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PLASMA FLUPHENAZINE LEVELS BY RADIOIMMUNOASSAY IN SCHIZOPHRENIC PATIENTS TREATED WITH DEPOT INJECTIONS OF FLUPHENAZINE DECANOATE

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1 Using a radioimmunoassay, plasma fluphenazine (FPZ) concentrations were examined in 33 schizophrenic patients during 38 intervals between injections of FPZ decanoate. Doses ranged from 12.5 to 150 mg and intervals from 1 to 5 weeks. At least three blood samples were taken between injections from each subject; also in 26 subjects additional samples were taken during the first 24 h post-injection.

2 FPZ was measurable in all plasma samples.

3 Each injection was followed by a rapid rise in plasma FPZ concentration to a maximum at 1–8 h. The height of this peak varied considerably. Within the next 12–36 h plasma FPZ fell to a level slightly above that found before injection and then remained stable until the next injection, thus confirming the steady release of FPZ from the depot over this period.

4 For the group, dose and mean plasma FPZ levels correlated strongly.

5 Despite this, there was a four-fold variation in plasma FPZ concentration among subjects receiving the same dose.

6 The FPZ level on the last day of an interval between injections was a satisfactory estimate of the mean FPZ level for the interval.

7 In one subject examined in this way, a positive correlation was found ($r = 0.76$) between plasma FPZ (by radioimmunoassay) and plasma prolactin levels.

Introduction

Treatment with depot injections of fluphenazine (FPZ) decanote provides good control of symptoms in most schizophrenic patients and reduces their rate of relapse (Hirsch, Gaind, Rohde, Stevens & Wing, 1973). Nevertheless, while receiving this treatment, as many as 37% of chronic schizophrenics relapse within 2 years of starting treatment and up to 40% show extrapyramidal side-effects (Johnson, 1977). Monitoring the concentration of FPZ in the plasma of these subjects might help lessen the relapse rate and reduce the incidence of extrapyramidal effects. Hitherto, such studies have been prevented by the lack of a satisfactory analytical technique. We have developed a radioimmunoassay (Wiles & Franklin, 1978) which can measure the concentrations of FPZ in the plasma of patients receiving depot preparations. We report here our preliminary findings on plasma FPZ concentrations in patients receiving the drug in a wide range of doses. These add to the information from studies using radioactive FPZ (e.g. Curry, Whelpton, de Schepper, Vranckx & Schiff, 1978). Moreover, comparison of the findings obtained with the two methods augments the evidence about the validity of the radioimmunoassay.

Methods

Subjects and sampling procedure

The subjects were 36 patients diagnosed as schizophrenics by their Consultant Psychiatrist: 25 male and 11 female aged between 23 and 59 years. Before participating in the study, all patients gave fully informed consent. A main group of 33 patients was studied; we also present data from three others who were examined in more detail.

1. *Thirty-three subjects already established on FPZ decanoate by injection* (*Table 1*). In these 33 patients

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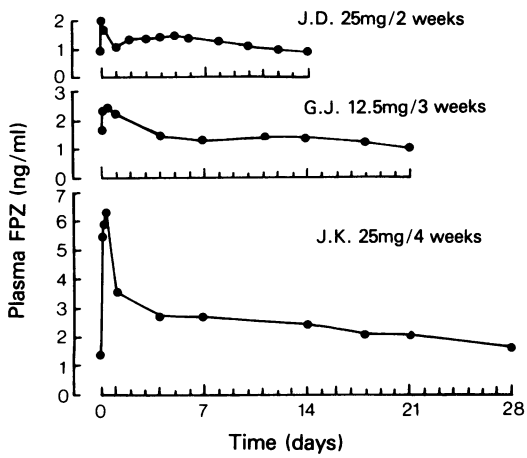

Figure 1 Plasma FPZ 'profiles' after depot injections of FPZ decanoate in three patients.

(23 men and 10 women), 38 intervals between injections were examined; of these all but two intervals followed the third or subsequent injection in a treatment schedule in which dose and interval were unchanged. The two exceptions (8 and 23) followed the second injection of a set regimen. Doses per injection varied between 12.5 and 150 mg and intervals ranged from 1 to 5 weeks, though 27 of the 33 were of 2 weeks. In grouping data, doses are expressed as mg/week as a way of taking account of variations in the interval as well as the dose. Expressed in this manner, doses ranged from 3.125 mg/week to 75 mg/week.

Blood samples for fluphenazine estimation were taken immediately before injection and at intervals up to the day of the next dose. The minimal sampling schedule was: pre-injection and then days 3 and 7 when the interval between injections was 1 week; and weekly if injections were given every 2, 3, 4 or 5 weeks. In many cases additional samples were taken on intervening days.

Blood samples were also taken during the 24 h immediately following an injection from 26 patients given 30 injections. Both the number of samples and the intervals varied. In 13 cases the schedule was: pre-injection (0); then at 0.5, 1, 2, 4 and 8 h post-injection. Following 11 injections only one blood sample was taken at 2 h ($n = 9$) or at 8 h ($n = 2$) post-injection; and following five infections, two or three samples were taken within this period. In one instance, blood samples were taken every 15 min, up to 2.5 h post-injection.

2. *Two subjects receiving FPZ decanoate as their first treatment with a neuroleptic.* Blood samples were taken before and at intervals after the first two doses.

3. *One man established on FPZ hydrochloride and then changed to FPZ decanoate.* This patient was originally taking FPZ hydrochloride 6 mg three times a day by mouth. He then changed to FPZ decanoate 25 mg every 2 weeks. Blood samples were taken weekly, before and 2 h after the morning dose of FPZ hydrochloride and at varying intervals during treatment with the depot preparation.

Method of estimation

Blood samples were taken into lithium heparin. Plasma was separated by centrifugation and stored at about −20°C until assayed. FPZ was estimated by radioimmunoassay. Each sample was assayed in triplicate. Although samples were kept between 2 weeks and several months before assay, unpublished control data have shown no change in plasma FPZ concentration measured by radioimmunoassay during 6 months frozen storage. Intra- and inter-assay precision (CV) were 10 and 12%, respectively. The antibody used cross-reacts strongly with 7-hydroxy FPZ (57%) and 8-hydroxy FPZ (98%) but reacts very weakly with FPZ sulphoxide (0.6%) and fluphenazine decanoate (0.9%). Plasma prolactin was measured using a modification of the homologous double antibody radioimmunoassay described by McNeilly & Hagen (1974). The intra- and inter-assay precision (CV) of this method were 4 and 8% respectively.

Results

Typical plasma FPZ patterns after an injection of FPZ decanoate are shown in Figure 1. Each of these three patients had been receiving doses of fluphenazine decanoate which had remained unaltered for at least 6 months. The injection was followed by a rapid rise in plasma FPZ to a maximum at 1–8 h. The drug concentration then fell within 24–48 h of injection to a concentration slightly above that found before the injection. Thereafter plasma FPZ levels were remarkably stable until after the next injection.

Results from the whole group follow the same pattern. Samples taken within 24 h of 31 injections invariably contained the highest FPZ levels found during the interval between injections. The time at which this peak occurred could be determined on 18 occasions. This varied between 1 and 8 h after the last injection with an average of 3.3 h (s.d. ± 1.8). The height of the post-injection peak also varied considerably (from 1.2 to 12.6 times the level found before the injection). It was on average 3.1 (s.d. ± 2.9, $n = 18$) times the pre-injection level. A 25 mg injection was followed by an increase of between 0.14 and 8.56 ng/ml ($n = 7$).

Table 1 shows plasma FPZ levels for 38 intervals

between injections taken from 33 subjects. In this table the term 'interval mean' plasma FPZ level, indicates the average drug concentration from all plasma samples taken during an interval between injections except those from within 24 h post-injection. Over the dosage range 6.25 to 50 mg/week there is a 3–4 fold variation in this interval mean FPZ level among subjects receiving the same dose. The average coefficient of variation within an interval was 18% (s.d. ± 10.2).

The FPZ level on the last day of an interval (i.e. the sample taken immediately before the next injection) was found to average 88.9% (s.d. ± 12.2) of the interval mean FPZ level (data from 35 intervals). It

Table 1 Plasma FPZ levels during 38 intervals between injections of FPZ decanoate in 33 schizophrenic patients

Interval* number	Sex M/F	Age (years)	Dose (mg/ weeks)	Interval (weeks)	Plasma FPZ (ng/ml) Interval mean† mean s.d.	(n)	CV%	Last day of interval mean s.d.	(n)
1	F	—	3.125	4	1.09 ± 0.16	(5)	14.68	0.92 ± 0.04	(2)
2	F	40	4.16	3	1.49 ± 0.35	(8)	23.49	1.38 ± 0.42	(2)
3	M	55	4.16	3	1.08 ± 0.18	(5)	16.67	0.86 —	(1)
4	M	42	5.0	5	0.67 ± 0.38	(9)	56.72	0.75 ± 0.53	(2)
5	M	54	6.25	4	2.31 ± 0.40	(8)	17.32	1.71 ± 0.26	(2)
6	M	55	6.25	4	2.34 ± 0.72	(8)	30.77	1,50 ± 0.20	(2)
7	F	37	6.25	2	2.06 ± 0.16	(3)	7.76	2.10 ± 0.20	(2)
8A	M	36	6.26	2	1.22 ± 0.10	(3)	8.19	1.16 ± 0.04	(2)
9B	M	38	6.25	2	0.90 ± 0.19	(3)	21.11	0.81 ± 0.13	(2)
10B	M	38	6.25	2	0.85 ± 0.39	(3)	45.88	0.63 ± 0.12	(2)
11	M	47	8.33	3	2.57 ± 0.61	(4)	23.69	2.19 ± 0.55	(2)
12	F	52	12.5	2	3.48 ± 0.21	(10)	6.03	3.26 —	(1)
13	M	55	12.5	2	3.16 ± 0.68	(6)	21.52	3.41 ± 0.94	(2)
14	M	48	12.5	2	2.64 ± 0.34	(6)	12.88	— —	—
15C	M	49	12.5	2	2.59 ± 0.63	(5)	24.32	1.99 —	—
16	F	44	12.5	2	2.33 ± 0.30	(4)	12.88	2.13 ± 0.10	(2)
17	M	50	12.5	2	2.22 ± 0.55	(4)	24.77	1.73 —	—
18A	M	36	12.5	2	1.43 ± 0.17	(4)	11.89	1.29 ± 0.10	(2)
19	M	27	12.5	1	1.28 ± 0.18	(3)	14.06	— —	—
20C	M	50	12.5	2	1.21 ± 0.21	(11)	17.35	0.93 ± 0.04	(2)
21D	M	38	12.5	2	1.11 ± 0.12	(4)	10.80	1.01 ± 0.04	(2)
22D	M	38	12.5	2	1.09 ± 0.13	(4)	11.91	0.98 ± 0.00	(2)
23	M	36	12.5	2	1.02 ± 0.07	(4)	6.75	0.96 ± 0.01	(2)
24	M	30	12.5	1	0.88 ± 0.16	(3)	18.18	0.80 ± 0.10	(2)
25	F	42	18.75	2	2.61 ± 0.58	(5)	22.22	2.44 ± 0.28	(2)
26	F	39	25	2	7.34 ± 0.63	(6)	8.58	6.90 ± 0.71	(2)
27E	M	59	25	1	4.69 ± 0.84	(3)	17.91	4.37 ± 0.89	(2)
28E	M	59	25	1	4.65 ± 0.62	(3)	13.36	4.42 ± 0.68	(2)
29	F	31	37.5	2	13.32 ± 0.20	(5)	15.02	16.40 —	—
30	F	32	37.5	2	3.64 ± 0.70	(5)	19.23	3.52 ± 0.31	(2)
31	M	36	50	2	16.76 ± 3.58	(4)	21.36	— —	—
32	M	23	50	2	13.75 ± 4.35	(6)	31.64	9.40 ± 2.88	(2)
33	M	49	50	2	11.31 ± 0.54	(5)	4.77	10.72 ± 0.11	(2)
34	M	23	50	2	7.41 ± 1.64	(6)	22.13	7.16 ± 3.22	(2)
35	M	29	50	2	6.88 ± 1.36	(6)	19.77	5.64 ± 1.29	(2)
36	M	42	50	2	5.10 ± 0.85	(5)	16.67	3.92 —	—
37	M	46	62.5	2	11.35 ± 1.38	(6)	12.16	9.60 ± 0.45	(2)
38	M	48	75	2	9.79 ± 1.78	(6)	18.18	7.92 ± 0.79	(2)

* The five patients in whom two intervals were studied are indicated by an alphabetical suffix on the interval number; with these exceptions, each interval number represents a different patient.
† Excludes first 24 h.

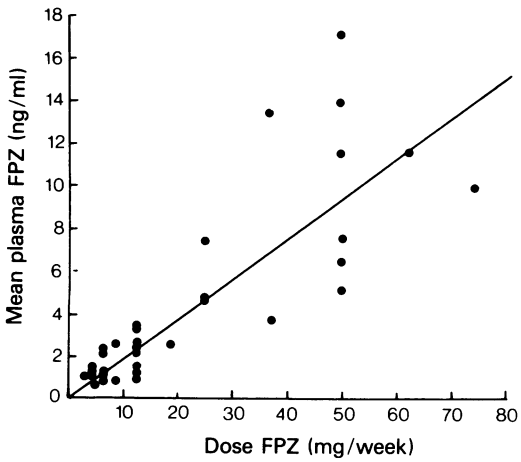

Figure 2 Correlation between dose of FPZ decanoate and plasma FPZ levels (38 intervals in 33 subjects) $r = 0.84$; $P < 0.001$.

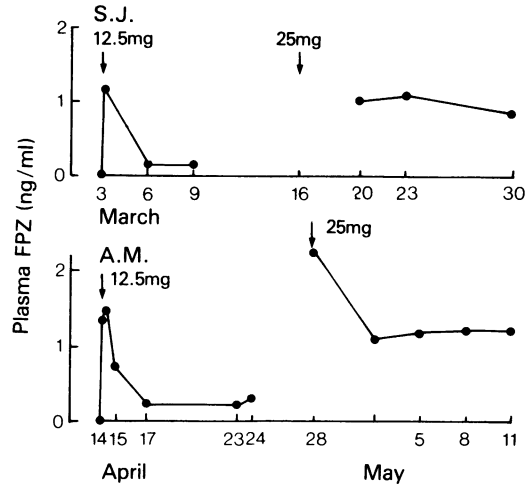

Figure 3 Plasma FPZ after the first two injections of FPZ decanoate.

was also strongly correlated with this mean ($r=0.96$, $P<0.001$).

We have less information about the variation from one interval to another in the same subject. Samples taken from two subjects on the last day of the interval on five occasions gave coefficients of variation of $\pm 13\%$ for one and $\pm 17\%$ for the other.

The weekly dose of FPZ decanoate and the interval mean plasma FPZ level were correlated positively: $r=0.84$, $P<0.001$, $n=38$, (Figure 2).

Figure 3 shows plasma FPZ levels in two patients who received FPZ decanoate as their first neuroleptic. In each case treatment started with a test dose of 12.5 mg. This was followed 2 weeks later by an injection of 25 mg. As predicted, no FPZ could be detected in plasma before the first injection. The 12.5 mg injection was followed by a rapid rise in plasma FPZ to 1.2 in one patient and 1.5 ng/ml in the other. Three days after the injection, very low FPZ levels (0.15 and 0.25 ng/ml) were found and these remained stable. In one patient (AM) a sample taken within 24 h after the subsequent injection of 25 mg FPZ showed an immediate rise in plasma FPZ but, thereafter, both subjects had stable day-to-day levels of about 1 ng/ml in response to the increased dose.

Figure 4 shows plasma FPZ and prolactin levels in a patient whose medication was changed from oral FPZ hydrochloride to injections of FPZ decanoate. With the oral dose of 6 mg three times per day, plasma FPZ levels reached 16 ng/ml. For convenience of administration, medication was then changed to FPZ decanoate 25 mg every 2 weeks. Average plasma drug levels of 1.6 ng/ml were found. Before the morning dose of FPZ hydrochloride, levels of prolactin were about eight times the upper limit for untreated male subjects; 2 h after this dose, levels had risen further, presumably in response to the increased drug level. When FPZ decanoate was substituted for FPZ–HCL, plasma FPZ levels fell, and so did plasma prolactin. There was a significant positive correlation between plasma FPZ and plasma prolactin levels ($r=0.76$, $P<0.001$, $n=20$).

Discussion

Fluphenazine was measurable by radioimmunoassay in all plasma samples taken after administration of FPZ decanate. In two subjects hitherto untreated, injections of 12.5 mg were followed by a short-lived increase to 1–1.5 ng/ml. Levels of 0.15 and 0.25 ng/ml were observed on subsequent days. Following a 25 mg injection, levels around 1 ng/ml were observed after the initial increase. These findings demonstrate the ability of the assay to detect appropriate changes in plasma FPZ when the dose was increased in both subjects, over the 0.15 to 1.5 ng/ml range.

The pattern of plasma FPZ levels between injections of the decanoate: the rapid but short-lived increase following injection and steady levels sustained until the next injection day, are similar to those found in the study of Curry *et al.* (1978) using radio-actively labelled FPZ decanoate. The day-to-day levels achieved by subjects receiving 25 mg injections in the present study are also of the same order of magnitude as those reported in that study, though somewhat higher. It is possible that 7-hydroxy FPZ may contribute to apparent FPZ levels as measured by this assay. For this reason the absolute levels which we report must be treated with

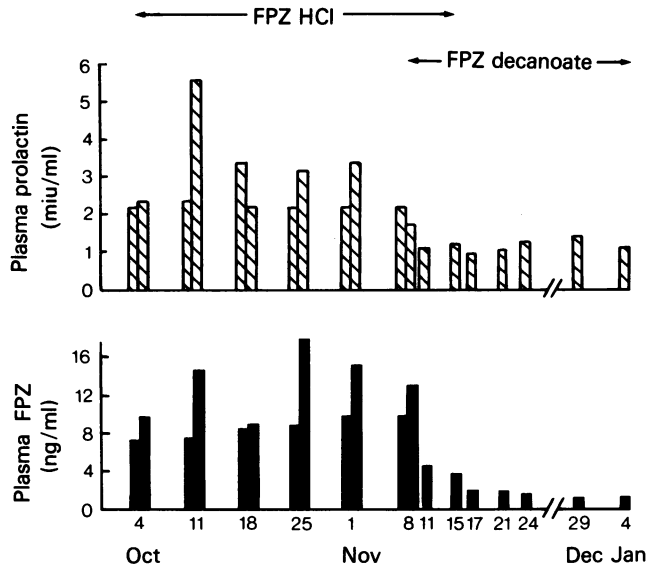

Figure 4 Plasma prolactin and FPZ levels in one patient (R.O.) during treatment with oral and depot FPZ. (During oral treatment adjacent histograms represent samples taken before and 2 h after the morning dose of FPZ–HCl).

some caution. However, the finding of a strong positive correlation between immunoreactive FPZ and plasma prolactin in subject R.O. may be considered evidence that this radioimmunoassay gives a reasonable estimate of those circulating components of the FPZ molecule which cause an important pharmacological effect in man.

The observation that higher FPZ levels are found during the 24 h following injection of the decanoate than at other times may have some clinical significance, particularly in regard to side-effects. Thus, acute dystonic reactions occur in the first 2–3 days after injection (Johnson, 1973), so that it is the habit of some psychiatrists to prescribe anti-cholinergic drugs to cover this period.

The contribution of FPZ decanoate to immunoreactive FPZ levels is likely to be small in view of the very weak cross-reaction of this compound with the antibody (0.9%). Although plasma concentrations of FPZ decanoate have not been measured in man we know that in dogs (Dreyfuss, Ross, Shaw, Miller & Schreiber, 1976) it is very rapidly hydrolysed to FPZ. In man, FPZ given orally or intramuscularly as the hydrochloride, disappears from plasma with a half-time of about 15 h (Curry, 1976). It has been shown in dogs (Dreyfuss *et al.*, 1976) that radioactivity was eliminated from plasma with equal rapidity following intravenous injections of [^{14}C]-FPZ or [^{14}C]-FPZ decanoate. Sustained release of FPZ was only obtained after intramuscular injection of FPZ decanoate in sesame oil. Therefore, the stable levels of FPZ described here between injections of FPZ decanoate, (excluding the first 24 h post-injection) probably result from the slow steady release of FPZ decanoate from the sesame oil depot.

The 3–4 fold variation in plasma FPZ levels among patients receiving the same dose of FPZ decanoate is smaller than that recorded when chlorpormazine is given by mouth, when a 10-fold difference has been reported (Wiles, Kolakowska, McNeilly, Mandelbrote & Gelder, 1976). This, together with the stronger correlation found between dose and plasma level of FPZ shows that the administration of neuroleptics of intramuscular depot injection produces more predictable plasma drug concentrations.

Out-patients receiving treatment with FPZ decanoate usually visit the clinic only on injection days. Consequently, there is practical value in the finding that a blood sample taken immediately before injection gives a plasma FPZ level which is reasonably representative of the entire interval between injections.

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