Liquid-Chromatographic Determination of Propisomide and Its Mono-N-dealkylated Metabolite in Plasma and Urine

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We describe a "high-performance" liquid-chromatographic assay for simultaneously determining propisomide and its mono-N-dealkylated metabolite in plasma and urine. After extraction with dichloromethane at alkaline pH, the unchanged drug, its metabolite, and the internal standard are separated by liquid chromatography on a reversed-phase column and the absorbance of the eluate is measured at 254 nm. Selectivity, sensitivity, and reproducibility are excellent. Results are similar to those by gas chromatography for propisomide but, in addition, the metabolite can be simultaneously measured in the same clinical sample. We also report results by this method for blood and plasma samples from a volunteer receiving a single 200-mg dose of propisomide.

Additional Keyphrases: chromatography, reversed-phase antiarrhythmic drugs

Propisomide, N,N-diisopropylaminoethyl-4-methyl-2-(2-pyridyl)-pentanamide (CM 7857), a new antiarrhythmic drug that currently is under clinical evaluation (Figure 1), has been very efficient in various experimental models of arrhythmia (1, 2), and has expressed an original electrophysiological pattern (3). The drug is well tolerated and shows promise in suppressing both atrial and ventricular arrhythmias in humans (4).

Species differences in its metabolism have been described elsewhere (unpublished results). Dogs and humans excrete the administered dose, predominantly unmetabolized, in the urine; the major metabolite identified in plasma and urine is the mono-N-dealkylated form (metabolite MND, Figure 1).4

A method for determining propisomide in biological samples by gas chromatography with nitrogen-selective detection (GC-NSD) has been developed (5). Here we describe a new "high-performance" liquid-chromatographic (HPLC) procedure with which one can simultaneously quantify propisomide and its metabolite in biological fluids. After comparing the concentrations of propisomide determined by HPLC and by GC-NSD in the same plasma samples, we report the results for quantifying, by HPLC, both the parent drug and its metabolite in whole blood and plasma from a volunteer who took a single 200-mg oral dose of propisomide.

Materials and Methods

Reagents. Propisomide, its MND metabolite, and the internal standard we used (Figure 1) were all supplied by Sanofi Research Center, Montpellier, France. The purity of each of these compounds, as assessed by thin-layer chromatography, exceeded 99%. Stock 100 μg/mL solutions of these compounds were prepared in acetonitrile. We prepared standard solutions of plasma or urine freshly daily by suitably diluting the stock solutions with blank plasma or urine.

The acetonitrile and dichloromethane were of analytical grade (Merck, Darmstadt, F.R.G.).

Extraction procedure. To 0.5 mL of whole blood or plasma add 10 μL of the 100 μg/mL internal standard solution in acetonitrile, 0.2 mL of alkaline buffer (1 mol of sodium carbonate per liter of NaOH solution, 0.5 mol/L), and 6 mL of dichloromethane. Shake the sample on a rotary mixer for 5 min, then centrifuge at 1500 × g for 15 min at 4°C. Discard the aqueous (upper) phase and add to the organic layer 4 mL of acidic buffer (potassium dihydrogen phosphate, 0.1 mol/L, adjusted to pH 2 with HC1). After shaking and centrifuging as before, discard the organic (lower) layer and make the aqueous phase alkaline by adding 0.2 mL of alkaline buffer (sodium carbonate, 1 mol/L, in 5 mol/L NaOH solution). Re-extract the compounds with 6 mL of dichloromethane, centrifuge as described above, then discard the aqueous layer and evaporate the organic phase under a mild stream of nitrogen. Dissolve the residue in 50 μL of the mobile phase, and inject 25 μL of this onto the column.

For analysis of urine samples, only one extraction of alkaline pH is needed, with no further cleanup. Mix 1 mL of urine with 1 mL of carbonate buffer (1 mol/L, pH 12) and 50 μL of the internal standard solution. Before analysis, extract with dichloromethane as described above.

HPLC. Our HPLC system consisted of a Model 6000-A pump, a Model U6K sample injector, and a Model M 440 ultraviolet spectrometer operated at 254 nm (all from Waters Associates, St Quentin-Yvelines, France). The chromatographic column was a radial-compression "Z" system, with the polyethylene cartridges filled with μBondapak C18 (Waters).

The mobile phase was acetonitrile/acetate buffer (0.2 mol/L, pH 4), 3/7 (by vol), delivered at a flow rate of 2.5 mL/min at room temperature.

Fig. 1. Chemical structures of propisomide (I), its major metabolite (II), and the internal standard used in this assay (III)

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4 Nonstandard abbreviations: MND, mono-N-dealkylated (metabolite); HPLC, "high-performance" liquid chromatography; GC-NSD, gas chromatography with nitrogen-selective detection.

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Calibration curves were prepared from samples of blank human plasma supplemented with known amounts of propisomide and of its metabolite (0.1 to 5 mg/L) and with the internal standard (5 mg/L). Peak-height ratios of the parent drug (or its metabolite) to the internal standard were plotted vs concentrations of the compound measured; standard curves were constructed by regression analysis. We included samples for new calibration curves with every set of unknown plasma samples.

Other procedures. To compare the performance of the HPLC method with that of the GC-NSD assay, we measured the concentrations of propisomide in split plasma samples collected from three patients being treated with short infusions of the drug, 4 mg/kg body weight. Blood samples (n = 44) were collected into heparinized tubes, and the plasma was separated by centrifugation and stored at -20 °C until analysis.

We also used the HPLC method to determine the concentrations of propisomide and its metabolite in samples of whole blood and plasma obtained from a volunteer who ingested 200 mg of propisomide in tablet form. One aliquot of each whole-blood sample was immediately frozen; the rest was centrifuged to separate the plasma, then stored frozen until analysis.

Results

Typical calibration curves for the parent drug and its MND metabolite show good linearity for both over the concentration range studied; in all cases, correlation coefficients exceeded 0.999 and the intercepts were not significantly different from zero. The detection limit of the method, defined as a signal/noise ratio equal to 10, is about 25 ng/mL for both compounds.

Figure 2 shows typical chromatograms for various plasma samples. Blank plasma assayed by this procedure shows no peaks that might noticeably interfere with the analysis.

Under the conditions specified, the retention times for propisomide, metabolite MND, and the internal standard are 3.5, 2.5, and 5.6 min, respectively.

We determined the efficiency with which the drug and its metabolite are extracted from plasma by comparing the peak-height ratios obtained for pure solutions of the two compounds with the ratios obtained after extraction, clean-up, and back-extraction. The overall mean analytical recoveries of the parent drug and the metabolite from plasma were 60% and 69%, respectively.

We assessed the reproducibility (CV) of the method by assaying, five times, each concentration used in standard curves. For propisomide, the CVs ranged from 1.4% to 6%; for the MND metabolite, from 1% to 4.4%. For the drug and metabolite in plasma, the CVs were low (<4.4%) for the usual range of concentrations found in plasma.

We measured propisomide in 44 plasma samples from three patients by both GC-NSD (y) and HPLC (x). The regression equation for the results was:

\[ y = 0.991x \pm 0.001; r = 0.986. \]

Figure 3 shows a typical concentration–time curve from our study of propisomide ingestion involving six human volunteers. The concentrations of the metabolite in plasma are about 0.1 of those of the parent drug. Table 1 summarizes the mean ratios of the concentrations in plasma and blood for the parent drug and its metabolite.

Discussion

The HPLC procedure described here is rapid, sensitive, specific, and reproducible. Results for propisomide concentrations in plasma are similar to those obtained by GC-NSD. However, the HPLC technique allows simultaneous assay of the drug and its major metabolite in the same biological samples, with use of a common internal standard. Gas-chromatographic methods would require an additional step for derivatization of the metabolite or separate analysis of the two compounds as reported for disopyramide, another
antiarrhythmic agent structurally close to propisomide (6–9).

The present method can be conveniently applied to the determination of the parent drug and its MND metabolite in plasma and urine samples from volunteers treated with 200 mg of propisomide (10). It is sensitive enough for routine applications in pharmacokinetic studies and drug monitoring. When we applied our method to the determination of the drug and its metabolite in whole blood and plasma, we found that the parent drug is preferentially distributed in plasma, whereas the metabolite is almost equally distributed between blood and plasma.

This HPLC method, like the gas-chromatographic assay, measures the total concentrations of propisomide in plasma. Because protein-binding of the drug in plasma reportedly (11) is concentration dependent within the range of therapeutic concentration, measurement of the concentration of the free drug may be important for achieving optimal therapeutic effect. Combining the HPLC method with use of equilibrium dialysis would provide determinations of total and free propisomide in plasma.

References

Paraproteinemia in Patients with Acquired Immunodefiency Syndrome (AIDS) or Lymphadenopathy Syndrome (LAS)

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Eight of 15 patients with acquired immunodeficiency syndrome (AIDS) and six of nine patients with lymphadenopathy syndrome (LAS) had paraproteins in their sera. Twelve of these 14 were IgG kappa; the other two had no demonstrable light chains. The relationship of the paraprotein to the pathogenesis of AIDS is not clear, but we discuss its relation to derangements of B-cell immune regulation and function and to B-cell tumors in AIDS patients.

Acquired immune deficiency syndrome (AIDS) is characterized by opportunistic infections and such disorders as Kaposi's sarcoma in certain high-risk groups. Within two years of diagnosis, the mortality rate is more than 60% (1–4). Another syndrome, which is known by several names—AIDS-related complex (ARC), pre-AIDS, or the lymphadenopathy syndrome (LAS)—is seen in the same risk groups and may be a precursor of AIDS.

The wide variety of immune abnormalities reported in AIDS cases includes lymphopenia, increased polyclonal serum immunoglobulins, inverted T4/T8 lymphocyte ratios, markedly depressed cellular immunity, and depressed in vitro response of lymphocytes to antigen and mitogen stimulation (5–7). However, to our knowledge, paraproteins have not been reported.

In our investigations, we noticed that two of our first three patients with AIDS had IgG paraproteins. This led us to study the sera of our other patients. We found that most of the patients with AIDS and LAS also had such abnormalities.

Materials and Methods

Serum was sampled from 24 patients—15 with AIDS and nine with LAS—all from central New York and all treated at the State University Hospital. All AIDS patients fulfilled the criteria of the Centers for Disease Control for AIDS: the patient had a tumor or opportunistic infection fairly predictable of an underlying defect in cellular immunity without a primary immunodeficiency, a malignancy, or a history of immunosuppressive therapy. All patients with LAS had...