_4$, after which dosing with FLU was resumed. FLU and FLU-$D_4$ plasma concentrations were determined by tandem mass spectrometry. Comparable estimates of apparent oral clearance were calculated from (i) a single dose of FLU, (ii) a pulse dose of FLU-$D_4$, and (iii) over a dosing interval at steady state. Average steady-state plasma concentrations were reliably predictable from a pulse dose of FLU-$D_4$.

## Introduction

A difficult task facing the psychiatrist is the determination of the optimal dose of neuroleptic for an individual patient in the acute phase of a psychosis because therapeutic effects of treatment with classical neuroleptics generally require 2−4 weeks to become optimal. Aggressive dosing in the early stages of treatment, however, may lead to the establishment of unnecessarily high maintenance doses.[1] Dysken and co-workers[2] suggested that a therapeutic window of 0.2 to 2.8 ng/mL trough concentrations of fluphenazine (FLU) in plasma was appropriate for schizophrenic patients. These early studies, however, gave no indication of the extent to which aberrantly high plasma levels are associated with inferior antipsychotic response.[3] A more recent and more sophisticated study by Van Putten and colleagues[4,5] employed logistic regression and survival analysis to examine relationships between plasma concentrations of FLU, estimated probability of improvement, and estimated probability of disabling side effects. Disabling side effects were defined as "side effects that significantly interfered with patients' functioning" or "side effects that outweigh therapeutic effects." The study showed that the probability of improvement increased as plasma FLU concentrations approached 4.0 ng/mL, but so did the probability of disabling side effects. It was found that the maximum probability of improvement without disabling side effects occurred at a plasma FLU concentration of 0.7 ng/mL.[5] Thus, patients who tolerate the drug well may benefit from plasma concentrations >1.0 ng/mL, whereas poorly responding patients with plasma levels of 4.0 ng/mL and above may benefit from a reduction in dosage. These data have provided a more rational basis for the use of plasma concentration data in FLU posology.

The acquisition of pharmacokinetic data from floridly psychotic acute schizophrenics, however, is difficult or impossible because these patients are usually too ill to be able to cooperate by providing samples of body fluids. It is certainly not appropriate to interrupt therapy to obtain single-dose pharmacokinetic data. In this situation, therefore, individualized pharmacokinetic information must be acquired after the clinical condition of the patient has been stabilized by treatment with a neuroleptic drug such as FLU. The use of a pulse dose of a stable isotopomer to assess the pharmacokinetics of a single dose of the drug while at steady state[6] is an appealing technique for two reasons: (i) there is no need to interrupt therapy or to disturb the steady state of active drug; and (ii) stabilized schizophrenic out-patients under maintenance therapy with oral FLU often take their medication at uneven time intervals, which renders uncertain any attempt to estimate pharmacokinetic parameters from plasma concentrations of unlabeled drug at steady state.

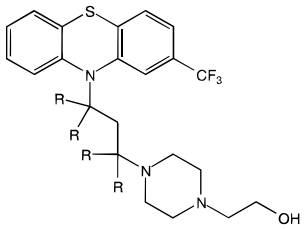


**Figure 1**—Structures FLU (R = H) and tetradeuterated FLU (R = $^2$H).

FLU is a lipophilic drug that exhibits after intravenous dosing in humans (Midha et al., unpublished data), a very large apparent volume of distribution (398 ± 202 L), a relatively short half-life (13.1 ± 4.1 h), and a systemic clearance (23.0 ± 12.3 L/h) of ∼25% of liver blood flow. Similar results were obtained after beagle dogs were dosed intravenously with FLU:[7] a large volume of distribution (51.0 ± 17.8 L), a relatively short half-life (6.0 ± 1.5 h), and a systemic clearance (5.8 ± 1.0 L/h) of ∼40% of liver blood flow. These data suggest that FLU undergoes extensive tissue distribution/binding in both humans and dogs. The present exploratory study in dogs was designed to test the hypothesis that exchange of isotopomer with unlabeled drug in tissue compartments would not impede the utility of the technique in practical terms. A tetradeuterated isotopomer (FLU-$D_4$, Figure 1) to be used in such studies was prepared in these laboratories[8] and was shown to be free of any isotopic metabolic effects in either humans[9] or dogs.[10]

## Materials and Methods

**Chemicals and Reagents**—FLU dihydrochloride was purchased from Sigma-Aldrich Canada, Ltd. (Ontario, Canada). FLU-$D_4$ was synthesized in our laboratories as described previously.[8] The isotopic purity of FLU-$D_4$ was >99%. Oral solutions containing FLU dihydrochloride equivalent to 1 mg/mL FLU base in distilled water were prepared daily. Oral solutions of the FLU-$D_4$ were

* To whom correspondence should be addressed. E-mail: Hubbard@duke.usask.ca.


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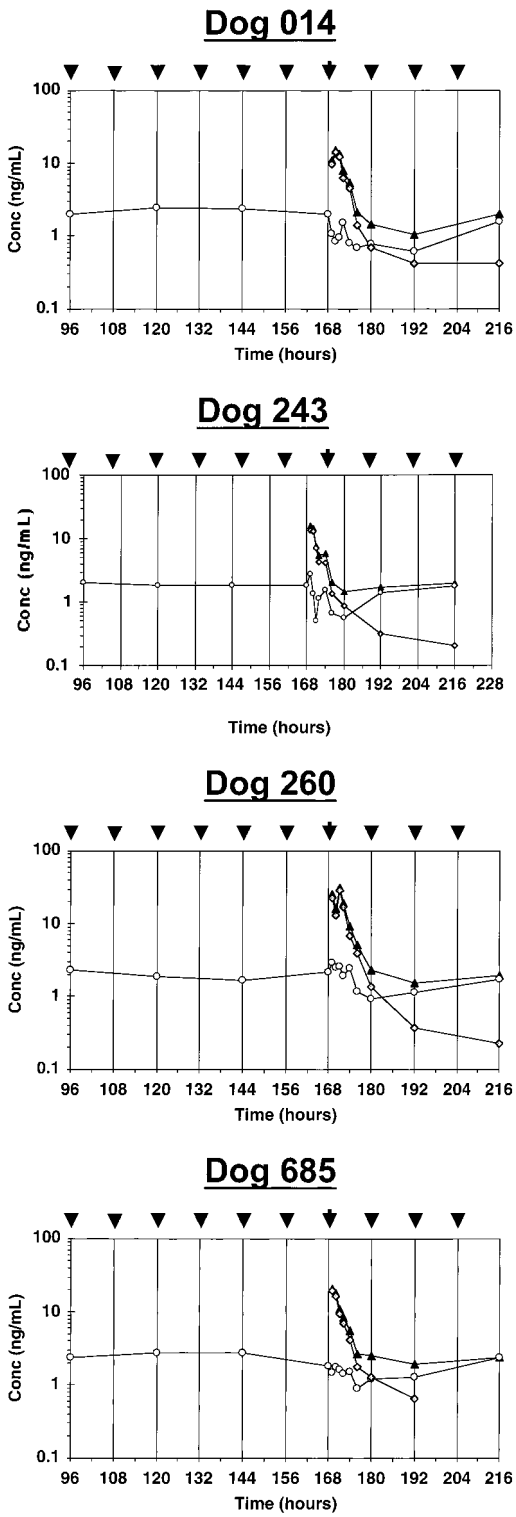


**Figure 2**—Steady-state trough plasma concentrations over the interval 96–216 h after the initial dose for FLU (circles), FLU-$D_4$ (diamonds), and total (FLU plus FLU-$D_4$, triangles) in four dogs. In each panel, black darts represent doses of FLU at 12-h intervals and the gray dart represents the pulse dose of FLU-$D_4$.

prepared in the same manner. Solvents and other chemicals were of analytical grade and were used without further purification.

**Animals and Study Design**—The study protocol was approved by the University of Saskatchewan Animal Care Committee. The experiments were carried out according to the Principles of Laboratory Animal Care (NIH Publication #85-23, revised 1985) and under conditions specified by the Canadian Council on Animal Care. Four pure bred female beagle dogs (ID nos. 014, 243, 260, and 685), ranging in weight (initially) from 7.0 to 9.5 kg (mean 8.1 ± 1.1 kg), were used. For each dose, the oral solution (2 mL, containing FLU or FLU-$D_4$ hydrochloride equivalent to 2 mg of the corresponding base) was trickled from a syringe (3 mL) into the right side of the dog's mouth over a period of 2−3 min. Immediately after administration of the oral solution, the right side of the mouth was slowly irrigated with 5 mL of distilled water. The oral pharmacokinetics of unlabeled FLU were determined from serial blood samples (5 mL) drawn via an indwelling jugular catheter at 0 (predose) and 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 36, and 48 h after a single oral dose. The animals were rested for 3 weeks, after which they were dosed to steady state with unlabeled FLU administered at 12-h intervals (09:00 and 21:00 h). After administration of 15 doses of FLU (168 h after the initial dose), the dogs were re-weighed and a pulse dose of FLU-$D_4$ was given in place of a regular dose of FLU. Blood samples (5 mL) were harvested immediately prior to administration of FLU-$D_4$ and at 1, 2, 3, 4, 6, 8, 12, 24, 36, and 48 h post FLU-$D_4$. (Unfortunately the blood samples at 36 h were lost because of a technical error). Administration of unlabeled drug was resumed for a total of three doses post FLU-$D_4$, as shown in Figure 2. Trough steady-state plasma concentrations were established as the arithmetic mean of minimum concentrations ($C_{min}$) of FLU plasma concentrations harvested at 72, 96, 120, 144, and 168 h after the initial dose and of total (FLU+FLU-$D_4$) $C_{min}$ plasma concentrations at 180, 192, and 216 h post initial dose. After collection of each sample, plasma was separated by centrifugation within 0.5 h and frozen (−20 °C) until analysis. Concentrations of FLU and FLU-$D_4$ in plasma were monitored by means of a validated tandem mass spectrometry technique[11] with a lower limit of detection of 25 pg/mL for both FLU and FLU-$D_4$, and overall coefficients of variation of 4.82% for FLU and 4.72% for FLU-$D_4$.

**Pharmacokinetic Analysis**—The plasma concentrations versus time data for both FLU-$D_4$ and FLU were analyzed by noncompartmental methods (WinNonlin Version 1.1). Steady-state trough plasma concentrations were estimated as the mean of eight measurements, five taken before administration of the pulse dose of FLU-$D_4$ and three taken post pulse dose. The accumulation index ($R_{ac}$)[12] was estimated according to eq 1:

$$R_{ac} = \frac{\mathbf{1}}{(\mathbf{1} - e^{-k\tau})} \tag{1}$$

where $\tau$ is the dosing interval, and $k$ is an estimated apparent terminal elimination rate constant following a single oral dose. The average plasma concentration of drug at steady state ($Cpss_{av}$) was calculated according to eq 2:

$$Cpss_{av} = \frac{AUC_\tau}{\tau} \tag{2}$$

where $AUC_\tau$ was estimated from the total plasma concentration (FLU plus FLU-$D_4$). Apparent oral clearances ($CL_O$) were estimated variously as (i) dose/$AUC_\infty$ from a single dose of FLU, (ii) dose/$AUC_\infty$ from a pulse dose of FLU-$D_4$, and (iii) dose/$AUC_\tau$ calculated from the sum of FLU and FLU-$D_4$ plasma concentrations in dogs at steady state. Apparent volumes of distribution ($V_d$) were estimated as the quotient of $CL_o$ and $k$.

A relationship between $CL_o$ and $Cpss_{av}$ was established as follows:[12]

$$Cpss_{av} \propto A$$

$$Cpss_{av} \times V_d = A$$

$$Cpss_{av} \times CL = A \times k = \frac{F \times \text{Dose}}{\tau}$$

$$Cpss_{av} = \frac{F \times \text{Dose}}{CL \times \tau} = \frac{\text{Dose}}{CL_o \times \tau} \tag{3}$$

where $A$ is the amount of drug in the body.

## Results and Discussion

FLU and FLU-$D_4$ (Figure 1) were shown previously to give virtually identical plasma concentration versus time

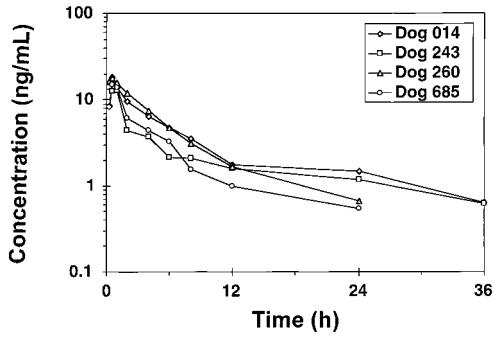


**Figure 3**—Plasma concentration versus time curves after single oral doses of FLU in four dogs.

profiles in schizophrenic patients[9] and in beagle dogs.[10] It was thereby concluded that the isotopomer was devoid of any isotopic metabolic effects in either humans or dogs.

After a single oral dose, plasma concentrations of FLU were measurable up to 24 h in two dogs and up to 36 h in the remaining two (Figure 3). The very short $t_{max}$ (0.25 h in 1/4 dogs and 0.5 h in 3/4 dogs) may have been attributable to rapid absorption through the buccal mucosae. This method of administration was chosen for two reasons. Earlier (unpublished) experiments had shown that oral administration of the drug in gelatin capsules to dogs led to erratic absorption characteristics. Administration of an oral solution was clearly desirable but it was not practicable to inflict gastric intubation on the animals twice a day for 1.5 weeks. Thus, the irrigated oral solution was the best compromise to give minimal variability. The apparent elimination half-life of FLU after the single oral dose was 12.75 ± 3.25 h, which is comparable to that obtained[13] after a single oral dose in humans, but at variance with an earlier study from our laboratories[7] in which the reported half-life was 6.0 ± 1.5 h after intravenous administration of FLU in four different beagle dogs (ID nos. 236, 244, 341, 960).

Once dosing to steady state with a lipophilic drug has commenced, drug from each successive dose undergoes exchange with drug from earlier doses sequestered in deep compartments. In the present study, dosing at 12-h intervals with unlabeled drug was continued until the dogs had received 15 doses, which in clinical terms would be a minimum number of doses after which rational dosage adjustments might be attempted. Moreover, it is possible that the deep compartments were fully loaded at this point. Individual plasma concentration versus time plots rather than averaged data are shown in Figure 2 to give an impression of (i) the steady state, (ii) the profile of the pulse dose of FLU-$D_4$, and (iii) the behavior of unlabeled FLU after the administration of the pulse dose of isotopomer.

Figure 2 shows a segment from 96 through 216 h of the steady state achieved by regular 12-h doses of 2 mg of FLU. At 168 h, one dose of FLU was replaced by a pulse dose of FLU-$D_4$ after which the mass spectrometer discriminated between FLU-$D_4$ (diamonds) and FLU (circles). Arithmetic mean trough steady-state plasma concentrations (mean ng/mL ± SD) were as follows: dog 014, 2.06 ± 0.29; dog 243, 1.77 ± 0.23; dog 260, 1.98 ± 0.29; and dog 685, 2.24 ± 0.47. In each case, the geometric mean steady-state concentration was close to the corresponding arithmetic mean (ng/mL): dog 014, 2.04; dog 243, 1.75; dog 260, 1.96; and dog 685, 2.19. The accumulation index (eq 1) was relatively low (mean ± SD = 2.089 ± 0.377).

It was possible to monitor FLU-$D_4$ for 48 h post pulse dose in 3 of the 4 dogs. Each animal showed undulations in the plasma concentrations of FLU after the pulse dose of FLU-$D_4$, suggesting that FLU arising from earlier doses of unlabeled drug appeared to rebound. Observation of this phenomenon indicates the presence of saturable distribution and/or tissue binding because rebound would not be expected to occur if distribution and/or tissue binding processes were linear. Saturable tissue binding has no effect on $CL_o$ but does affect $V_d$ and therefore the apparent elimination rate constant. As anticipated, therefore, estimates of apparent volume of distribution after a pulse dose of FLU-$D_4$ at steady state tended to be smaller than those estimated from the single dose (Table 1) because the latter was beginning the process of loading deep compartments. Maximum change in apparent volume of distribution should occur when the deep compartments are fully loaded. Failure to reach statistical significance (ANOVA) was probably due to low statistical power in this pilot study. The effects of saturable tissue binding, however, might explain why the terminal portions of the plasma concentration versus time curves of FLU-$D_4$ do not appear to be log linear (Figure 2). Consequently the data points at 48 h post FLU-$D_4$ were not used in estimates of apparent elimination rate constants. The weight-corrected apparent oral clearances estimated from the pulse dose were very similar to those estimated by dose/$AUC_\tau$ or from the single oral dose in each dog (Table 1).

**Table 1— Comparison of Apparent Oral Clearance and Volume of Distribution after a Single Oral Dose of Fluphenazine or a Pulse Dose of Isotopomer in Dogs at Steady State on Fluphenazine**

| | $CL_o$(L/kg/h) | | | $V_d$(L/kg) | |
|---|---|---|---|---|---|
| dog | single dose[a] | pulse dose[a] | steady state[b] | single dose[c] | pulse dose[c] |
| 014 | 1.81 | 2.86 | 2.92 | 44.67 | 42.15 |
| 243 | 2.82 | 3.60 | 3.45 | 52.73 | 40.05 |
| 260 | 2.12 | 2.17 | 2.02 | 28.81 | 15.75 |
| 685 | 2.77 | 2.83 | 3.02 | 45.84 | 46.59 |
| mean ± SD | 2.38 ± 0.50 | 2.86 ± 0.59 | 2.86 ± 0.60 | 43.01 ± 10.11 | 36.14 ± 13.86 |

[a] Dose/AUC∞. [b] Dose/AUC$\tau$ (where AUC$\tau$ estimated from total FLU + FLU-D4 over a dosing interval). [c] $CL_o/k$ (where $CL_o$ estimated as dose/AUC∞).

**Table 2— Comparison of Observed $Cpss_{av}$ (ng/mL) at Steady State with Values Calculated from Single Dose Data and from a Pulse Dose of Isotopomer**

| | | predicted steady state | |
|---|---|---|---|
| dog | observed steady state[a] | single dose[b] | pulse dose[b] |
| 014 | 6.0 | 10.0 | 6.1 |
| 243 | 5.7 | 6.9 | 6.5 |
| 260 | 11.8 | 8.5 | 11.0 |
| 685 | 7.4 | 5.6 | 7.9 |
| mean ± SD | 8.9 ± 2.2 | 7.7 ± 1.9 | 8.4 ± 2.4 |

[a] AUC$\tau/\tau$ (where AUC$\tau$ estimated from total FLU + FLU-D4 over a dosing interval). [b] Dose/$CL_o$*$\tau$ (where $CL_o$ estimated as dose/AUC∞).

Table 2 shows good within-dog agreement between $Cpss_{av}$ values estimated from the pulse dose and those observed in the steady state. Pharmacokinetic parameters calculated from the steady state, however, may not be of value for patients in whom the dosing interval may vary, whereas a pharmacokinetic parameters calculated from a pulse dose of the isotopomer should be applicable in such patients.

$Cpss_{av}$, however, is not affected by saturable tissue binding. One would therefore expect predictions of $Cpss_{av}$ from a single dose of FLU calculated by eq 3 to be similar to those estimated from a pulse dose of FLU-$D_4$ at steady state (eq 2).

## Conclusions

The results from this study suggest that the oral pharmacokinetics estimated from a pulse dose of FLU-$D_4$ would

be a viable method applicable to patients at steady state on oral FLU. The technique is potentially useful in schizophrenic patients who respond to treatment with potent neuroleptics but are sensitive to side effects and therefore require doses at the low end of the therapeutic range.

## References and Notes


1. Tupin, J. Focal neuroleptization: An approach to optimal dosing for initial and continuing therapy. *J. Clin. Psychopharmacol.* **1985**, *5*, 15S−21S.
2. Dysken, M. W.; Javaid, J. I.; Chang, S. S.; Schaffer, C.; Shahid, A.; Davis, J. M. Fluphenazine Pharmacokinetics and Therapeutic Response. *Psychopharmacology* **1981**, *73*, 205−210.
3. Van Putten, T.; Marder, S. R.; Wirshing, W. C.; Aravagiri, M.; Chabert, N. Neuroleptic plasma levels. *Schizophr. Bull.* **1991**, *17*, 197−216.
4. Van Putten, T.; Aravagiri, M.; Marder, S. R.; Wirshing, W. C.; Mintz, J.; Chabert, N. Plasma fluphenazine levels and clinical response in newly admitted schizophrenic patients. *Psychopharmacol. Bull.* **1991**, *27*, 91−96.
5. Midha, K. K.; Hubbard, J. W.; Marder, S. R.; Marshall, B. D.; Van Putten, T. Impact of clinical pharmacokinetics on neuroleptic therapy in patients with schizophrenia. *J. Psychiatr. Neurosci.* **1994**, *19*, 254−264.
6. Eichelbaum, M.; von Unruh, G.; Somogyi, A. Application of stable labeled drugs in clinical pharmacokinetic investigations. *Clin. Pharmacokinet.* **1982**, *7*, 490−507.
7. Luo, J.-P.; Hubbard, J.; Midha, K. Studies on the mechanism of absorption of depot neuroleptics: fluphenazine decanoate in sesame oil. *Pharmaceut. Res.* **1997**, *14*, 1079−1084.
8. Sardessai, M. S.; Brander, M. J.; Midha, K. K.; Hawes, E. M. Synthesis of deuterium labeled fluphenazine utilizing borane reduction. *J. Labelled Compd. Radiopharm.* **1986**, *23*, 317−327.
9. Hadad, S.; Hubbard, J. W.; McKay, G.; Hawes, E. M.; Shrikhande, S.; Midha, K. K. Evidence for the lack of a human metabolic isotope effect of a deuterium analogue of fluphenazine. *Pharmaceu. Res.* **1995**, *12*, 1388−1390.
10. Edom, R.; McKay, G.; Hawes, E.; Midha, K. In *Proceedings of the 37th ASMS Conference on Mass Spectrometry and Alled Topics*; Miami Beach, Florida, 1989; pp 684−685.
11. Hadad, S.; Edom, R.; McKay, G.; Hawes, E. M.; Midha, K. K. Simultaneous measurement of fluphenazine and [H-2(4)]-fluphenazine in plasma using solids probe tandem mass spectrometry. *J. Mass Spectrom.* **1995**, *30*, 849−856.
12. Rowland, M.; Tozer, T. *Clin. Pharmacokinet. Concepts Applications*, 2nd ed.; Lea & Febiger: Philadelphia and London, 1989.
13. Midha, K. K.; Hawes, E. M.; Hubbard, J. W.; Korchinski, E. D.; McKay, G. Variation in the single dose pharmacokinetics of fluphenazine in psychiatric patients. *Psychopharmacology* **1988**, *96*, 206−211.


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