Studies on the Mechanism of Absorption of Depot Neuroleptics: Fluphenazine Decanoate in Sesame oil

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Purpose. The purpose of the present study was to investigate the pharmacokinetic characteristics of fluphenazine (FLU) and its decanoate (FLU-D) after intravenous and intramuscular administration to dogs.

Methods. A group of four beagle dogs was used in all intravenous and intramuscular experiments, with washout periods of no less than three months between doses.

Results. After intravenous FLU-D, the pharmacokinetics of the prodrug (mean ± SD) were as follows: Clearance (CL) 42.9 ± 6.3 L/h; terminal half-life ($t_{1/2}$) 3.5 ± 0.8 h; volume of distribution (Vd) 216 ± 61 L. The fractional availability of FLU was 1.0 ± 0.2. After intravenous FLU, the volume of distribution of FLU (51 ± 17.8 L) was some 4 fold less than that of the prodrug. Simulations (Stella II) suggested that the rate limiting step was slow formation of FLU from the prodrug in the tissue compartment. After intramuscular FLU-D in sesame oil, the apparent $t_{1/2}$ of FLU was 9.7 ± 2.0 days whereas after intramuscular FLU base in sesame oil, the apparent $t_{1/2}$ was only 7.7 ± 3.4 h showing that the absorption of FLU itself from the intramuscular site and proximal lymph nodes is relatively rapid.

Conclusions. The rate limiting step after intramuscular FLU-D appeared to be the slow partitioning of the prodrug out of the sesame oil at the injection site and in proximal lymph nodes.

KEY WORDS: fluphenazine decanoate; prodrug; fluphenazine; pharmacokinetics; single dose.

INTRODUCTION

Fluphenazine decanoate (FLU-D), a long-acting injectable phenothiazine ester, is widely used in the maintenance treatment of schizophrenia and other forms of psychosis. It is formulated as an oil based intramuscular "depot" injection with which patients are typically injected once every 2–3 weeks (1). After intravenous administration of fluphenazine (FLU) in human, the disposition half-life was about 13 h (unpublished data), whereas after intramuscular administration of FLU-D in sesame oil, the terminal half-life of FLU was 7–10 days (2,3). In the latter case, the pharmacokinetics of FLU are said to be rate limited by absorption of FLU from the depot, although the absorption of FLU-D and the formation of FLU are related, but are not governed by identical pharmacokinetic processes. Moreover, enzyme-mediated hydrolysis of FLU-D can occur in various parts of the body including the intramuscular injection site, the lymphatic system, and blood (4). Hydrolysis mediated by the ubiquitous esterases is also likely to occur in a variety of other tissues into which the prodrug might be distributed after presystemic absorption. Recently, FLU-D has been found in the plasma of schizophrenic patients under maintenance therapy (5), although concentrations of the prodrug were extremely low and at present, there are no pharmacokinetic data on the ester. Thus, impact of the pharmacokinetics of the prodrug on those of fluphenazine remains to be established.

The goal of the present study was to understand the mechanism of absorption of the depot by investigation of the pharmacokinetic characteristics of both the drug (FLU) and the prodrug (FLU-D) after intravenous and intramuscular administrations to dogs. To the best of our knowledge, this is one of the few studies to quantify both plasma FLU and FLUD and the first to examine the pharmacokinetic relationship between the formation of FLU and the absorption of its prodrug.

MATERIALS AND METHODS

Chemicals and Reagents

FLU·2HCl was purchased from Sigma Co. (U.S.A.). FLU-D dissolved in sesame oil (100 mg/mL) was obtained from Bristol Meyers Squibb (Montreal, Canada). 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 and the research adhered to the standards of the Canadian Council on Animal Care and the "Principles of Laboratory Animal Care" (NIH publication #85-23, revised 1985). Four pure bred female Beagle dogs were used, ranging in weight (initially) from 6.2 to 8.4 kg (mean 7.18 ± 0.93 kg). The dogs tended to gain weight during the course of the experiments which took approximately 18 months to complete. Therefore the animals were re-weighed before each xenobiotic treatment. The following series of four treatments were conducted with the four dogs with a washout period of at least 3 months between treatments: (i) FLU·2HCl dissolved in water (25 mg mL^{-1}, equivalent to 0.63 mg kg^{-1} of fluphenazine base) was injected slowly into the cubital vein. (ii) A solution of FLU-D in ethanol (25 mg mL^{-1}, equivalent to 0.74 mg kg^{-1} FLU base) was injected slowly into the cubital vein. (iii) A solution of FLU-D in sesame oil (100 mg mL^{-1}) was injected intramuscularly into the semimembranous and semitendinous groups of muscles on both left and right sites of each of four dogs at the dose of 10 mg kg^{-1}. (iv) FLU base in sesame oil (25 mg mL^{-1}) was injected intramuscularly in the same manner as (iii) at the dose of 1 mg kg^{-1}. All injections were administered by a competent veterinary technician.

Serial venous blood samples (8 mL) were drawn as follows: (i) after intravenous FLU·2HCl, at 0 (predose), 1, 5, and 10 min. and 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 48 h; (ii) after intravenous dosing of FLU-D, at 0 (predose), 5 and 10 min. and 0.5, 1, 2, 4, 6, 12, 24, 36, 48, 72, 96, 148, 240, 336 h; (iii) following intramuscular dosing of FLU-D, daily for the first 5 days and then every second day until the end of the third week. Thereafter, blood samples were drawn weekly until the end of the sixth week. (iv) After intramuscular injection of FLU base

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in sesame oil, at 0 (predose), 10 min. and 0.5, 1, 2, 4, , 8, 12, 24, 36, 48, 72 h. Each blood sample was centrifuged immediately after collection, and the separated plasma was stored at −20°C until analysis.

Analysis of FLU and FLU-D in Plasma

Plasma level analyses were carried out by a highly sensitive HPLC procedure with coulometric detection (5). The limits of quantification of FLU and FLU-D were 0.25 ng mL^{-1} for each analyte with coefficients of variation less than 11%. Quality control samples were prepared by adding known amounts of FLU or FLU-D to separate aliquots of blank plasma. The quality control samples were stored with the test samples at −20°C and analyzed blindly at the same time as the test samples to establish sample stability and assay accuracy and precision.

Pharmacokinetic Analysis

The plasma concentrations versus time data for both FLU-D and FLU were analyzed by noncompartmental methods (6). The maximum plasma concentration (C_{max}) and time to reach maximum plasma concentration (t_{max}) were obtained directly from the raw data. In the four drug treatments, the terminal phase rate constant (λ) for both FLU-D and FLU was determined by linear regression of the terminal phase of the plasma concentration versus time curve. The corresponding half-life ($t_{1/2}$) was determined by the expression $0.693/\lambda$. The area under plasma concentration time curve (AUC_{last}) up the last quantifiable concentration (C_{last}) was determined by the linear trapezoidal rule and the area was extrapolated to infinite time (AUC) by addition of the quotient of C_{last} and λ. The AUCs were expressed as nmol mL^{-1} h^{-1} in order to calculate the fractional availability of FLU.

Clearances (CL) and apparent clearances (CL_{ap}) were calculated from the appropriate molar dose and AUC expressed in terms of nmol·$h^{-1}$$mL^{-1}$. Apparent volumes of distribution (Vd) were calculated as quotients of CL and λ. The fraction of fluphenazine decanoate absorbed as intact prodrug (F) was calculated as the quotient of CL (after intravenous prodrug) and CL_{ap} (after intramuscular prodrug). Fractional availabilities (F_m) of fluphenazine after administration of prodrug were estimated as the quotients of appropriate values of CL and CL_{ap}.

Compartmental pharmacokinetics were examined by means of Topfit. Simulations were carried out on a Power Macintosh 7200/90 computer by means of the Stella II program (High Performance Systems Inc., Hanover, NH). This program incorporates automatically appropriate differential equations for the various processes, rate constants for which were set as follows: K12 FLU = 0.165; K12 FLU-D = 0.06; K13 FLU-D = 0.05; K1H FLU-D = 0.07; K21 FLU = 0.194; K21 FLU-D = 0; K31 FLU-D = 0; K32 FLU-D = 0.001; Kel FLU = 0.1155; Kf FLU in C1 = 0.03; Kf FLU in C2 = 0.016; Kf Flu in Liver = 0.11.

RESULTS

Plasma concentrations versus time profiles of FLU and/or FLU-D are shown in Figures 1 and 2. Some mean pharmacokinetic parameters are summarized in Table 1. In the estimations of AUC, the area in the tail amounted to <2% of AUC_{last} for all individual plasma concentration versus time profiles. Following intravenous administration of FLU (Figure 1), the mean terminal $t_{1/2}$ for FLU was 6.0 ± 1.5 h and the mean clearance of FLU was 5.8 ± 1.0 L h^{-1}

Following intravenous injection of FLU-D ester (Figure 1), the prodrug rapidly disappeared from plasma with a mean terminal $t_{1/2}$ of 3.5 ± 0.8 h. The clearance of the prodrug was 42.9 ± 6.3 L h^{-1}, about 8 fold higher than that of FLU, while the apparent volume of distribution of the prodrug was 216 ± 61 L suggesting FLU-D had more extensive distribution/tissue binding than FLU. The mean apparent terminal half-life of FLU estimated from the terminal phase was 43.3 ± 6.6 h. The apparent clearance of FLU after intravenous administration of FLU-D was almost identical to the clearance of FLU after intravenous dosing of FLU which suggests that FLU was cleared from the body in a similar manner in both cases. Moreover, the mean fraction of prodrug converted into FLU after intravenous dosing with FLU-D was unity, indicating that the prodrug was metabolized completely to FLU.

After intramuscular administration of FLU base in sesame oil (Figure 2), the time to reach maximum plasma concentration (2.1 ± 1.4 h) corresponded to relatively high maximum plasma concentrations of the drug (124.1 ± 44.6 ng/mL) and the mean apparent terminal half-life (7.7 ± 3.4 h) was similar to that observed after intravenous administration of FLU (Table 1). In contrast, after intramuscular administration of the decanoate ester in sesame oil (Figure 2), the Cmax of unchanged FLU-D was relatively low (2.54 ng mL^{-1}), but the prodrug disappeared very slowly from plasma such that it was possible to measure plasma FLU-D concentrations for more than 400 h after dosing. The mean apparent terminal half-life of FLU-D was 151.2 h (Table 1) which is 43× longer than the terminal half-life of FLU-D following intravenous dosing with the prodrug. The apparent clearance of FLU-D (144 ± 26.5 L h^{-1}) was 3 fold greater than that after intravenous dosing of the prodrug (43 L h^{-1}). The fractional availability of FLU-D after intramuscular injection was estimated as 0.3 ± 0.05. The formation of plasma FLU after intramuscular administration of its prodrug was very slow as indicated by a mean tmax of 108.0 ± 41.6 h. The C_{max} of plasma FLU ranged from 9.46 to 14.42 ng mL^{-1} with a mean of 11.87 ng mL^{-1}.

DISCUSSION

To the best of our knowledge, the present study in beagle dogs was the first to describe pharmacokinetic relationships between fluphenazine and its decanoate ester after (separate) intravenous administrations of drug and prodrug. Fluphenazine exhibited a rather short terminal half-life (6.0h), but a relatively large volume of distribution (51L) after intravenous injection, which suggests that tissue binding plays an important role in the elimination of the drug. In comparison, however, the phenomenon is even more marked in the disposition of the prodrug which exhibited a mean terminal half-life of only 3.5 hours, and an enormous mean volume of distribution of 216L. These data show that the relatively long apparent terminal half-life of fluphenazine (42.9 h) after intravenous administration of the prodrug (Figure 1) is not rate limited by elimination, but by formation of fluphenazine from the prodrug in various body tissues. Earlier *in vitro* data provided support for this interpretation in that the decanoate ester was hydrolyzed relatively slowly

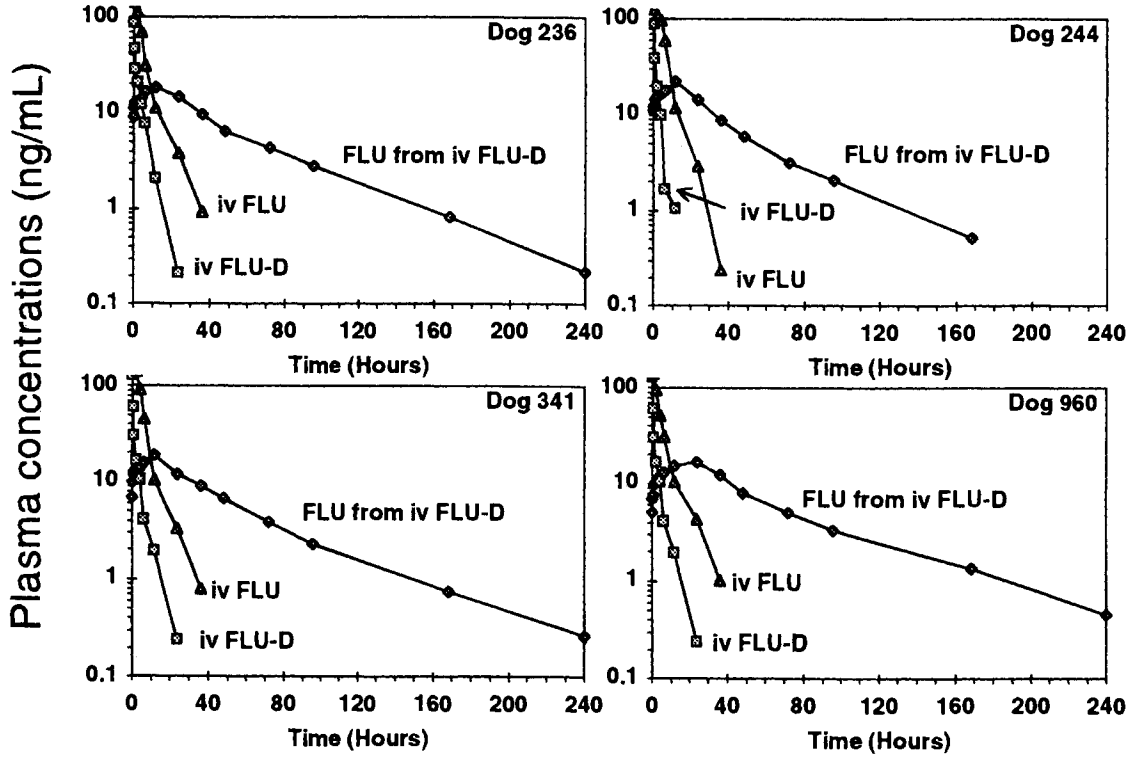

Fig. 1. Plasma concentration versus time profiles in four dogs after (separate) intravenous administrations of (i) FLU 2HCl in water (triangles) and, after a washout period of at least 3 months, (ii) FLU-D in ethanol (squares). Concentrations of FLU arising from FLU-D are depicted as diamonds.

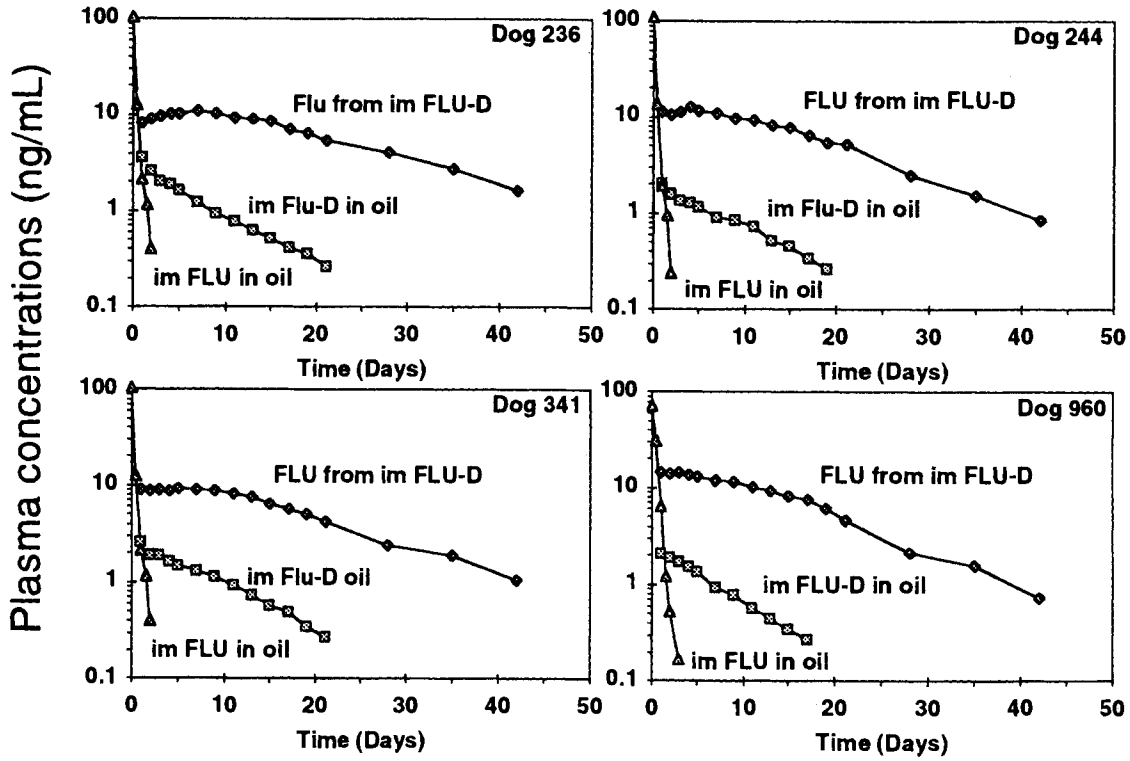

Fig. 2. Plasma concentration versus time profiles in four dogs after (separate) intramuscular administrations of (i) FLU base in sesame oil (triangles) and, after a washout period of at least 3 months, (ii) FLU-D in sesame oil (squares). Concentrations of FLU arising from FLU-D are depicted as diamonds.

Table 1. Summary of Non-compartmental Pharmacokinetics of Fluphenazine and Fluphenazine Decanoate

Treatment	Fluphenazine				Fluphenazine decanoate			
	CL (L/h)	$t_{1/2}$ (h)	V_d (L)	F_m[a]	CL (L/h)	$t_{1/2}$ (h)	V_d (L)	F[b]
Intravenous FLU·2HCl[c]	5.8 ± 1.0	6.0 ± 1.5	51.0 ± 17.8	—	—	—	—	—
FLU-D[d]	5.9 ± 0.7[e]	43.3 ± 6.6	—	1.0 ± 0.2	42.9 ± 6.3	3.5 ± 0.8	216 ± 61	—
Intramuscular FLU[f]	10.9 ± 1.9[e]	7.7 ± 3.4	118.8 ± 42.7	—	—	—	—	—
FLU-D[f]	8.8 ± 0.7[e]	233 ± 49	—	0.7 ± 0.03	144 ± 27[e]	151 ± 17	—	0.3 ± 0.05

[a] Fm of FLU after administration of prodrug were estimated as the quotients of appropriate values of CL and CL_{ap}.
[b] F of FLU-D was estimated as CL/CL_{ap}.
[c] In water for injection.
[d] In ethanol.
[e] CL_{ap}.
[f] In sesame oil.

in dog (or human) plasma but much more quickly in dog muscle homogenates (4).

A model (Figure 3) was developed in order to explore the pharmacokinetic processes involved in the disposition of FLU-D and the formation of FLU after intravenous administration of the prodrug. The kinetics of fluphenazine in the simulations were described by a two compartment model since the drug was observed to fit a two compartmental model (Topfit) after intravenous injection of FLU in these dogs. Thus experimentally determined mean values for K12 FLU, K21 FLU, and Kel FLU were used in the simulation.

The situation with FLU-D was more complicated because the model must provide mechanisms for both rapid production of FLU and sustained plasma levels of the drug. Since *in vitro* data have indicated a slow rate of hydrolysis of FLU-D in dog plasma (4), a liver compartment was included in the model in order to provide for a rapid production of FLU and relatively high plasma concentrations of the drug at early time points. FLU-D was also lost from plasma by partitioning into a well perfused tissue compartment (C2) and a poorly perfused (fatty) deep compartment (C3). The rate constants for these various processes (Kf FLU in C1, K1H FLU-D, K12 FLU-D, and K13

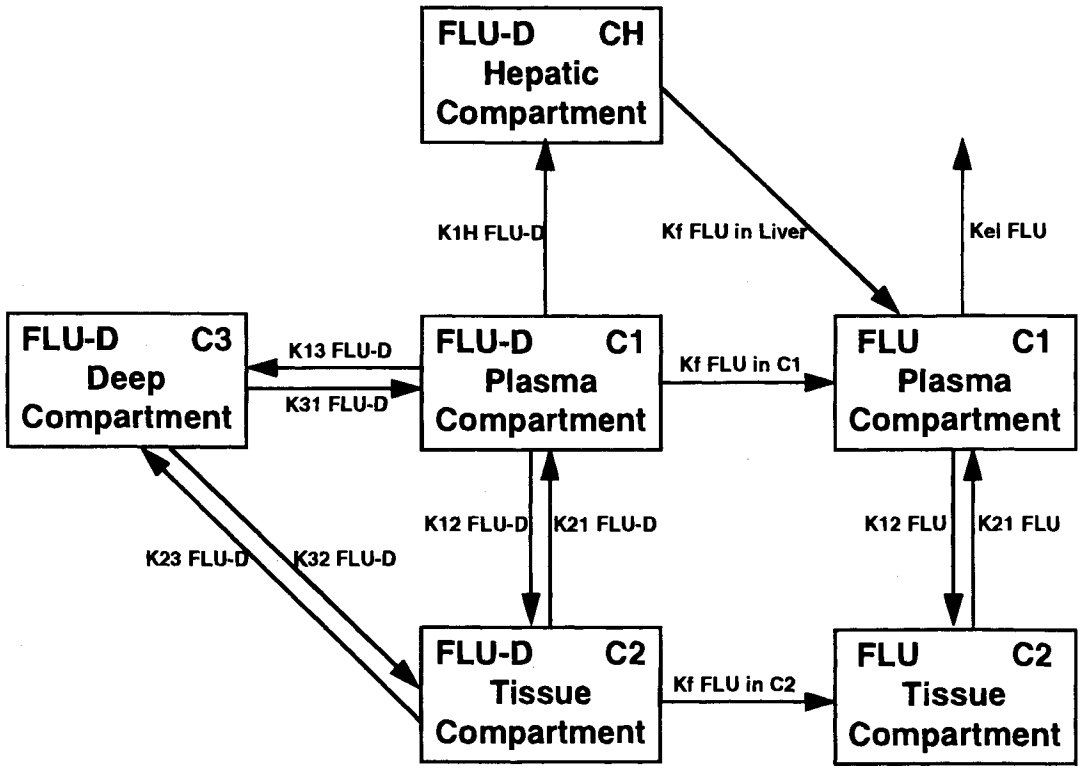

Fig. 3. A pharmacokinetic model for the disposition of FLU-D and the formation of FLU after iv administration of FLU-D. For clarity FLU-D and FLU are shown in separate boxes for compartments C1 and C2.

FLU-D) were determined by trial and error in such a manner that their sum was equal to the observed mean elimination rate constant of FLU-D. Inclusion of the fatty deep compartment (C3) was essential in view of the huge Vd, and the highly lipophilic nature of the prodrug. Thus FLU-D was sequestered in C3 in such a manner that 240 h after intravenous administration, the last sample with quantifiable analytes, 19% of the dose is predicted by the model to remain in the deep compartment. The slow release of prodrug from the deep compartment and subsequent hydrolysis in more highly perfused tissues would lead to prolonged appearance in plasma of extremely low concentrations of FLU which may well be below the lower limit of quantitation of the assay. Consequently, the present results are not in contradiction with the finding that the area in the tail was less than 2% of AUC_{last}, but are in keeping with the notion that the observable pharmacokinetics of highly lipophilic drugs tend to be limited by assay sensitivity and experimental design (7). The rate limiting step in the observed apparent elimination profile of FLU, however, was its slow formation from the prodrug in tissue compartment C2.

Figure 4 shows that the model provided a reasonable fit of the observed data and also provided a mechanism for the appearance of extremely low plasma concentrations of FLU over a prolonged period of time, as observed in patients after cessation of treatment with FLU-D (8). The simulations were also consistent with the hypothesis that FLU-D distributes rapidly into fatty tissues following intravenous administration, and that slow release of the prodrug from these "deep pools" then provides a ready source of substrate for the ubiquitous easterases in the body.

The impact of the decanoate ester group on the intramuscular pharmacokinetics is dramatically illustrated in Figure 2. After intramuscular injection of FLU base in sesame oil, the terminal half-life of the drug was comparable to that obtained after iv administration of FLU, whereas the intramuscular half-life of FLU-D was 43 fold longer than the corresponding intravenous value. Moreover, the apparent half-life of FLU after intramuscular FLU-D was 30 fold longer than the value obtained after intramuscular FLU in sesame oil. These data suggest that the highly lipophilic prodrug partitions out of the sesame oil into surrounding tissues at a markedly slower rate than does FLU. Earlier studies have shown that the nature of the oil also has a big impact on the pharmacokinetics of the drug. In one such study, the plasma concentrations of perphenazine were lower but more sustained after administration of perphenazine decanoate in sesame oil compared with the levels obtained when the same two patients were treated with perphenazine decanoate in viscoleo which is lighter than sesame oil (9). The half-life for disappearance of sesame oil from intramuscular injection sites in rats was shown to be about 9 weeks (10). Hydrolysis of FLU-D at intramuscular injection sites has been shown to be slow (11,12), probably because the oil protected the ester from exposure to esterases. Recently, the involvement of lymph nodes has been demonstrated in the absorption of FLU-D from intramuscular oily depot sites. *Ex vivo* experiments in rats has demonstrated high concentrations of FLU-D and FLU in iliac and hypogastric lymph nodes after intramuscular injection of FLU-D in sesame oil into the femoral muscles. For example, concentrations of fluphenazine in the proximal lymph nodes were 216× higher than those in plasma at 1 day post dose, and 1560× higher at 21 days post dose. Moreover, both drug and prodrug were measurable in these lymphatic tissues 28 days post dose when neither was detectable in plasma. These (unpublished) data suggest that the lymphatic system is involved in the presystemic absorption of FLU after intramuscular administration of its decanoate ester in sesame oil. A pharmacokinetic model (Stella II) for intramuscular administration of FLU-D in oil has been developed for use with intrinsic data obtained after intravenous administration. Use of the model, however, requires quantitative data on the role of the lymphatic system in the presystemic input of FLU and FLU-D. In future experiments, we shall attempt to obtain such data from acute experiments in dogs in which lymph from the thoracic duct will be sampled after IM administration of the prodrug in sesame oil. The present data suggests that the long half-life of FLU in plasma after intramuscular FLU-D may be rate limited either by the extremely slow release of prodrug from oil at the injection sites and proximal lymph nodes, or by the slow formation of FLU from prodrug seeping slowly out of deep (fatty) compartments.

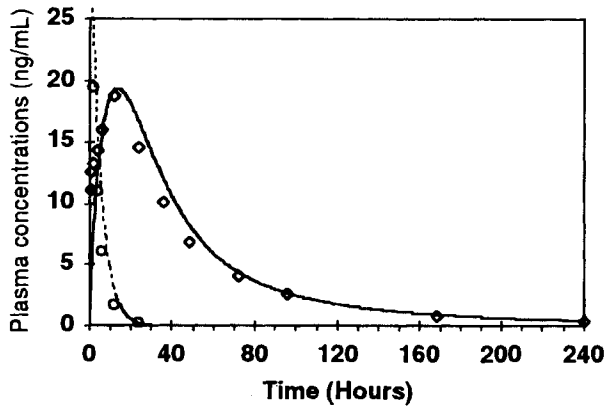

Fig. 4. Simulations (Stella II) of mean plasma levels of FLU (solid line) and FLU-D (dashed line) versus time profiles after iv administration of FLU-D to dogs: Open diamonds, FLU data points; open circles FLU-D data points.

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