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Stereoselective disposition of flupentixol: influence on steady-state plasma concentrations in schizophrenic patients

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SUMMARY

Steady-state plasma concentrations of cis(Z)-flupentixol (active principle) and trans(E)-flupentixol (inactive) were measured in 41 patients at least on one occasion. Results indicate that concentrations of the trans-isomer are significatively higher. This demonstrates that the two isomers are not handled in the same way by the organism. This may be relevant if plasma level monitoring is performed using non-specific analytical methods.

INTRODUCTION

When a drug is commercialized as a mixture of an active and an inactive isomer, great care must be taken if pharmacokinetic data obtained with a non-specific method are analysed (1). The same is true for the development of a "drug level monitoring program" (2).

We had previously developped a gas-chromatographic assay for the determination in plasma of flupentixol, an incisive neuroleptic (Fig. 1). The oral form of the drug (Fluanxol®) is commercialized as a 1 to 1 mixture of cis(Z)-flupentixol (active isomer) and trans(E)-flupentixol (inactive), whereas the depot form (Fluanxol® Depot) is pure cis(Z)-flupentixol. Based on previously published statements we had accepted the fact that the cis(Z) to trans(E) ratio remains constant around one (3, 4).

The present work was prompted by the fact that when we compared the results of plasma level monitoring of Fluanxol® using a "nearspecific" radioimmunoassay and a "trans-cis non-specific" gas-chromatographic assay, we observed differences between the two methods which could not be explained by simple methodological differences (5). A new series of fused silica capillary columns allowed to separate and quantitate the cis and trans isomers. We have thus decided to investigate the steady-state plasma concentrations of these two isomers after oral administration of the drug to patients.

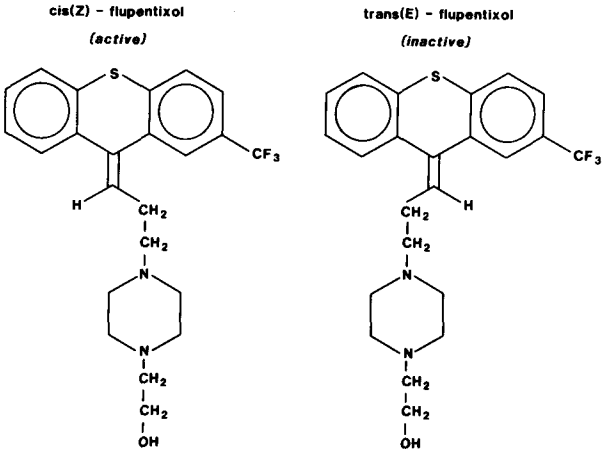

Fig. 1: Structural formulae of the two isomers of flupentixol.

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PATIENTS AND METHODS

Patients

Steady-state plasma samples of 41 patients treated orally with Fluanxol® for at least 7 days with the same dosage regimen were analysed. The daily dosage in this population varied from 4 to 15 mg. In addition, data obtained in 9 patients on two or more occasions were included in order to estimate the intra-individual variability. All these patients were suffering from an acute episode of schizophrenia and they were in-patients. Compliance was assessed by the clinical staff and could also be evaluated by comparing the measured plasma concentrations with the values of our data base.

Blood sampling

Blood samples were drawn by venipuncture into Venoject® tubes containing EDTA as anticoagulant. Steady-state concentrations were defined as those measured at 8 a.m., i.e. 10 hours after administration of the last dose of Fluanxol®.

Analytical method

Material and reagents. Plasma concentrations of flupentixol were determined by a modified gas-liquid chromatography method (5) on a Hewlett-Packard model 5890 gas chromatograph equipped with a NP detector. The standard curves were prepared with cis(Z)-flupentixol and trans(E)-flupentixol supplied as dihydrochlorides. The internal standard was a chlorinated analogue of flupentixol without the double bond in the side chain to the ring structure (Lu 9-215, a gift from Lundbeck & Co, Denmark). The solvents and reagents were of analytical grade (Merck-Darmstadt). Separation was obtained on a 30 m (0.53 mm i.d., 0.88 µm film thickness) fused silica column HP-1 (crosslinked methyl silicone) from Hewlett-Packard. Retention times and peak heights were calculated with an automatic peak integrator (Hewlett-Packard 3992 A).

Standard solutions. The 1 µg/ml standard solutions were prepared daily by diluting 1:1000 a stock solution containing 1 mg/ml of the two isomers and the internal standard in methanol. The stock solutions, stored at −20°C, were stable for 3 weeks. The calibration curves were prepared for each analysis at a concentration of 1, 2, 4 and 6 ng/ml for each isomer.

Extraction procedure. Two milliliters of plasma (unknown samples and plasma containing known amounts of the drug) were transferred to 20 ml glass tubes. The internal standard was added to all samples to give a final concentration of 15 ng/ml. Each sample was analysed in duplicate and the mean of the two determinations was used for further calculations. 200 µl of 4N NaOH and 5 ml of heptane/2-propanol (9:1) were added to each tube. After mechanically shaking the tubes for 20 minutes, the two phases were separated by centrifugation at 3500 rpm for 10 minutes. The upper organic layer was transferred to 10 ml glass tubes and 1 ml of 0.1N HCl was added. The tubes were shaken for 10 minutes and centrifuged for 5 minutes. The organic layer was discarded. The acid layer was transferred to 10 ml glass tubes containing 0.1 ml of 6N NaOH (pH = 13) and the drug was extracted into 0.5 ml of the extraction solvent. Following centrifugation, the organic layer was transferred to 1 ml microvials and evaporated to dryness at 40°C under a gentle stream of nitrogen. The residue was dissolved by mixing (Vortex-Genie) with 10 µl methanol.

Chromatographic conditions. One µl of the methanolic solution was injected into the gas chromatograph. The injection port and detector were maintained at 285°C. A temperature program was used for the oven (T1 = 245°C, T2 = 280°C with a rate of 2°C/min). Helium, air and hydrogen flow rates were 10, 85 and 3.5 ml/min respectively. Typical chromatograms are shown in Figure 2.

Assay performances. The lower limit of detection was 0.5 ng/ml using a 2 ml plasma sample. The within-day coefficients of variation for identical samples were 10.4 and 6.6% for cis(Z)-flupentixol and 9.0 and 6.2% for trans(E)-flupentixol at 1 ng/ml and 4 ng/ml respectively. The recovery of the compounds was determined by comparison with the direct injection of the compounds into the chromatograph. The recoveries of the two isomers and the internal standard were constant over a range of 1 to 6 ng/ml and averaged 75%.

The peak separation of cis(Z)- and trans(E)-flupentixol was calculated using the following formulae (6):

Capacity factor : $k1 = (t1 - t0)/t0$ and $k2 = (t2 - t0)/t0$

Separation factor: $S = k2/k1$

Resolution factor: $R = 2*d/(a1 + a2)$

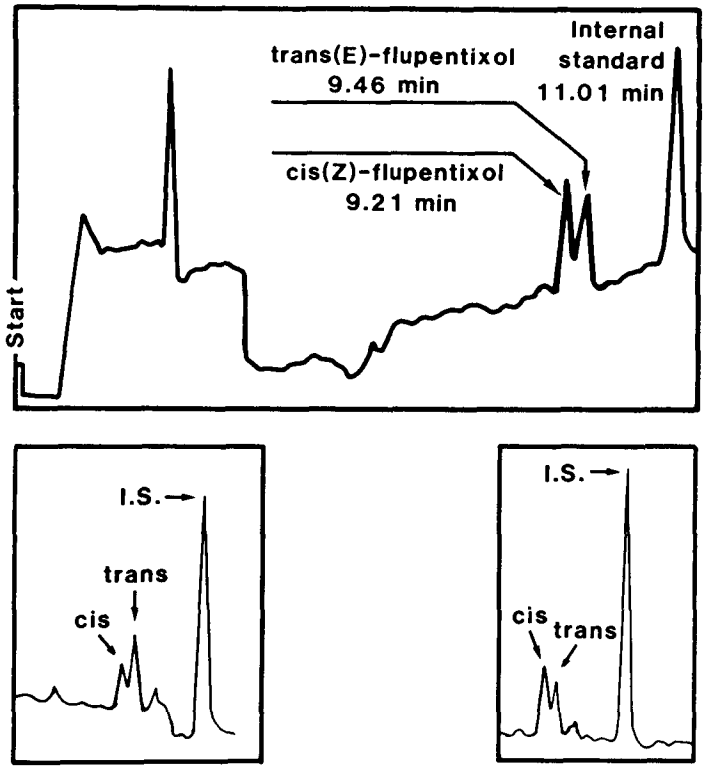

Fig. 2: Chromatograms of cis(Z)- and trans(E)-flupentixol.
Upper panel: pure solution of the two isomers at a concentration of 2 ng/ml and of the internal standard at 15 ng/ml. Right lower panel: spiked plasma at the same concentrations as above. Left lower panel: plasma sample from one patient receiving daily 12 mg of Fluanxol®; cis(Z)-flupentixol was 1.7 ng/ml and trans(E)-flupentixol was 3.0 ng/ml.

where t1 and t2 are the retention times and t0 the retention time of a non-retained compound, a1 and a2 are the peak width measured on the base line and d is the difference between t2 and t1.

From the first chromatogram presented in Figure 2, the following values were obtained:

$t0 = 2.49$ min, $t1 = 9.21$ min, $t2 = 9.46$ min
$k1 = 2.70$ $k2 = 2.80$ $S = 1.037$

R can be estimated at 0.75. Accordingly, the separation of the two isomers can be appreciated to be fair.

Statistical methods

The values for the cis(Z)- and trans(E)-flupentixol concentrations were first divided by the administered dose in order to eliminate biases introduced by the fact that patients received daily doses of Fluanxol® ranging from 4 to 15 mg.

The difference between the cis(Z)- and trans(E)-flupentixol plasma concentrations, normalized by dose and paired by patient was first tested by the non-parametric Wilcoxon's signed rank test. The 95% confidence limits for the mean difference were calculated from the Student's t-distribution. The closeness of the association between the two isomers was estimated through Spearman's ranks correlation coefficient and an estimation of the trans(E)/cis(Z) ratio was found from the slope of the regression line through the origin. Finally, intra- and inter-individual variabilities were compared by an analysis of variance, assuming a random effect model. The methods cited are described by Snedecor and Cochran (7). The statistical analysis was performed with the SPSS procedures on a CDC-Cyber computer (8).

RESULTS

Figure 3 shows the histograms of the "normalized" concentrations for cis(Z)- and trans(E)-flupentixol.

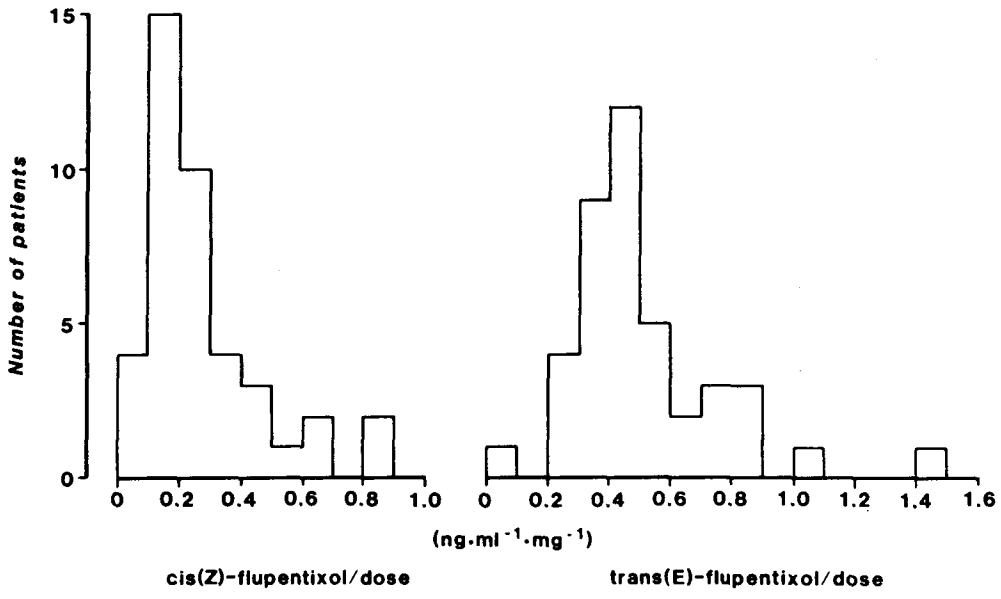

Fig. 3: Distribution of the "normalized" concentrations for cis(Z)- and trans(E)-flupentixol in 41 patients.

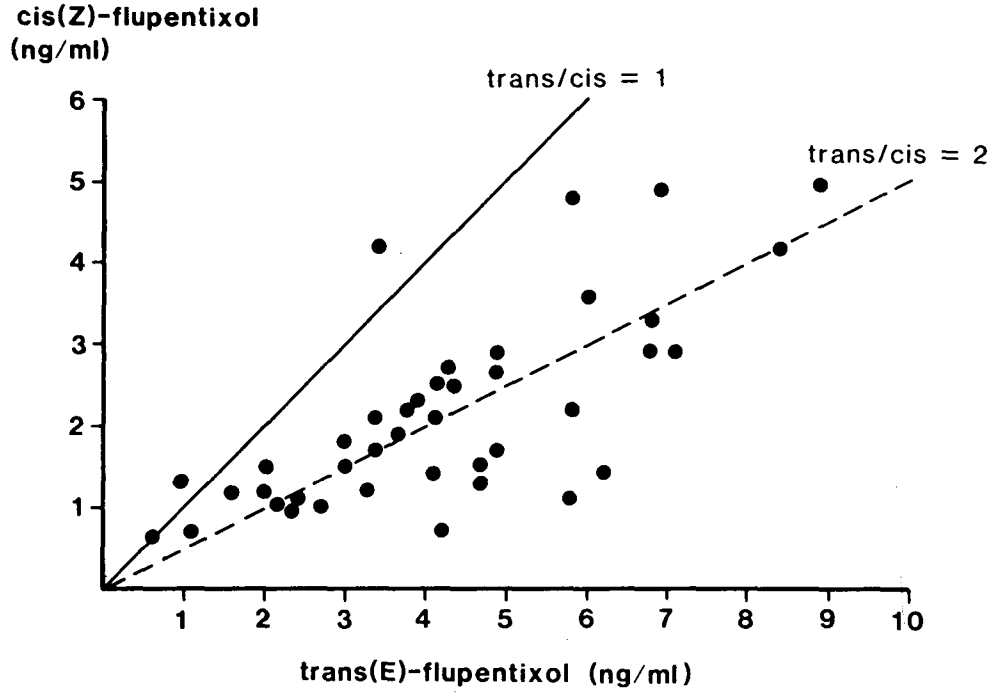

Fig. 4: Steady-state concentrations of cis(Z)- and trans(E)-flupentixol in 41 patients. In this population sample, 59% of the patients had a trans(E)/cis(Z) ratio ranging from 1.5 to 2.5, with extreme values of 0.77 and 6.0.

Both distributions significantly depart from normality and exhibit positive skewness ($p < 0.05$). Wilcoxon's matched-pairs signed-rank test indicates that the difference between the two isomers is significantly assymetrical about zero ($z = -5.34$, $p \ll 0.001$). One patient had the same concentration for the two isomers, two patients had a lower trans(E)-flupentixol level and in 38 out of 41 cases (93%), the trans(E)-concentration exceeded the cis(Z)-concentration.

The analysis of variance for 9 patients for whom more than one plasma determination was available shows that the inter-individual variance of the normalized difference between trans(E)- and cis(Z)-flupentixol is significant ($F = 5.196$, $p < 0.005$). The homogeneity of the intra-individual variance is confirmed by Cochran's C-test ($P = 1$). Estimation of the variance components indicates a 0.58 ratio of intra/inter-individual variances. It is, accordingly, unlikely that the measured differences between the two isomers arise from a methodological artefact.

Figure 4 shows that high plasma concentrations of one isomer tend to be associated with high values of the other. This is confirmed by the rank correlation coefficient between the two isomers which is 0.724 ($p < 0.005$). Nevertheless, the ratio of the trans(E)/cis(Z)-concentration clearly differs from 1 at steady-state. In our population, 59% of the patients had a ratio between 1.5 and 2.5, with extreme values of 0.77 and 6.0.

DISCUSSION

The present results clearly demonstrate that the trans(E)/cis(Z) steady-state plasma concentration ratios do not remain constant around one, but that the inactive trans(E)-isomer is usually present at higher concentrations than the active cis(Z)-isomer. The difference of these findings with previously published statements indicating that the two isomers were eliminated at the same rate (3, 4), are to be found in the data of Muusze et al. (9). They studied by thin layer chromatography the behaviour of Fluanxol® in 2 patients at different times (4 hours after each dose of a t.i.d. regimen at steady-state). There was considerable intra- and inter-individual variability and the trans/cis ratio varied between 0.4 and 1.3. Although Muusze et al. (9) pointed to the fact that the concentration ratio deviated from unity in one of the two patients, the available data were not sufficient to draw any further conclusion. Based on these results it was hypothesized that the trans(E)/cis(Z) ratio remains constant around 1 (3, 4).

From our results it cannot be determined whether differential isomer absorption or metabolism is responsible for the observed differences. As a matter of fact, steady-state concentrations of a drug following linear kinetics (which seems to be the case for flupentixol), are governed by the systemic availability, as well as by the rate of administration and the clearance. Since flupentixol seems to be absorbed from the gastro-intestinal tract by passive diffusion, it is likely that the "portal availability" is the same for the two isomers since gastro-intestinal absorption appears to be complete (5). Accordingly, the differences in steady-state concentrations are most probably the consequence of differences in the pre-systemic and the systemic clearances of cis(Z)- and trans(E)-flupentixol in man. This fact should, however, be confirmed by further specifically designed studies.

Whatever the cause might be, it is important to take this fact into consideration for the development of a "drug level monitoring" procedure for Fluanxol®. As far as the radio-immunoassay for cis(Z)-flupentixol is concerned, a cross reactivity with the trans(E)-isomer of 7% has been reported (10). This is probably of little significance when the trans(E)/cis(Z) ratio is close to one, but might represent a source of error when this ratio reaches higher values.

On the other hand, it is necessary to admit that the clinical experience we have accumulated using a non-specific method (11) has been positive as far as the management of patients is concerned. This might be due to the fact that very little is presently known about the true "therapeutic concentration range" of cis(Z)-flupentixol and that the detection of very low or very high concentrations of the drug is already a marked progress as compared to the "blind" use of this very active compound.

As a practical consequence of our present findings, we have introduced this specific GC-method in our routine "blood level monitoring program" and a projective study is presently underway with the scope to define more precisely the "optimal therapeutic range" of cis(Z)-flupentixol.

CONCLUSIONS

Our past experience and the present results show that more work is needed before a totally coherent approach to the therapeutic monitoring of Fluanxol® will emerge. It is clear, however, that any further studies must take into consideration that, on the one hand, the two isomers are eliminated at different rates and that each individual will show his own pattern of "differential disposition" and, on the

other hand, the fact that the oral formulation is a mixture of the two isomers, whereas the parenteral form contains only the active principle.

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