Radioimmunoassay for Flupenthixol in Plasma

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We describe a radioimmunoassay for the neuroleptic drug flupenthixol, suitable for routine monitoring of its concentration in blood. Antibodies for the assay were raised in a sheep against a 7-carboxyflupenthixol/ovalbumin conjugate. The resulting assay, with [3H]flupenthixol as the label, is capable of detecting 2.0 μg of flupenthixol per liter, in a 100-μl plasma sample. The antiserum shows no cross reactivity with tricyclic drugs and low interference from the major metabolites of flupenthixol. Concentrations in plasma after a single oral dose of flupenthixol have been followed in one volunteer. Peak values were reached after 3 h. Determinations of flupenthixol after fortnightly intramuscular depot injections of the sustained-release preparation, flupenthixol decanoate, showed the extent of fluctuations during this period.

Additional Keyphrases: monitoring therapy • drug assay • schizophrenia • drug metabolites • concentrations after administration of sustained-release form

Flupenthixol [4-(3-(2-trifluoromethyl)-9H-thioxanthene-9-yldene)propyl]-1-piperazineethanol; also "flupentixol"] is a potent neuroleptic drug used in the treatment of schizophrenia (1, 2). A frequent problem with schizophrenic patients is to ensure that they are taking the prescribed medication. Frequent measurement of the drug in the blood would indicate whether or not this is the case and may demonstrate why a particular patient is not responding to treatment. In addition, wide individual variations in rates of absorption and excretion, genetically determined differences in rates of metabolism, and interactions with other drugs all create difficulties in predicting plasma drug concentrations after a given dose. Such determinations in these cases would indicate whether or not the dosage should be adjusted to maintain the appropriate concentration in plasma.

Until the development of the radioimmunoassay presented here, no convenient, simple method for determining flupenthixol in blood has been described. The little information available regarding this drug has come from in vivo experiments with radioactively labeled flupenthixol (3, 4). This paper describes an assay for flupenthixol and discusses the results obtained on measuring the drug in the plasma of two volunteers.

Materials and Methods

Preparation of immunogen: Antibodies to flupenthixol were raised in a sheep against a conjugate of 7-carboxyflupenthixol and ovalbumin, prepared as follows. 7-Carboxyflupenthixol (39.5 mg) was dissolved in distilled water (12.5 ml) together with ovalbumin (64.7 mg). The water-soluble carbodiimide, 1-ethyl-3-diisopropylaminocarbodiimide HCl (129.0 mg), was then added and the solution was stirred overnight at room temperature. Unconjugated flupenthixol was removed by dialysis against distilled water and the purified dialysand solution was freeze-dried. The number of haptenic flupenthixol molecules coupled to each carrier protein molecule was determined by ultraviolet analysis of the diffusate (5); the ratio was found to be about 45:1.

Immunization techniques: A ewe was immunized with the conjugate (2.5 mg) dissolved in distilled water (0.5 ml) and emulsified with two volumes of Freund's complete adjuvant. Booster immunizations, containing similar amounts of immunogen, emulsified with two volumes of Marcol 52/10% Arlacel A adjuvant (6), were given at monthly intervals. Venous blood was sampled from the external jugular vein seven to 10 days after each booster, allowed to clot, and the serum stored at 4 °C.

Assay procedure: Table 1 shows the protocol for the assay.

Tritiated flupenthixol (specific activity = 0.7 kCi/mol; 92.05 nmol/liter) was used as the label and all dilutions of standard, antiserum, and label were made with the assay buffer, phosphate-buffered saline (pH 7.4, 0.1 mol/liter phosphate, and containing 10 g of ethylenediaminetetraacetate and 10 g of gelatin per liter). The dilution of antiserum binding 50% of the
**Table 1. Protocol for Flupenthixol Antiserum Dilution and Standard Curves**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Total counts tube</th>
<th>Non-specific binding tube</th>
<th>Antiserum dilution curve</th>
<th>Standard tube</th>
<th>Sample tube</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>250</td>
<td>250</td>
<td>200</td>
<td>50</td>
<td>150</td>
</tr>
<tr>
<td>[³H]Flupenthixol</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Standard</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Sample</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Normal plasma</td>
<td>50</td>
<td>50</td>
<td>—</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Antiserum</td>
<td>—</td>
<td>—</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Incubate for 48 h at 4 °C

| Buffer                  | 200               | —                         | (—)                      | —             | —           |
| Dextran-coated charcoal | —                 | 200                       | 200                      | 200           | 200         |

Centrifuge for 5 min at 4 °C, 2500 rpm take 500-μl aliquot for liquid scintillation counting

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added label in the absence of unlabeled flupenthixol, as determined from antiserum dilution curves, was that used in the assay.

Standard solutions for use in the assay and in cross-reactivity studies were prepared immediately before use in the assay buffer at drug concentrations ranging from 1.56 to 400 μg/liter, from a stock solution containing 40 mg of drug per liter of ethanol.

The reagents added to the assay tubes in the order indicated in the protocol. After the antiserum was added, the contents of the tubes were mixed and incubated at 4 °C and 48 h. The tubes were then placed in iced-water and cold dextran-coated charcoal was added, for phase separation. The suspension contained 12.5 g of Norit A charcoal and 1.25 g of Dextran T-70 (Pharmacia) per liter of the assay buffer. The contents of the tubes were then mixed on a vortex-type mixer and the tubes were centrifuged at 2500 rpm for 5 min at 4 °C; aliquots of the supernates were then taken for liquid scintillation counting.

**Analysis of plasma samples**: Plasma flupenthixol was measured in two subjects, in one (D.R.) after a single oral dose of 12 mg of flupenthixol (usual dose: 3 mg three to four times a day) and in the other (J.J.) after intramuscular depot injections (see below) of flupenthixol decanoate. Blood specimens were collected into heparinized tubes at frequent intervals after administration and the plasma was transferred to plastic vials and stored at −40 °C until analysis. All specimens from each subject were analyzed in the same assay.

**Calculation of results**: For the antiserum dilution curves the mean count/minute (cpm) for each set of duplicates, after subtraction of the mean nonspecific bound (NSB) counts/minute, was expressed as a percentage of the mean total counts/minute at each dilution of antiserum, i.e.,

\[
\left( \frac{\text{mean sample cpm} - \text{mean NSB cpm}}{\text{mean total cpm}} \right) \times 100 = \% \text{ total binding}
\]

The titre of the antiserum was then calculated from a plot of % total binding vs. final dilution of antiserum, and was that dilution giving 50% total binding.

The calculations for the standard curve and unknown samples were similar except that they were expressed as per cent zero binding, i.e., per cent mean counts/minute in the zero standard tubes. The standard curve was plotted as % zero binding vs. concentration of standard and the concentrations in the unknown samples were read from the curve.

**Results**

Antiserum dilution curves indicated that the serum from the sheep could be used at a final dilution of the 120-fold. The avidity of this antiserum, calculated by using a modified Scatchard plot, was found to be 1.23 × 10⁸ liter/mol. The sensitivity of the assay, calculated from the mean standard curve (Figure 1) by the method of Albano and Ekins (6), was 2.06 μg/liter. The mean within-assay coefficient of variation, determined by
setting up 20 standard curves in one assay, was 6.8%. The between-assay CV, estimated by including duplicate aliquots of the same sample in five separate assays, was 10.4%. The mean (n = 10) analytical recovery of flupenthixol added to normal plasma (25 μg/liter) was 92.7%.

Our cross-reactivity studies indicated that the antiserum recognized both the cis and trans isomers of flupenthixol (Table 2), both of which are present in the commercial preparation. The derivative (7-carboxyflupenthixol) used in the preparation of the immunogen showed 77.5% cross reactivity, indicating that the antibodies are not directed against the linkage between the hapten and the carrier (8). The closely related drug fluphenazine shows a similar degree of cross reactivity, but other tricyclic drugs do not appear to react with the antibodies.

Figure 2 gives data on the concentration of flupenthixol in plasma after a single oral dose. The values increased steadily for the first 2 h and then fluctuated about this peak value for the next 5 h. The plasma flupenthixol values then declined gradually during the next seven days with an apparent biological half-life of about 1.5 days.

Plasma flupenthixol determinations after intramuscular depot injections also showed considerable variation (Figure 3). After an initial injection of 20 mg, the values remained low, reaching 6.5 μg/liter after seven days; 40-mg injections were then given at 14-day intervals, during which time concentrations of drug in plasma increased to a peak after seven days, and decreased to the original value before the next injection.

**Discussion**

Our assay procedure for flupenthixol in blood is capable of detecting as little as 2.0 μg/liter. It can be directly applied to 100-μl plasma samples. Cross-reactivity studies indicate that the assay is specific for flupenthixol, showing no interference from tricyclic drugs or the antiparkinsonian drug, orphenadrine, which is commonly administered at the same time. Cross reactivity with the two metabolites, flupenthixol sulfoxide and Lu 5-051, is low; it is not yet clear whether or not these metabolites are pharmacologically active.
frequency of the dosage to be adjusted to maintain a constant value and will confirm that the patient is taking his medication.

We thank Dr. A. Jorgensen (Ludbeck Ltd., Copenhagen) for supplying the [3H]flupenthixol and the 7-carboxyflupenthixol used in preparing the immunogen. We also thank the volunteers who took part in the pharmacokinetic studies.

References