Radioimmunoassay for Chlorpromazine in Plasma

K. K. Midha, J. C. K. Loo, J. W. Hubbard, M. L. Rowe, and I. J. McGilvery

A radioimmunoassay for chlorpromazine in plasma is described. The antisera was obtained by immunizing rabbits with a conjugate of bovine serum albumin and N-[2-carboxyethyl]desmethylchlorpromazine. It is specific for chlorpromazine and its minor active metabolite, N-desmethylchlorpromazine. Other known active or inactive chlorpromazine metabolites and other psychotropic drugs tested do not cross react with the antisera. Less than 34 pg of the drug can be detected in 200 μL of plasma. As many as 100 samples can be processed in a day by one technician. Concentrations of chlorpromazine can be measured in 200-μL samples of plasma collected as late as 48 h after a single oral 25-mg dose.

Various chemical methods (1–7) have been reported for analysis for chlorpromazine in biological fluids. These procedures require extraction of the drug from biological fluids and (or) its derivatization, and (or) the use of expensive and sophisticated instrumentation; moreover, while useful as reference methods, they are not easily amenable to routine clinical monitoring, and in most cases they are too insensitive for use in single-dose pharmacokinetic studies.

Another important technique for determining plasma concentrations of chlorpromazine is radioimmunoassay (RIA), which may combine high sample turnover with the required specificity and sensitivity (8). Two RIA methods for the analysis of chlorpromazine have been reported. The method of Shostak et al. (9) is not described in sufficient detail. The method of Kawashima et al. (10) requires a 24-h incubation, and it has been applied only to rat-brain homogenates and rat plasma.

The present paper describes a specific, sensitive (170 ng/L), and rapid RIA procedure, which allows quantitation of concentrations of chlorpromazine in plasma for as long as 48 h after a single 25-mg oral dose of the aqueous solution (as the hydrochloride).

Materials and Methods

Synthesis of Immunogen

The synthesis of the immunogen [the bovine serum albumin conjugate of N-[2-carboxyethyl]desmethylchlorpromazine] is described elsewhere (10).

Eight New Zealand white rabbits, four months old, were each given one intradermal injection of 1.0 mg of the immunogen emulsified with 0.25 mL of Freund's complete adjuvant and 0.25 mL of isotonic saline. Thereafter, the rabbits were immunized at two-week intervals with the same amount of immunogen emulsified with incomplete adjuvant rather than complete adjuvant. All rabbits produced sera with adequate titer after the fourth and subsequent injections (10).

Apparatus

We used a Beckman liquid scintillation counter, Model LS-150, equipped with automatic quench compensation device (Beckman Instruments Inc., Fullerton, CA 92634).

Reagents and Standards

Rabbit antisera to chlorpromazine, in 1.5-mL aliquots, was lyophilized in glass vials and stored at −20°C. The contents of each vial were reconstituted with 22.5 mL of distilled water before use.

Tritiated chlorpromazine was prepared by catalytic exchange with tritium gas (Nuclear Research Centre, Negev, Beer-Sheva, Israel). The specific activity of the tracer was 5.67 kCi/mol.

The following reagents were used without modification.

Phosphate buffer, pH 7.4, 0.2 mol/L.

Dextran-coated charcoal suspension, containing 0.2 g of bovine serum albumin per liter (Bio-R.I.A., Montreal, Canada, H3M 3A2).

Liquid scintillator: Dilute 150 mL of BBS-3 solubilizer (Beckman Instruments Inc., Fullerton, CA 92634) to 1 L with toluene containing, per litre, 0.2 g of 1,4-bis(2-[5-phenylxazolyl])benzene and 6 g of 2,5-diphenylxazole (both from BDH Chemicals, Toronto, Canada, M8Z 1K5).

Plasma chlorpromazine standards: Five milligrams of chlorpromazine hydrochloride was dissolved in 40 mL of 50 mmol/L aqueous hydrochloric acid. Serial dilutions in plasma were made to provide working standards of 0.68, 1.37, 2.75, 5.50, 11.0, 22.0, and 33.0 μg/L (0.034, 0.068, 0.137, 0.275, 0.55, 1.10, and 1.65 ng in the final assay tube). For determination of chlorpromazine concentrations in plasma after a single 25-mg oral dose, the plasma standards were further diluted to provide working standards of 0.17, 0.34, 0.68, 1.37, 2.75, 5.50, and 8.25 μg/L.

Working tracer solution: Stock [3H]chlorpromazine, 1 μL, in ethanol was added to 100 mL of the phosphate buffer. This solution was prepared daily.

Procedures

Sample dilution: This will depend on the expected concentration in plasma. For analysis for subjects receiving 25-mg doses of the drug, the plasma sample volume required varied between 50 and 200 μL. The samples were diluted to a final volume of 200 μL in pooled drug-free plasma. The samples were often assayed at several dilutions in order to confirm accuracy.
Table 1. Cross Reactions of Chlorpromazine Antiserum

<table>
<thead>
<tr>
<th>Compound tested</th>
<th>Cross reaction, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorpromazine</td>
<td>100</td>
</tr>
<tr>
<td>7-Hydroxychlorpromazine</td>
<td>0.8</td>
</tr>
<tr>
<td>6-Hydroxychlorpromazine</td>
<td>1.0</td>
</tr>
<tr>
<td>N-Desmethylchlorpromazine</td>
<td>100.0</td>
</tr>
<tr>
<td>Didesmethylchlorpromazine</td>
<td>7.0</td>
</tr>
<tr>
<td>Chlorpromazine sulfoxide</td>
<td>0</td>
</tr>
<tr>
<td>Chlorpromazine-N-oxide</td>
<td>1.2</td>
</tr>
<tr>
<td>7-Hydroxychlorpromazine sulfoxide</td>
<td>0</td>
</tr>
<tr>
<td>Diazepam</td>
<td>0</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>0</td>
</tr>
<tr>
<td>Imipramine</td>
<td>0.9</td>
</tr>
<tr>
<td>Thoridazaine</td>
<td>2.0</td>
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</table>

Assay procedure: The assay should be done in subdued light. To 12 × 75 mm polypropylene tubes, add plasma or samples or standards. Add 5 μL of the diluted antiserum to the side of the tube in a fashion such that the serum adheres to the side wall. Add about 20 000 dpm (0.6 mL) of the working tracer solution. Vortex-mix and incubate at 4 °C for 45 min. Add 0.8 mL of charcoal suspension (stored at 4 °C), vortex, and incubate at 4 °C for an additional 10 min. Centrifuge at 4 °C and 6000 × g. Decant the supernate into 15 mL of liquid scintillation fluid and count the radioactivity in a suitable counter.

Calculate the concentration of unknown samples by use of a standard curve that is described by the following equation:

\[
\log_{10} Y = m \log_{10} C + I
\]

where \(Y\) = (percentage bound/percentage bound at zero concentration), \(C\) = the quantity of drug in ng, \(m\) = slope, and \(I\) = the intercept.

Results

Figure 1 shows the standard curve (n = 101), which is definable by the following equation:

\[
\log_{10} Y = -0.50 \log_{10} C + 1.25 \quad (r^2 = 0.99)
\]

Specificity: We assessed the cross reactivity of available metabolites of chlorpromazine and other psychotropic drugs by the criteria of Abraham (11); the data are shown in Table 1. Except for N-desmethylchlorpromazine the other metabolites and drugs tested do not cross react significantly with chlorpromazine.

Table 2. Intra- and Inter-assay Variance

<table>
<thead>
<tr>
<th></th>
<th>Mean, ng</th>
<th>SD, ng</th>
<th>n</th>
<th>CV, %</th>
</tr>
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<tbody>
<tr>
<td>Intra-assay</td>
<td>1.81</td>
<td>0.14</td>
<td>19</td>
<td>7.66</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>0.07</td>
<td>19</td>
<td>6.56</td>
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<td></td>
<td>0.59</td>
<td>0.04</td>
<td>19</td>
<td>7.29</td>
</tr>
<tr>
<td></td>
<td>0.30</td>
<td>0.02</td>
<td>19</td>
<td>6.99</td>
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<td>0.121</td>
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<td>19</td>
<td>6.74</td>
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<td>0.066</td>
<td>0.007</td>
<td>19</td>
<td>6.12</td>
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<td></td>
<td>0.042</td>
<td>0.004</td>
<td>19</td>
<td>6.30</td>
</tr>
<tr>
<td>Inter-assay</td>
<td>1.06</td>
<td>0.035</td>
<td>19</td>
<td>9.68</td>
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<tr>
<td></td>
<td>0.68</td>
<td>0.045</td>
<td>19</td>
<td>7.29</td>
</tr>
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<td></td>
<td>0.30</td>
<td>0.026</td>
<td>19</td>
<td>8.76</td>
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<tr>
<td></td>
<td>0.12</td>
<td>0.007</td>
<td>19</td>
<td>6.08</td>
</tr>
<tr>
<td></td>
<td>0.063</td>
<td>0.004</td>
<td>19</td>
<td>5.78</td>
</tr>
<tr>
<td></td>
<td>0.047</td>
<td>0.005</td>
<td>19</td>
<td>11.09</td>
</tr>
</tbody>
</table>

Sensitivity: The detection limit is less than 34 pg, which corresponds to 0.17 μg/L if 200 μL of plasma sample is used; this sensitivity is several fold that of existing chemical methods.

Precision: The inter- and intra-assay variation was determined by using plasma standards prepared in accordance with the procedure described. The data are summarized in Table 2. Note that intra-assay variances do not differ greatly from inter-assay variances.

Influence of plasma volume on standard curves: Standard curves were prepared by use of 50- or 200-μL of volumes of plasma. The slopes and intercepts derived were identical; evidently plasma volume does not affect the assay. Plasma was drawn from 10 different subjects and the \(B_0\) values were estimated in triplicate; we found no differences, which indicates that endogenous materials in plasma do not interfere with the assay.

Analytical recovery: Tritium-labeled chlorpromazine was added to plasma samples containing cold drug and incubated with buffer in accordance with the procedure described; the supernates were then decanted into scintillation fluid and the radioactivity measured. The percentage recovery at 0.17 and 1.25 ng/L was 95.4 ± 0.6 and 93.7 ± 1.4, respectively. We stress that by using the decanting technique a little of the sample adheres to the side of the tube; thus, actual recoveries may be higher. We did another experiment in which radioactive tracer was added directly to the polypropylene tube without using plasma; we found that about half of the radioactive tracer was adsorbed on the surface of the tube. Clearly, all standards at the low nanogram range should be prepared in plasma.

Capacity of the procedure: One technician can assay 60 to 100 samples, in duplicate, per day.

Concentrations in plasma measured in healthy volunteers after administration of 25 mg of chlorpromazine are illustrated in Figure 2. Note that the assay can detect the drug in specimens collected as late as 48 h after a single oral dose.

Discussion

Several reports (12–18) stress the need for specific, sensitive, and rapid assays of chlorpromazine and (or) its metab-
olites as an aid to the clinician in determining drug dosage for the individual patient. In most clinical studies of this drug the electron-capture gas chromatographic procedure of Curry (1) or modifications of it (13, 15, 18-21) have been used. However, several problems have been noted (2) when this method has been applied to routine determinations. The radioimmunoassay we describe here is suitable for routine clinical monitoring of plasma concentrations of chlorpromazine. The antiserum used in the present assay discriminates chlorpromazine from its major metabolites (16-19) formed by sulfoxidation and hydroxylation, but not from its N-monodesmethylated metabolite. The concentration of this metabolite in patients' plasma is much lower than that of chlorpromazine (16-19), too low to influence the clinical interpretation of data on concentrations in plasma.

We acknowledge the excellent technical assistance of Messrs. C. Charette and J. K. Cooper.

References