Monitoring Clorazepate Dipotassium as Desmethyldiazepam in Plasma by Electron-Capture Gas–Liquid Chromatography

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We describe a procedure for determining clorazepate dipotassium as its decarboxylated, pharmacologically active metabolite, desmethyldiazepam, in 100 µL of plasma, with use of electron-capture gas–liquid chromatography and with methyltritazepam as the internal standard. The procedure is a one-tube, one-step extraction without derivatization and is accurate, reproducible, and rapid. The sensitivity limit is 20 µg/L. Within-run and between-run CV's (concentration, 3.5 mg/L) were 2.9 and 3.5%, respectively. Within-run CV's for 1.5 and 1.0 mg/L concentrations were 3.9 and 4.3%, respectively. For a 1.0 mg/kg per day dose of clorazepate dipotassium, the mean steady-state concentration of desmethyldiazepam in plasma was 1.037 mg/L.

Additional Keyphrases: monitoring therapy • epilepsy • anti-anxiety therapy

Clorazepate dipotassium (Tranxene, Abbott) is used for the symptomatic relief of anxiety associated with anxiety neurosis, in other psychoneuroses in which anxiety symptoms are prominent features, as an adjunct in disease states in which anxiety is manifested, and for symptomatic relief of acute alcohol withdrawal. For these purposes the recommended daily dose is from 15 to 60 mg. Recently, it has also been used for treatment of certain types of epilepsy, in which case the dosage can approach 300 mg (1). Monitoring therapeutic concentrations of anticonvulsants in the blood is of great importance in the clinical management of epilepsy (2).

Clorazepate dipotassium (7-chloro-1-3-dihydro-2-oxo-5-phenyl-1H-1,4-benzodiazepine-3-carboxylic acid, monopotassium salt, monopotassium hydroxide) (I) spontaneously decarboxylates to desmethyldiazepam (7-chloro-1,3-dihydro-5-phenyl-2H-1,4-benzodiazepine-2-one) (II) at below pH 4, but is relatively stable at pH 7.4 and above (3). Thus, in the acidic environment of the stomach, I becomes II, which is also the active metabolite of diazepam III (Valium, Roche) (Figure 1).

Desmethyldiazepam can be measured by gas–liquid chromatography (GLC) with electron-capture (EC) detector (4, 5) and by nitrogen-sensitive detector (6).

We describe here a simple procedure (applicable to routine use) for analyzing desmethyldiazepam by use of a linear EC-GLC instrument and a highly convenient one-step extracting method originally developed by Weinfeld et al. (7) for determination of diazepam and desmethyldiazepam in patients being treated with diazepam. Emphasis here is on the determination of steady-state concentration of desmethyldiazepam in plasma after administration of relatively high doses of clorazepate dipotassium to patients for seizure control. We made no attempt to estimate intact clorazepate dipotassium, because 4 to 12 h after a dose no clorazepate can be detected (3).

Materials and Methods

Gas Chromatography

We used a Hewlett-Packard gas-chromatograph, Model 5738A, equipped with a 63Ni electron-capture detector, Model 18713A, linear electron-capture control electrometer, Model 5709A, integrator, Model 3380A, and automatic sampler, Model 7671A. A coiled borosilicate glass column, 1.2 m long, 4 mm i.d., containing a pretested preparation of 3% OV-17 on 60–80 mesh Gas-Chrom Q (Applied Science Labs., Inc., State College, PA 16801) was used. The column was conditioned at 325 °C for 4 h at “no flow” state and then at 275 °C overnight with argon–methane (90/10 by vol) at 40 mL/min. Analysis conditions: Oven temp., 240 °C; injection port temp. 300 °C, and EC detector at 400 °C. The flow rate was adjusted so as to obtain retention times of about 4.0 and 6.0 min for desmethyldiazepam and methyltritazepam (the internal standard), respectively. At the beginning of each working day, the column was primed by injecting five drug-free blank plasma extracts or previously used calibrator plasma extracts, to avoid peak-tailing for desmethyldiazepam.

Reagents

All solvents were pesticide grade or “Glass Distilled” (Burdick & Jackson, Muskegon, MI 49442). Saturated potassium chloride (reagent grade), approx. 4.8 mol/L, was prepared in distilled water. Analytically pure desmethyldiazepam and methyltritazepam were generous gifts from Hoffmann-La Roche, Nutley, NJ 07110. The drugs were used as received, after drying in a desiccator at reduced pressure.

Standard Solutions

Place 10.00 mg of desmethyldiazepam in a 10-mL volumetric flask, dissolve it in 1.0 mL of absolute ethanol, and dilute to volume with benzene, to yield a stock solution (1.0 g/L). Prepare a working solution, 10 mg/L, by diluting 1.0 mL of this stock solution 100-fold with benzene. Place 10 to 50 µL (100 to 500 ng) into a series of tubes and evaporate the solvent in a gentle stream of nitrogen. Add 0.1 mL of drug-free blank plasma, vortex mix. These standards are now ready to be run along with patients' specimens for extraction.

Place 10 mg of methyltritazepam (internal standard) in a 100-mL volumetric flask, dissolve it in 10 mL of absolute ethanol, and dilute to volume with benzene to yield a 100 mg/L stock solution. From this, prepare a 10 mg/L solution, and dilute a 250-µL portion of this to 100 mL with benzene to give a 25 µg/L solution.

Methods

Into 13 × 100 mm test tubes (Corning 9810) with ungeared pentyhead stoppers place 0.1 mL of plasma and 0.1 mL of saturated potassium chloride solution (for specimens containing trace quantities of desmethyldiazepam, up to 1.0 mL of plasma and an equal volume of saturated KCl can be used.

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without altering the extraction procedure), and mix. Add 1.0 mL of benzene containing 25 ng of methylnitrazepam as the internal standard. Extract the mixture on a Fisher Roto-Rack (Model 343) at 40 rpm for 15 min. Centrifuge the tubes at 2500 rpm for 10 min. Transfer 0.1 mL of this benzene extract into 100-μL vials (Hewlett-Packard 4330-0540), and stopper them with Teflon-lined vial caps (H-P 4080-87130). Along with the patients’ plasma prepare tubes for a working standard curve for 0 to 500 ng/0.1 mL of plasma. Inject 10 μL of each extract into the gas chromatograph. Chromatograph extracts the same day they are made.

Results

Peak identification: When 10 μL of each of the above-prepared calibration specimens was injected (1, 2, 3, 4, and 5 ng on the column), the response was linear to concentration. Figure 2 shows a standard curve for various concentrations of desmethyldiazepam extracted from plasma.

Analytical recovery: The recovery efficiency of desmethyldiazepam and methylnitrazepam at physiological pH has already been studied in detail (7). Under research conditions, we were able to confirm the near-100% recovery. Nevertheless, under routine clinical laboratory conditions, recovery varied, and at times was as low as 77%. We believe this variation is attributable to difficulty in maintaining strictly constant and optimal conditions in routine operation. Therefore, each working day the patients’ results were based on recalibration of freshly extracted standards included in each run.

Figure 3 shows typical chromatograms of extracted blank plasma, of the drug added to blank plasma, and of a patient’s plasma containing the drug.

Reproducibility and accuracy: The linearity of the procedure was confirmed each working day by extracting 0.1 mL of plasma containing 100, 200, 300, 400, and 500 ng of desmethyldiazepam per 0.1 mL, together with 1.0 mL of benzene containing 25 ng of methylnitrazepam as the internal standard. The ratio of integrated peak area of desmethyldiazepam to that of the internal standard was linearly related to concentration (n = 10 for each point). Replicate analyses of 20 to 30 samples each of plasma containing high, medium, and low concentrations (predicted ranges based on earlier exploratory work) of desmethyldiazepam showed the proce-
Table 1. Typical Mean Steady-State Desmethylazepam Concentrations in Plasma of Eight Patients on a Relatively High Dosage of Clorazepate Dipotassium

<table>
<thead>
<tr>
<th>Dosage, mg/kg body wt.</th>
<th>Mean steady state µg/L of plasma</th>
<th>1 mg of drug/kg per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.8</td>
<td>3469</td>
<td>1239</td>
</tr>
<tr>
<td>1.0</td>
<td>1530</td>
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</tr>
<tr>
<td>3.2</td>
<td>2819</td>
<td>881</td>
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<tr>
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<td>1674</td>
<td>728</td>
</tr>
<tr>
<td>2.4</td>
<td>1978</td>
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</tr>
</tbody>
</table>

* All patients also had therapeutic plasma concentrations of phenytoin, carbamazepine, and/or valproic acid.

Discussion

We have used this procedure for six months and find it to be accurate, reproducible, and rapid. We included specimens supplemented with desmethylazepam at every two patients' sample intervals, in duplicate, with duplicate injections of each extract. Our results led us to institute this method for routine clinical laboratory use at Lafayette Clinic.

Oxazepam [see Figure 1 (IV)] is a major urinary metabolite of clorazepate dipotassium as well as of other 1,4-benzodiazepines-2-ones. During chronic administration of 20 mg of diazepam three times daily for 10 weeks, the reported (7) plasma concentration of oxazepam in plasma was as high as 290 µg/L. Blank plasma samples, supplemented with oxazepam, produced no interfering peaks (retention time <1.5 min). We have observed (when 1.0 mL of plasma was used) what might be an oxazepam peak in some of our patients' plasma. What appears to be a poor extraction efficiency under our conditions coupled with our routine use of only 0.1 mL of plasma for extraction prevents our reporting anything definitive on the relation (if any) between the concentration of desmethylazepam in plasma and the presence of oxazepam.

Table 1 shows typical steady-state desmethylazepam concentrations for some patients on a relatively high dosage of clorazepate dipotassium. Based on these initial data, for 1.0 mg/kg daily dose of clorazepate dipotassium, the mean (n = 8) steady-state concentration of desmethylazepam is 1.037 mg/L of plasma.

References