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Note

Chromatographic assay of 10-[3-(3-hydroxypyrrolidinyl)propyl]-2-trifluoromethyl phenothiazine using electrochemical detection

SHINJI SHIBANOKI, TAIZO KUBO and KOICHI ISHIKAWA*

Department of Pharmacology, Nihon University School of Medicine, Oyaguchi-Kami Machi, Itabashi, Tokyo 173 (Japan)

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10-[3-(3-Hydroxypyrrolidinyl)propyl]-2-trifluoromethyl phenothiazine hydrochloride (E-0663, I) is a novel phenothiazine derivative, of which the chemical structure is shown in Fig. 1. The compound has been expected to act as a long-lasting antipsychotic agent. The present study was carried out to develop a simple procedure for determining the pharmacokinetics of the drug in biological samples.

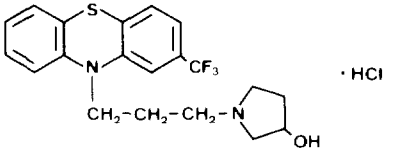

Fig. 1. Chemical structure of 10-[3-(3-hydroxypyrrolidinyl)propyl]-2-trifluoromethyl phenothiazine hydrochloride (E-0663, I).

Electrochemical detection (ED) has been employed for the determination of substances metabolically related to monoamine transmitters in the brain [1—3] and biological fluids [4], combined with high-performance liquid chromatography (HPLC). The detector was reported to be effective for the quantitative determination of some drugs that possess phenolic hydroxyl groups [5—7] or a heterocyclic structure [8, 9]. In a previous report, we also demonstrated a simple procedure for the assay of chlorpromazine (CPZ) in the brain and blood of mice [10]. The HPLC—ED technique was therefore also applied to the assay of compound I.

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EXPERIMENTAL

Chemicals

Compound I was a generous donation from Eisai (Tokyo, Japan). Promethazine (PMZ) hydrochloride, the chromatographic internal standard, were obtained from Shionogi Pharmaceuticals (Osaka, Japan). The other chemicals for extraction and chromatography were all purchased from Wako Pure Chemicals (Osaka, Japan).

Apparatus

A liquid chromatographic system (L-2000, Yanagimoto, Kyoto, Japan) was used with a six-port injector (7125, Rheodyne, Berkeley, CA, U.S.A.) and a glassy carbon electrochemical detector (VMD-101, Yanagimoto). The analytical column was an Ultrasphere-ODS reversed-phase column (average particle size 5 μm; 250 × 4.6 mm I.D.; Altex Scientific, Berkeley, CA, U.S.A.). To protect the analytical column, a short ODS column (10 × 4.5 mm I.D.) was also used. The detector potential was set at 800 mV vs. the Ag/AgCl reference electrode.

The chromatographic mobile phase consisted of a mixture of 0.1 *M* acetate buffer (pH 3.5)—acetonitrile—tetrahydrofuran—pyridine (100:200:3:0.3). The flow-rate was set at 1.5 ml/min.

Animals

ICR mice weighing ca. 30 g were used. All animals were kept in a room with a controlled temperature (23 ± 0.5°C), humidity (55 ± 5%) and light cycle (12 h illumination with the light being turned on at 07:00). They were injected intravenously with 10 mg/kg compound I, and sacrificed by decapitation at different times after the injection. Brain samples were obtained as quickly as possible after decapitation. The samples were stored in a deep-freeze (−80°C) until assay. Blood samples were collected from the carotid artery when the animals were decapitated. The whole blood was also stored in a deep-freeze.

Extraction procedure

The brain sample that had been stored was homogenized in a glass-stoppered tube containing 1 ml of 1 *M* sodium hydroxide and the internal standard (PMZ). A 5-ml aliquot of an organic solvent mixture of heptane—isoamyl alcohol (99:1) was added to the tube. The tube was vortexed for 1 min and then centrifuged for 3 min to separate the organic layer, of which 4 ml were transferred to another tube. Then, 100 μl of 0.1 *M* hydrochloric acid were added to the tube and the mixture was vortexed for 1 min. After a brief centrifugation, a portion of the hydrochloric acid layer was employed as the chromatographic sample. In the case of the blood samples, 200 μl of the sample were transferred to a glass-stoppered tube containing the internal standard and 500 μl of 1 *M* sodium hydroxide. The tube was agitated vigorously and then 5 ml of the organic mixture were added. Subsequent procedures were the same as for the brain samples. The final hydrochloric acid layer was injected into the HPLC system.

RESULTS AND DISCUSSION

HPLC—ED represents a useful chromatographic approach for substances possessing phenolic hydroxyl groups and a heterocyclic structure. While the major application is in the determination of substances metabolically related to monoamine transmitters in the brain [1–3] and biological fluids [4], some recent reports have demonstrated applications to the pharmacokinetics of morphine [5], benzodiazepines [8] and tricyclic antidepressants [9]. The detector has been reported to be applicable to the determination of CPZ, a phenothiazine, in the brain as well as in the blood [10]. It was also expected therefore that compound I, a novel phenothiazine derivative, could be measured by HPLC—ED if appropriate conditions for the chromatographic separation and detector potential were selected.

The electrochemical response depends upon the applied potential. Although a higher potential yields a greater response, the background noise is also increased. Therefore, the optimal potential should be determined for each substance. Fig. 2 shows hydrodynamic voltammograms for compound I and PMZ. Although the responses differed from each other, the potential that yielded the maximum response was almost the same, being ca. 750 mV for both the phenothiazines.

The critical factor in the separation of compound I from the internal standard was the concentration of acetonitrile in the mobile phase. The retention time of phenothiazines was shortened by increasing the concentration of the organic solvent (Fig. 3). A higher concentration (> 60%) of acetonitrile made it impossible to separate the internal standard and compound I. Delayed retention was also insufficient for chromatography since the peaks became broader, leading to a decreased peak height. An acetonitrile concentration of 55% was optimal. Under such conditions, one chromatographic run was finished within 7.5 min. A typical chromatogram is illustrated in Fig. 4. No endogenous substances interfered with the assay of phenothiazine at an applied potential of 750 mV.

The recovery and precision were investigated by adding known quantities of compound I to blank samples of whole brain and analysing the samples

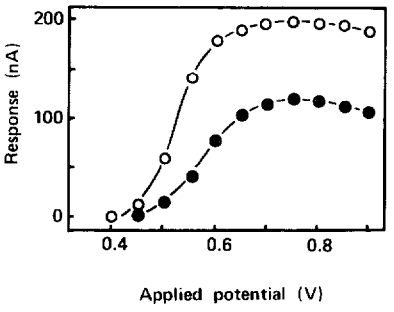


Fig. 2. Hydrodynamic voltammogram of compound I (•) and PMZ (internal standard for the assay; ○).

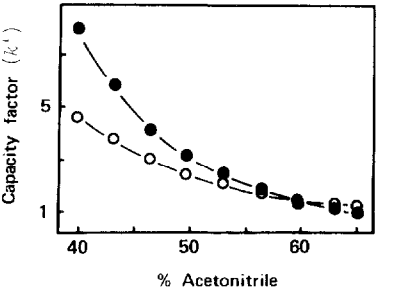
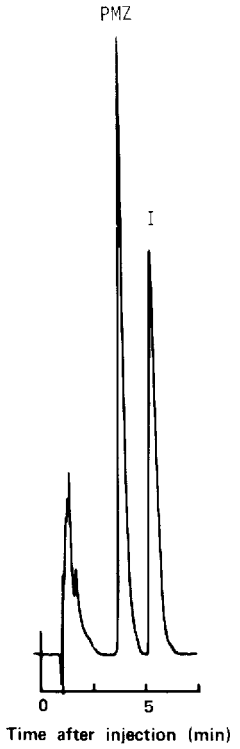


Fig. 3. Effect of concentration of acetonitrile on the retention time of compound I (•) and PMZ (○).



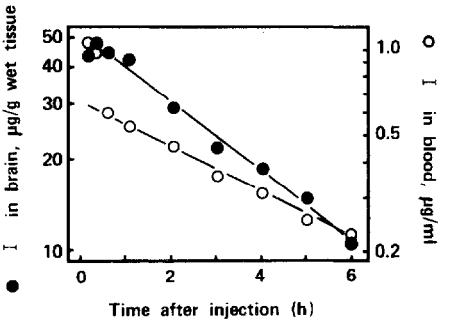


Fig. 4. Typical chromatogram obtained from the mouse brain injected intravenously with 10 mg/kg compound I 1 h before sacrifice. See text for the procedures of extraction and chromatography.

Fig. 5. Disappearance curves of compound I from the brain (•) and blood (○). The biological half-lives were estimated to be 3 and 2.5 h in the brain and blood, respectively. The regression coefficients for linearity were calculated to be −0.993 and −0.991, respectively.

according to the procedure described. The absolute recovery of compound I and PMZ with the present extraction from brain samples, after adjusting for solvent loss, were estimated at 85 ± 2 and 88 ± 3%, respectively. The coefficients of variation were calculated to be ca. 4% for compound I. The quantitative determination was based on peak heights. The ratio of the peak heights for the phenothiazine and the internal standard were compared with samples and standards taken through the entire extraction procedure. With this procedure, the ratios of the phenothiazine and the internal standard varied linearly with the amount of drug added in the range between 2 ng and 1 μg. This made it possible to calculate the concentration from a simple measurement of the ratio.

The time course of compound I disappearance was examined after a 10 mg/kg intravenous injection (Fig. 5). The maximum intracerebral concentration of the drug was obtained at 15 min after the injection. The biological half-lives were estimated to be ca. 3 and 2.5 h for the blood and brain, respectively. These values were remarkably longer than those of CPZ, for which the half-lives were 1.1 and 1.3 h, respectively [10]. This may be related to the long-lasting action of the drug on monoamine metabolisms.

The present method provides a simple and sensitive procedure for the determination of the novel phenothiazine compound, 10-[3-(3-hydroxypyrrolidinyl)propyl]-2-trifluoromethyl phenothiazine, in the brain and blood of mice by a combination of the extraction with an organic mixture and HPLC—ED. This method may be applicable in clinical studies to monitor the concentration of the drug in the blood.

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