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## The determination of the steady-state pharmacokinetic profile of fluphenazine decanoate by gas chromatography/mass spectrometry detection

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This study uses the highly sensitive method of gas chromatography/mass spectrometry to compare the basic steady-state pharmacokinetic parameters of two fluphenazine decanoate formulations. Sixteen stable outpatients participated in a two-way crossover design study of the bioavailability of a new formulation of FPZ Dec, i.e., 10 mg/ml, to the standard 25 mg/ml formulation. When compared to a 1 ml injection of the standard formulation (25 mg/ml) over a two-week, steady-state period, we found bioequivalence as evidenced by similar mean areas under the curve (hrs × ng/ml). We did find that the injection volume of the same dose (2.5 ml of a 10 mg/ml formulation) results in a statistically significantly higher maximum serum level of parent fluphenazine. A tendency toward faster time to peak level was observed with the 10 mg/ml formulation but the difference was not statistically significant. Both of these differences are considered too small to be clinically significant. In a subgroup of 10 patients, pre-injection serum fluphenazine levels correlated significantly (Pearson  $r = 0.78$ ,  $p < 0.05$ ) with serum prolactin levels.

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*Key words:* Fluphenazine decanoate; Injection volume; Pharmacokinetic; (Schizophrenia)

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### INTRODUCTION

The purpose of this report is to describe the steady-state pharmacokinetic parameters of fluphenazine decanoate (FPZ Dec) using the highly sensitive method of gas chromatography/mass spectrometry (GC-MS) and to relate the findings to the formulation used and serum prolactin levels. Knowledge about the basic steady-state pharmacokinetic parameters of FPZ Dec is important because this depot neuroleptic preparation is used on a maintenance basis. Such knowledge will aid in the understanding of the relationship of serum levels to

clinical outcome (Marder et al., 1986). Previous studies of the steady-state serum pharmacokinetic profile of parent fluphenazine over the course of an injection interval using fixed-dose designs have utilized gas liquid chromatography (GLC) (Nasrallah et al., 1978; Chang et al., 1985) and radioimmunoassay (RIA) (Altamura et al., 1985; Glazer, 1988). Although these studies describe plasma levels occurring at relatively low ranges, i.e. 0.2–16.0 ng/ml, they are less clear about basic pharmacokinetic parameters. For example, only one study (Glazer, 1988) has reported group data pertaining to area under the serum formulation time curve (AUC), maximum and minimum serum formulations (C<sub>max</sub> and C<sub>min</sub>), time to reach C<sub>max</sub> (T<sub>max</sub>) and the Fluctuation Index. In that study, pharmacokinetic parameters were estimated with an RIA method that suffered from the prob-

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lem of cross-reactivity between the parent fluphenazine and several metabolites. Since no previous study has utilized the more sensitive and specific method of GC-MS, we decided to characterize with this analytical method the two-week steady-state pharmacokinetic profile of parent fluphenazine.

The steady-state pharmacokinetic profile of a depot neuroleptic formulation is a function in part of the length of the injection interval and the concentration and volume administered. To date there are few if any data clarifying the effect of concentration and volume on fluphenazine's pharmacokinetic profile. We had the opportunity to examine this question via a study that was designed to compare the bioavailability of a new formulation of FPZ Dec to the standard formulation. The study design kept the injection interval and dose constant while varying the concentration and volume.

The measurement of the correlation of serum neuroleptic levels with serum prolactin levels may enhance the understanding of the clinical effects of these medications (Brown, 1984). For example, the understanding of the relationship between fluphenazine blood levels and clinical response is limited in part because there are few data pertaining to its biological activity and that of its metabolites. Since the GC/MS method differentiates parent fluphenazine from its metabolites, the correlation of this measurement with serum prolactin would help to ascertain the parent compound's central biological activity.

## MATERIALS AND METHODS

### *Patients*

Patients were selected from the outpatient clinics of the Connecticut Mental Health Center in New Haven, Connecticut, and the Brentwood VA Hospital in Los Angeles, California. These patients were participants in a collaborative study among two facilities and the Squibb Institute for Medical Research. The study compared in a two-way cross-over design the bioavailability of a new formulation of FPZ Dec, i.e., 10 mg/ml, to the standard 25 mg/ml formulation.

To be eligible for the study, patients had to be 18–59 years of age, had to have a DSMIII diagno-

sis compatible with chronic neuroleptic maintenance, and had to be receiving FPZ Dec in a dose of 25 mg every 2 weeks for at least 3 months. Patients were excluded if they had been receiving medication other than antiparkinsonian agents, if they were actively abusing substances within 2 months prior to the study, and if they had medical illnesses that would affect the pharmacokinetics of the study drug, e.g. liver or kidney disease. Monthly urine samples were screened for drugs of abuse, and patients were dropped from the study if these tests were positive. A total of 39 patients were enrolled. Of these, 16 completed the study and provided evaluable data — 17 were dropped for protocol violations, unstable clinical condition or personal reasons; in 6 patients who finished the study the data were incomplete because of technical problems with the assay (inadequate sample volume, etc.). The randomization of the two formulations was preserved in spite of this dropout rate.

Following the pharmacokinetic study, we decided to measure serum prolactin levels at the pre-dose timepoint in 10 patients from the New Haven cohort and from whom we had enough serum available for analysis. Seven of these 10 patients had completed the pharmacokinetic study.

### *Procedure*

After signing informed consent and undergoing a complete medical evaluation, patients were randomly assigned to receive 25 mg doses of FPZ Dec every 2 weeks over a 14-week period. The drug was administered in one of two formulations: 1 ml of the 25 mg/ml formulations, or 2.5 ml of the 10 mg/ml formulation. Seven doses were employed to assure steady-state conditions (Midha et al., 1987). Kinetic parameters were measured after the seventh dose, i.e., weeks 13 and 14 of the administration of each formulation. Following these initial 14 weeks, the alternate formulation was given over the subsequent 14-week period without a washout period. Drug was administered intramuscularly (using Z-track injection technique) in the same anatomical site, the deltoid muscle, in the morning.

Blood samples were obtained prior to the 6th and 7th (time 0) doses, then, 2, 4, 6, 24, 48, 72, 168, 240 and 336 hr following the 7th dose. These time points were selected based on the results of a prior study (Glazer, 1988). The blood was stored

at room temperature in the dark for at least 45 min after collection, then it was centrifuged and frozen and protected from exposure to ultraviolet and visible light.

#### *Assay method*

An automated, capillary column gas chromatography method utilizing electron impact mass selective detection was developed at the Squibb Institute for Medical Research for the determination of fluphenazine in the serum (Jemal et al., 1987). This analytical method utilizes a simple and reproducible purification scheme, incorporated deuterated fluphenazine as an internal standard and a stable tert-butyl dimethylsilyl derivative for high resolution gas chromatography coupled to an electron impact mass-selective detector. The limit of quantitation is 50 pg/ml of fluphenazine in serum.

Serum prolactin was determined by a double antibody radioimmunoassay for which the within and between assay coefficients of variation were 6.7% and 8.6%.

#### *Data analysis*

The following steady-state pharmacokinetic parameters were determined for each patient: area under the serum concentration time curve (AUC), maximum serum concentration (C<sub>max</sub>), time to reach C<sub>max</sub> (T<sub>max</sub>) and the Fluctuation Index, calculated as (C<sub>max</sub>-C<sub>min</sub>)/(AUC/dose interval) where C<sub>min</sub> is the minimum concentration and the dose interval was 336 hr (Gibaldi, 1984). A general linear modelling procedure accounting for treatment, patient and sequence was used to compare these parameters for the two formulations. General linear modelling procedures were also used to predict serum prolactin levels.

## RESULTS

Of the 16 patients with evaluable data at the end of the study, 13 (81%) were men, and 10 (63%) were white. Their mean age was 40 yr (range 28-59), mean height 172 cm (range 152-185) and mean weight 79 kg (range 57-100). Serum prolactin measures were obtained from a sub-group of 10 patients (including 3 noncompleters) from the New Haven cohort. The characteristics did not

differ significantly in the subgroup and the 23 patients who did not complete the study.

Figure 1 displays the 2-week mean serum concentrations that we observed. Except for a brief period of time within the first 24 hr after administration, the mean fluphenazine levels for each formulation were relatively constant throughout the 2-week dose interval. The levels observed prior to the sixth dose were similar to the those observed just prior to and 336 hr following the seventh dose, and the data indicated that steady state levels had been reached. Specifically, prior to the sixth injection, seventh injection (0 hr) and 336 hr after the seventh injection the 1.0 ml formulation resulted in mean serum levels of 0.81 (SE=0.09), 0.80 (SE=0.11) and 1.0 (SE=0.13) ng/ml, while the 2.5 ml formulation resulted in mean serum levels of 0.93 (SE=0.18), 0.91 (SE=0.10) and 0.89 (SE=0.11) ng/ml. Generally, the 2.5 ml injection of the new formulation yields slightly higher levels of fluphenazine.

Table 1 compares the 2 week mean ( $\pm$ ) SEM serum values and ranges for the steady-state bioavailability parameters that we observed with the two formulations. The mean AUC for the 10 mg/ml formulation was slightly but not significantly higher than that for the 25 mg/ml formulation (375 vs 341 hr/ng/ml). The mean C<sub>max</sub> value for the 10 mg/ml formulation (1.9 ng/ml) was 21% higher than that for the standard formulation (1.5 ng/ml), and this difference was not statistically significant ( $p < 0.05$ ). The mean C<sub>min</sub> value for the 10 mg/ml formulation (0.8 ng/ml) was slightly higher than the value for the standard formulation (0.7 ng/ml), but this difference was not statistically significant. The mean T<sub>max</sub> was less following the 10 mg/ml formulation (65 hr vs 34 hr) but this difference is not statistically significant. T<sub>max</sub> occurred within 24 hr in 13 of the 16 patients receiving the 10 mg/ml formulation as compared to 11 of 16 patients following the 25 mg/ml formulation. The remaining patients had T<sub>max</sub> values between 72 and 224 hr (10 mg/ml formulation) and between 72 and 336 hr (25 mg/ml formulation). The mean serum level is better characterized by the median T<sub>max</sub> values than the means (4 and 6 hr for the 10 and 25 mg/ml formulations respectively). As expected, the mean of the individual patient C<sub>max</sub>, C<sub>min</sub> and T<sub>max</sub> values are not identical to the value calculated for the average serum concentration shown in Fig. 2. The differ-

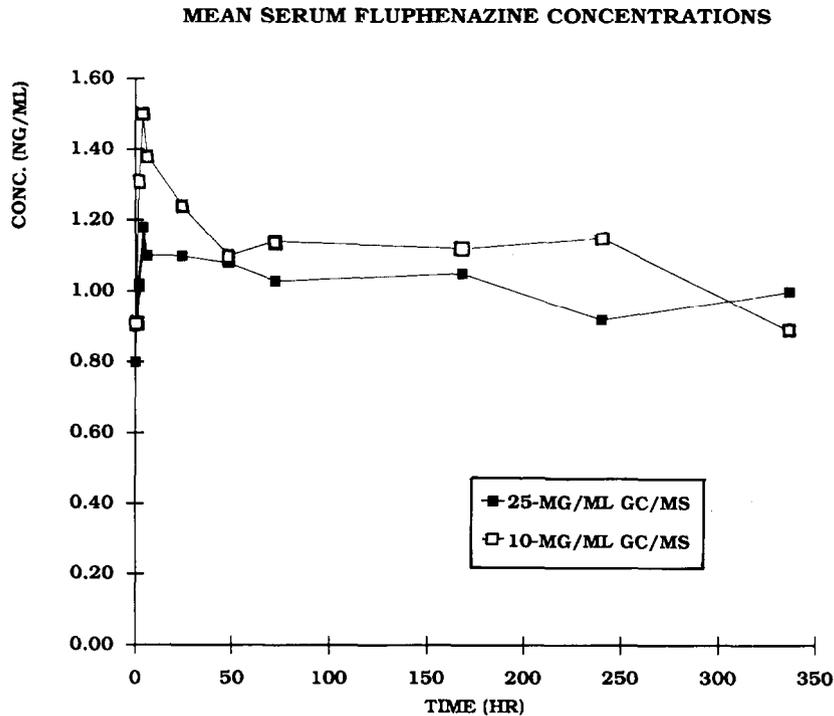


Fig. 1. Mean serum steady-state concentrations of fluphenazine administered in two formulations (see legend) of FPZ Dec over a two week interval.

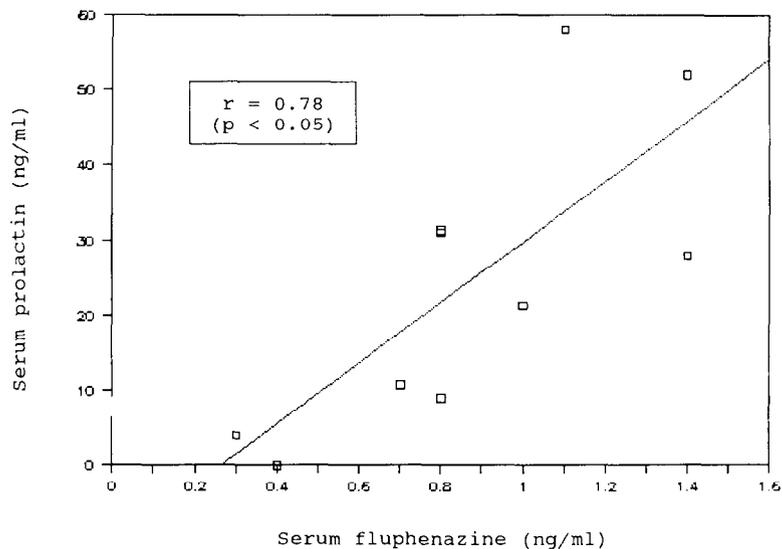


Fig. 2. Scattergram depicting GC/MS-determined serum fluphenazine levels and RIA determined serum prolactin levels obtained just prior to two-week injection interval in a subsample of the study population.

ence is caused by the patient to patient variability of  $T_{max}$ . The mean fluctuation index following the 10 mg/ml formulation was 44% greater

( $1.12 \pm 0.13$  vs  $0.78 \pm 0.07$ ) than the 25 mg/ml formulation, and this difference was statistically significant ( $p < 0.05$ ).

TABLE 1

Individual and mean ( $\pm$ SE) steady-state bioavailability parameters

Patient number	$AUC_{0-336}$ (hr·ng/ml)		$C_{max}$ (ng/ml)		$T_{max}$ (hr)		$C_{min}$ (ng/ml)	
	25 mg/ml	10 mg/ml	25 mg/ml	10 mg/ml	25 mg/ml	10 mg/ml	25 mg/ml	10 mg/ml
101	422.40	661.20	1.50	2.70	6	24	1.20	1.70
102	352.98	255.23	1.50	1.10	6	6	0.79	0.63
105	292.31	276.10	1.40	1.10	4	2	0.67	0.61
108	252.51	341.75	1.10	1.90	4	4	0.60	0.72
109	585.42	616.84	2.70	3.10	24	168	0.59	0.85
111	381.78	539.16	2.10	2.60	336	240	0.55	0.92
112	309.18	246.08	1.50	1.60	168	2	0.57	0.60
116	190.45	243.08	0.87	1.99	4	4	0.39	0.48
201	431.76	569.00	1.80	2.10	2	2	0.61	1.40
206	334.03	186.51	1.30	1.00	4	6	0.70	0.35
207	420.30	515.20	1.70	2.60	72	4	0.95	1.10
208	380.90	251.52	1.60	1.30	336	4	0.75	0.43
209	384.72	241.91	1.80	1.30	48	4	0.62	0.60
215	231.47	263.19	0.90	1.70	4	2	0.57	0.56
218	317.96	599.20	1.10	2.80	2	72	0.69	1.30
224	163.60	188.23	0.61	1.70	24	2	0.21	0.37
Mean $\pm$ SE	341 $\pm$ 26	375 $\pm$ 43	1.5 $\pm$ 0.1	1.9* $\pm$ 0.2	65 $\pm$ 28 <sup>a</sup>	34 $\pm$ 17 <sup>b</sup>	0.7 $\pm$ 0.1	0.8 $\pm$ 0.1
Range	164–585	187–661	0.6–2.7	1.0–3.1	2–336	2–240	0.2–1.2	0.4–1.7

\*Significantly different ( $p < 0.05$ ) from the standard (25 mg/ml) formulation.<sup>a</sup>Median = 6 hr.<sup>b</sup>Median = 4 hr.

The mean serum prolactin level for the 10 patients receiving the 25 mg/ml formulation measured at time 0 of the 2 week pharmacokinetic curve was 24.5 ng/ml (range 3.8–58.1). The results of univariate regression analyses with prolactin levels treated as the dependent variable and age, sex, race, height, weight and time 0 serum fluphenazine levels each treated as independent variables indicated that only the GC-MS assay predicted prolactin levels significantly ( $p < 0.05$ ). Serum prolactin and fluphenazine levels are plotted by individual patient in Fig. 2. The Pearson correlation between serum fluphenazine and prolactin levels was 0.78, ( $p < 0.05$ ).

## DISCUSSION

The study described here is the first to demonstrate the fixed dose, steady-state pharmacokinetic profile of fluphenazine using the GC/MS assay method.

Furthermore, the study is the first to explore the effect of concentration and volume of dose on this pharmacokinetic profile. We are confident that our patients reached steady state after at least 12 weeks of treatment because the fluphenazine levels observed prior to the sixth dose were similar to the those observed just prior to and 336 hr following the seventh dose. Other than an initial peak in fluphenazine levels described by a several other investigators (Jann et al., 1985), mean fluphenazine levels were approximately constant throughout the 2-week dose interval. The relatively constant levels account for the variability of the  $T_{max}$  values.

In comparing the basic pharmacokinetic parameters resulting from the 1 ml (25 mg/ml) and 2.5 ml (10 mg/ml) formulations, we found that the same dose administered in the larger volume resulted in a significantly higher mean maximum serum concentration of fluphenazine ( $C_{max}$ ) and mean fluctuation index. Additionally, the latter formulation resulted in a higher  $C_{min}$  and a shorter mean time

to peak level ( $T_{max}$ ); however, these differences were not statistically significant. If the therapeutic range of fluphenazine falls within the range of 0.2–2.8 ng/ml that has been suggested by some authors (Knudsen, 1985), the magnitude of the differences in the basic pharmacokinetic parameters, e.g., a mean  $C_{max}$  of 1.9 ng/ml vs 1.5 ng/ml, is probably clinically insignificant. Thus, we conclude that the bioavailability of the new formulation of FPZ Dec is clinically comparable to the standard formulation.

The apparent effect of concentration and volume on these parameters deserves some consideration. One possible explanation for our results is that a larger depot reservoir in the intramuscular space with the 10 mg/ml formulation results in a greater surface area exposure to the blood supply and a more rapid delivery to the system, i.e., circulation, hence a higher  $C_{max}$  and smaller  $T_{max}$ .

The question of clinical relevance of serum levels of parent fluphenazine and its active metabolites remains to be elucidated. The association between neuroleptic blood concentration and prolactin levels may shed light on this question. Previous investigations in patients treated with FPZ Dec at steady state (Altamura et al., 1985; Nasrallah et al., 1978; Brown and Silver, 1985) or following discontinuation (Wistedt et al., 1981) have yielded weakly positive associations between the blood neuroleptic level and prolactin. Kitamura et al. (1988) reported a significant ( $r=0.45$  for men and  $0.44$  for women) relationship between Day 0 plasma fluphenazine determined by an RIA and prolactin in 100 patients treated at steady state with varying doses of FPZ Dec. The more definitive GC/MS assay utilized in our study leads to the suggestion of a significant correlation ( $r=0.78$ , ( $p<0.05$ )) between serum parent fluphenazine levels interval and serum prolactin levels. This association is consistent with previous studies and supports the view that parent fluphenazine, among other possible metabolites, has biological activity associated with central dopaminergic function. It would be of interest to know if such a correlation exists for metabolites of fluphenazine such as the 7-hydroxy compound that have been thought to be biologically active. Indeed, Hoffman et al. (1989) reported that the 7-hydroxyl and N-oxide metabolites of fluphenazine hydrochloride demonstrated  $D_2$  and  $\alpha_1$  binding activity while the sulfoxide metabolite did

not. Further studies of associations between fluphenazine levels determined by other types of assay methods and serum prolactin levels would help in the understanding of the validity of these assay methods.

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