Liquid-Chromatographic Determination of Two Antidepressants, Trazodone and Mianserin, in Plasma

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Plasma containing trazodone or mianserin was extracted. The organic phase containing trazodone was evaporated and the residue was reconstituted in dilute acid. Mianserin was back-extracted from the organic phase with dilute acid. Both drugs were chromatographed on μBondapak C18 columns, with phosphate/acetonitrile as the mobile phase. Peak-height ratios of drug/internal standard were linearly correlated with concentrations between 25 and 200 μg/L for trazodone, and between 25 and 200 μg/L for mianserin, with respective between-run CVs of 4.7% and 7.6%. Detection limits were 5 ng. Of some common drugs and metabolites examined, nortriptyline co-elutes with the internal standard used in the trazodone assay, while flurazepam co-elutes with mianserin. Concentrations of trazodone in 26 patients ranged from 73 to 1678 μg/L. For two geriatric patients, concentrations were about 2000 μg/L. For two overdose patients, they were about 5000 μg/L. The concentration of mianserin was 27 μg/L for a volunteer treated with a single 40-mg oral dose.

Additional Keyphrases: therapeutic drug monitoring · chromatography, reversed-phase

Trazodone (TRA), 2-[3-[4-(m-chlorophenyl)-1-piperazinyl]-propyl]-s-triazolo(4,3a)pyridin-3-(2H)-one hydrochloride, has recently been approved by the Food and Drug Administration for use as an antidepressant. Mianserin (MIA), 1,2,3,4,10,11b-hexahydro-2-methylidenobenzoc[f]pyrazino[1,2-a]azepine hydrochloride, is currently used for clinical research in this country, but has been used clinically for treating depression and depression associated with anxiety in Europe, Australia, and New Zealand (7–9). According to recent reviews of the pharmacological properties of TRA (3) and MIA (7), they do not have anticholinergic side effects and their cardiovascular toxicity is relatively low (6–9). However, TRA may cause ventricular ectopy (10). Therapeutic and toxic concentrations in plasma have not been established for MIA and TRA, but even overdoses were reportedly well tolerated (3, 7, 11).

Ankier et al. (2) developed a liquid-chromatographic (LC) assay for TRA and used it to estimate the pharmacokinetic parameters. Ether was the extractant, and the internal standard, 2-[3-[4-(m-chlorophenyl)-1-piperazinyl]-propyl]-5-methyl-4-phenyl-triazol-3-(2H)-one (TRA-IS) (Figure 1), was added only after the extraction. Caccia et al. (12) described two separate gas-chromatographic assays for TRA and an active metabolite of it, 1-(m-chlorophenyl)piperazine.

Materials and Methods

Materials

Instrumentation. The chromatograph we used consisted of a Model 5000 binary pump (Varian Associates, Walnut Creek, CA 94931) equipped with a Model 7125 injector (Rhodeyne, Berkeley, CA 94710) and a column heater. Effluent was monitored at 214 nm. The column used for both assays was a 4.6 mm × 30 cm μBondapak C18 column, coupled to a guard column packed with Bondapak/Corsil C18 (3.9 mm × 2.3 cm) (Waters Associates, Milford, MA 01757).

Reagents. TRA hydrochloride (Desyrel) was obtained from Mead Johnson & Co., Evansville, IN 47721. TRA-IS was kindly donated by Dr. G.T. Ward from Roussel Lab., Wiltshire, U.K. MIA was from Organon, Oss, Holland. Clomipramine (Cl-IMI), the internal standard for the MIA assay, was from Ciba-Geigy, Summit, NJ 07901.

Prepare stock solutions of the drugs and internal standards (1 g/L, free-base) by dissolving the appropriate amounts in 10 mL of methanol. To prepare the standard solution of TRA in plasma (10 mg/L), evaporate 1 mL of the stock TRA solution in a 100-mL silanized volumetric flask, and dissolve the residue in "drug-free" plasma. Transfer aliquots to micro-cap polypropylene tubes (Sarstedt, Princeton, NJ 08540). These may be stored at −20 °C for as long as three months before use in analysis. Prepare standard solutions of MIA, Cl-IMI (both at 10 mg/L), and TRA-IS (20 mg/L) by diluting aliquots of the stock solution with methanol.

Acetonitrile, methanol, and n-hexane were "UV" grade, distilled in glass (Burdick and Jackson Labe, Muskegon, MI...
The droxide, 49442). Isoamyl alcohol, orthophosphoric acid, sodium hydroxide, and potassium dihydrogen phosphate were "Baker-Analyzed" reagent grade (J. T. Baker Chemical Co., Phillipsburg, NJ 08865).

For the precision studies, we prepared the "quality control" samples by adding 40 µL of the stock methanolic solution of TRA or 1 mL of the standard methanolic solution of MIA to two separate 100-mL siliconized volumetric flasks. The methanol was evaporated under nitrogen and 100 mL of "drug-free" plasma was then added to each residue, giving final concentrations of 400 µg/L for TRA and 100 µg/L for MIA.

Sample Collection

Blood was sampled from 29 patients who had been treated with TRA for at least three days (5 × t1/2 = 50 h), either just before the next dose (50 to 300 mg, given two or three times daily) or 12 h later to assess "trough" (steady-state) TRA concentrations in plasma. For sampling we used lavender-top Vacutainer Tubes (Becton Dickinson, Rutherford, NJ 07070) containing EDTA but with no tri(2-butxyethyl) phosphate plasticizer in the rubber stopper. For monitoring MIA, a volunteer ingested 40 mg of MIA; blood was sampled 2 h later.

Procedures

TRA assay is a two-step extraction procedure—organic extraction and evaporation/reconstitution. To a series of silanized test tubes containing 1 mL of "drug-free" plasma, add (with vortex-mixing) 25, 100, 250, 500, 1000, and 2000 ng of TRA, by pipetting aliquots of the standard solution of TRA in plasma. To these standards, "quality control" samples, and patients' plasma samples, add (with vortex-mixing) 2000 ng (100 µL) of TRA-IS standard solution, 0.5 mL of 2 mol/L NaOH, and 5 mL of n-hexane/isoamyl alcohol (99/1 by vol). Extract in a rotary mixer for 15 min, centrifuge for 5 min, then transfer the organic (upper) phase to another series of test tubes. After evaporating the organic phase at 40 °C, dissolve the residue with 400 µL of H3PO4 (0.5 g/L). Inject 100-µL aliquots into the chromatograph, which is operated in the following arrangement: column, µBondapak C18 with Bondapak/Corasil guard column; mobile phase, phosphate (50 mmol/L, pH 4.7)/acetonitrile (72/28 by vol); flow rate, 2.5 mL/min; detection wavelength, 214 nm at 0.01 to 0.05 Å (full scale); injection volume, 100 µL; and temperature, 60 °C. Plot peak-height ratios (TRA/TRA-IS) vs TRA concentrations. Estimate the TRA concentrations in the "quality control" samples and patients' samples from this plot.

MIA assay is similar to our previously published procedure (15), except that the amount of internal standard, Cl-IMI, is decreased to 200 ng.

Results

Figure 2 shows the chromatograms of plasma extracts containing TRA. The respective retention volumes of TRA and TRA-IS were 20 and 40 mL. "Drug-free" plasma chromatograms (Figure 2A) did not show any interference peaks with TRA. Peak-height ratios were linearly correlated with TRA concentrations from 25 to 2000 µg/L (r² = 0.9994, y = 0.0023x - 0.0309). Sensitivity, defined as signal-to-noise ratio of 3, was 5 ng. Table 1 shows the precision and recovery data. The TRA concentration in a plasma sample from a patient receiving 150 mg of TRA daily was 384 µg/L (Figure 2C). During the past 12 months, we monitored 29 TRA-treated patients receiving 50- to 300-mg daily doses and two overdose patients. Their TRA concentrations may be divided into four groups, as follows: (a) n = 1, 0 µg/L, indicative of non-compliance; (b) n = 26, range = 73 to 1678 µg/L, and mean = 639 µg/L, steady-state (trough) concentrations; (c) n = 2, 1983 and 1938 µg/L, two geriatric patients receiving 300-mg daily doses; and (d) n = 2, about 5000 µg/L, the overdose patients. The steady-state concentrations did not follow gaussian distribution, but tended to cluster between 100 and 600 µg/L.

Figure 3 shows the chromatograms of plasma extracts containing MIA. The column retention volume of MIA was 9 mL. The chromatogram of "drug-free" plasma (Figure 3A) did not show any interference peak with MIA. Peak-height ratios (MIA/Cl-IMI) were linearly correlated to MIA concentrations from 25 to 200 µg/L (r² = 0.9995, y = 0.0085x - 0.0091). Sensitivity, as defined previously, was 5 ng. Table 1 shows the precision and recovery data. A volunteer's plasma concentration of MIA was 27 µg/L (Figure 3C).

Table 2 shows that nortriptyline interferes with TRA-IS, while flurazepam interferes with MIA.

Discussion

Both TRA and MIA are new "second-generation" antidepressants, for which therapeutic and toxic ranges are as yet unestablished. At present, monitoring of TRA and MIA may help to define the "therapeutic range" for a particular patient, to check for noncompliance, to check for possible causes of side effects (important for geriatric patients), and to ascertain the drug elimination in overdose cases. Thus we undertook this study.
study of standard solutions of TRA in plasma showed between-run CVs < 10%. Moreover, these solutions, if frozen at -20 °C, are stable for as long as three months.

For monitoring suspected overdose cases involving TRA, the preparation of an additional patient's sample diluted with "drug-free" plasma is necessary. Although nortriptyline co-elutes with TRA-IS, co-administration of nortriptyline and TRA to a patient is unlikely, and this co-elution would not cause an interference problem in the assay. As a precaution for monitoring patients whose medication is being changed from amitriptyline or nortriptyline to TRA, a "wash-out" period of two to three weeks should be allowed before monitoring for TRA.

Patients' Studies

During the past nine months, we used this assay to monitor patients treated with TRA. One group (n = 25) showed substantial concentrations of TRA in plasma (mean, 639 μg/L), similar to concentrations reported in the other studies as follows: 50-mg single dose (2), TRA = 800 μg/L at 1 to 1.5 h, and 100 μg/L at 25 to 30 h; 50-mg single dose (16), TRA = 700 μg/L at 2 h; 200-mg single dose (16), TRA = 2500 μg/L at 2 h; 50-mg single dose (17, 18), TRA = 550–600 μg/L; 25 mg two or three times daily, steady-state within four days (18), TRA = 500 or 900 μg/L, respectively; and 417-mg mean daily dose for responders (19) TRA = 1510 μg/L. Two geriatric patients, receiving 300 mg of TRA daily, showed high plasma concentrations of the drug of about 2000 μg/L, probably because of decreased metabolism of TRA. In two overdose patients, plasma concentrations of TRA were exceedingly high, about 5000 μg/L; as did the overdose cases reported in Europe (3, 11), both recovered with supportive therapy.

From our experience of monitoring another tetracyclic, maprotiline, by a similar procedure (15), the present assay for MIA could easily be adapted for use in both pharmacokinetics studies and routine monitoring. The procedure is simple, involving reversed-phase LC and detection at 214 nm, both of which are readily within the capabilities of most clinical laboratories.

We thank Dr. G. R. McKinney of Mead Johnson Pharmaceutical Co. for supplying TRA and Dr. R. Pinder of Organon for supplying MIA.

References

2. Ankier SI, Martin BK, Rogers MS, et al. Trazodone—a new antidepressant (16), TRA = 2500 μg/L at 2 h; 50-mg single dose (17, 18), TRA = 550–600 μg/L; 25 mg two or three times daily, steady-state within four days (18), TRA = 500 or 900 μg/L, respectively; and 417-mg mean daily dose for responders (19) TRA = 1510 μg/L. Two geriatric patients, receiving 300 mg of TRA daily, showed high plasma concentrations of the drug of about 2000 μg/L, probably because of decreased metabolism of TRA. In two overdose patients, plasma concentrations of TRA were exceedingly high, about 5000 μg/L; as did the overdose cases reported in Europe (3, 11), both recovered with supportive therapy.

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