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Note

High-performance liquid chromatographic determination of imipramine and desipramine in human serum

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Quantitative determination of antidepressants in blood from depressive patients has been suggested to be essential for pharmacotherapeutic and clinical investigations of depression [1—3]. The present studies were also attempted to establish a routine laboratory method capable of assaying simultaneously imipramine and its active metabolite, desipramine, in serum from the patients. Reversed-phase high-performance liquid chromatographic (HPLC) methods have now been evaluated to be time saving for routine monitoring of drugs as compared with methods such as normal-phase HPLC and gas chromatography. Since several reversed-phase HPLC methods for imipramine have already been presented from other laboratories [3—7], we re-examined such procedures. In our hands, the application of these methods produced chromatograms that did not allow quantitation of imipramine and desipramine, because of interference from unidentified peaks.

In the present methods following precipitation of proteins in serum with methanol, a Sep-Pak C_{18} cartridge (Waters Assoc., Milford, MA, U.S.A.) was used for purification of the HPLC sample. Our chromatographic quantitative analyses of imipramine and desipramine were simple and reproducible, demonstrating each single peak on the chromatogram with a peak of clomipramine as internal standard. Practically, the concentrations of both drugs were measured versus time in serum from patients and volunteers who were orally administered imipramine for two weeks.

EXPERIMENTAL

HPLC conditions

The equipment used was a Waters Model 6000A solvent delivery system,

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Model U6K universal injector, Data Module, Model 440 ultraviolet detector fixed at 254 nm. A reversed-phase column (15 cm × 4.6 mm I.D.; particle size 5 μm), Cosmosil $5C_{18}$ (Nakarai Chemicals, Kyoto, Japan) was employed. The operating conditions were as follows: mobile phase, acetonitrile—1% triethylamine (pH 6.0, adjusted with phosphoric acid) (38:62) at a flow-rate of 1 ml/min; injection volume, 50 μl; detector sensitivity, 0.005 a.u.f.s.

Sample preparation

Human serum was conventionally prepared by centrifugation and kept frozen at −20°C until the following procedures. Serum (1 ml) was transferred into a polypropylene centrifuge tube, and then 200 ng of clomipramine were added as internal standard, followed by addition of methanol (4 ml). The mixture was shaken for 1 min and centrifuged for 3 min at 11,000 *g*. An aliquot (4.3 ml) of the resultant supernatant was applied on a Sep-Pak C_{18} cartridge. After the cartridge was washed by 4 ml of 50% methanol, imipramine, desipramine and clomipramine were eluted with 2 ml of acetonitrile—1% triethylamine · HCl (9:1). When 200 ng of each drug were added to serum, approx. 65% of the amount was recovered in the eluate. The eluate was collected in a glass tube and evaporated under a stream of nitrogen gas. The residue was dissolved with 100 μl of the mobile phase and an aliquot (50 μl) was injected into the HPLC system.

Materials

Imipramine and clomipramine were donated by Dainihon Pharmaceutical (Osaka, Japan). Desipramine was a gift from Ciba-Geigy (Basel, Switzerland). Acetonitrile, methanol and triethylamine, which were purified for HPLC, were obtained from Nakarai Chemicals (Kyoto, Japan). The conventional distilled water was further purified by Milli-Q Reagent Water System.

RESULTS AND DISCUSSION

When a solution containing imipramine (25 ng), desipramine (25 ng) and clomipramine (100 ng) was subjected to HPLC, a chromatogram as shown in Fig. 1 was obtained with retention times of 7.6, 5.4, and 14.1 min, respectively, and each peak was sharp and symmetrical. Serum was obtained from a healthy volunteer receiving no drugs. To a portion of the serum were added three kinds of antidepressant as described above.. An HPLC sample was prepared from the serum with or without the drugs and injected for HPLC. As shown in Fig. 2, the peaks of desipramine, imipramine and clomipramine in human serum were quite distinguishable in the chromatogram and each retention time was in good agreement with that in Fig. 1. However, a small unidentified peak (peak a in Fig. 2) was observed near the peak of imipramine, whether the serum contained drugs or not.

Calibration curves were produced as follows. Working solutions were prepared by dissolving 10, 20, 30, 50, 100, 200, 300, and 500 ng each of imipramine and desipramine in 1 ml of human serum, which contained 200 ng of clomipramine. These solutions were processed, using the HPLC conditions as described in the experimental section. The ratios of the peak area of either

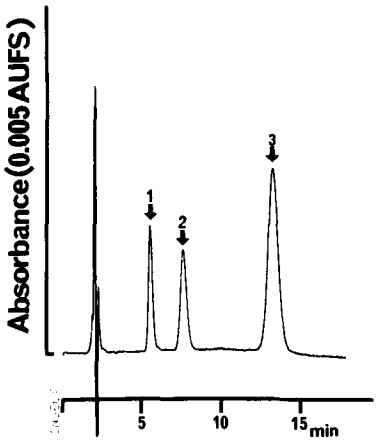

Fig. 1. Chromatogram of imipramine and desipramine with clomipramine. The standard HPLC conditions were used except that the mixture containing imipramine (25 ng), desipramine (25 ng) and clomipramine (100 ng) was directly injected into the HPLC system. The arrows at 1, 2 and 3 indicate the peaks of desipramine, imipramine and clomipramine, respectively. Each retention time is described in the text.

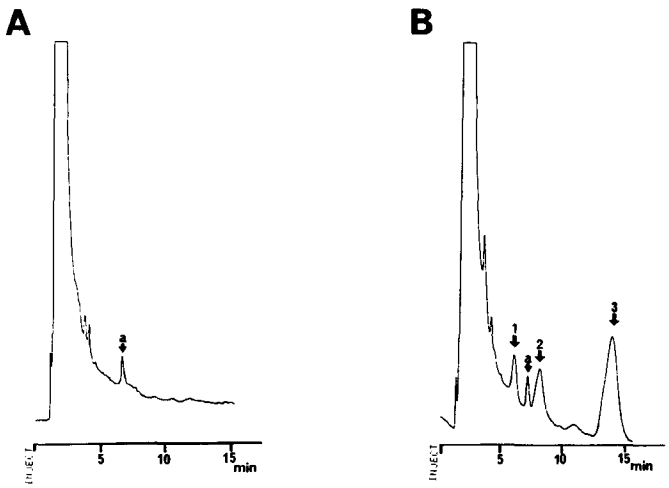

Fig. 2. Chromatograms of human serum in the absence (A) and presence (B) of antidepressants. The serum was obtained from a healthy volunteer with no medication. A 1-ml portion of the serum was spiked with desipramine (50 ng) and imipramine (50 ng) with clomipramine (200 ng). The sera with and without the drugs were used as HPLC samples. The other experimental conditions are described in the text. The arrows at 1, 2 and 3 are defined as described in Fig. 1. The arrow at "a" is an unidentified peak, of which the retention time was 6.8 min.

imipramine or desipramine to the area of clomipramine on the chromatogram were plotted versus the concentrations of imipramine or desipramine in the working solution. The curves exhibited linear relationships over concentrations of both drugs between 10 and 500 ng/ml, with a detection limit each of approx. 10 ng/ml. The reproducibilities of the calibration curves were determined by performing eight replicate analyses on aliquots of human serum, to which the tricyclic drugs were added. In four different concentrations (50, 100,

200, and 500 ng/ml) of the drugs in serum and the coefficients of variation were less than 7%, except that the variation was 11% for the lowest concentration of desipramine. For the quantitative determination of desipramine with better reproducibility than in the present methods, the use of two internal standards might be needed.

Two healthy volunteers and two depressive patients were orally administered imipramine for two weeks. The dose was 75 mg per day, divided into three equal doses at intervals of 8 h. Blood was sampled between 9.00 and 10.00

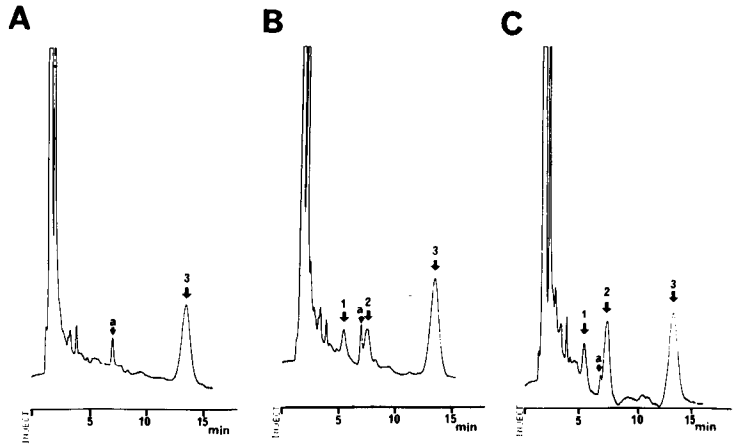

Fig. 3. Chromatograms of the serum from a healthy volunteer with no medication (A), and during the first (B) and second (C) weeks of continuous dosing with imipramine. Detailed experimental conditions are described in the text. The arrows in the chromatograms are as defined in Fig. 2.

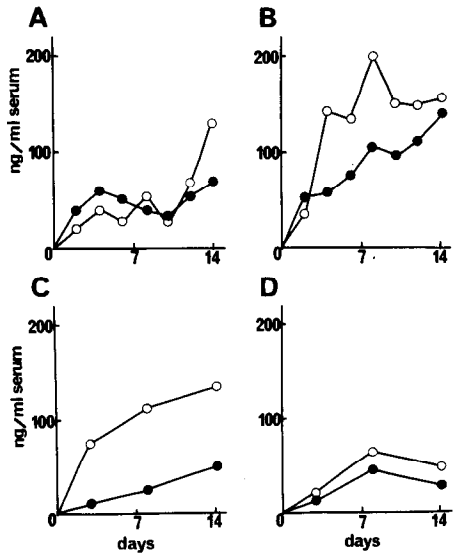

Fig. 4. Serum levels of imipramine (○) and desipramine (●) during the continuous dosing of imipramine. The abscissa shows the days after starting the administration. Two volunteers (A and B) and two depressive patients (C and D) were orally given the drug as described in the text. The other experimental conditions are described in the Experimental section.

a.m. Fig. 3 demonstrates chromatograms of the serum from one of the volunteers during the first and second weeks after the start of imipramine administration. The concentrations of imipramine and desipramine in the serum were calculated to be 53 and 40 ng/ml, respectively, in the first week. In the second week the values increased to 130 for imipramine and 70 for desipramine. The concentrations of both drugs in serum from two volunteers and two patients were quantitatively determined at various times during continuous dosing. The results are shown in Fig. 4, suggesting interindividual variation of the ratio of desipramine to imipramine and on the rates of increases in their serum levels.

Using a Sep-Pak C_{18} cartridge and a reversed-phase column, the present procedures were simple and reproducible with fewer unidentified peaks in the chromatogram. The usefulness of C_{18} bonded-phase disposable columns such as Sep-Pak C_{18} cartridges has been demonstrated for gas chromatographic analyses of tricyclic antidepressants [8, 9]. Also in the present methods the Sep-Pak C_{18} procedure was essential for the purification of the HPLC sample from serum. But a small unidentified peak (peak a) was always present near the peak of imipramine in the chromatogram and was suggested to originate from human serum itself. In the present methods the hydroxylated derivatives of imipramine and various kinds of minor tranquillizers such as benzodiazepines were washed out by 50% methanol from the cartridge and no peaks attributable to them were observed in the chromatogram.

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