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Note

Determination of a novel calcium entry blocker, AJ-2615, in plasma using solid-phase extraction and high-performance liquid chromatography with electrochemical detection

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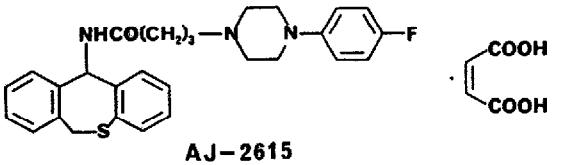
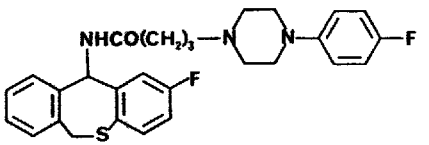
11-[4-[4-(4-Fluorophenyl)-1-piperazinyl]butyryl]amino-6,11-dihydrodibenzo-[*b*,*e*]thiepin maleate (AJ-2615, AJ, Fig. 1) [1] is a novel calcium entry blocker that exhibits gradual and long-lasting antihypertensive activity and myocardial protective effects in experimental models [2].



AJ–2615



Internal Standard

Fig. 1 Structures of AJ-2615 and the internal standard.

This paper describes a sensitive and simple method for the determination of AJ in plasma by the combination of solid-phase extraction and high-performance liquid chromatography (HPLC) with electrochemical detection (ED). The application of this method to a single-dose pharmacokinetic study in animals demonstrates its utility.

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EXPERIMENTAL

Materials

AJ and 11-[4-[4-(4-fluorophenyl)-1-piperazinyl]butyryl]amino-2-fluoro-6,11-dihydrodibenzo[*b*,*e*]thiepin (internal standard, I.S., Fig. 1) were synthesized in the research laboratories of Dainippon Pharmaceutical. Bond Elut C_{18} cartridges (200 mg) were obtained from Analytichem International (Harbor City, CA, U.S.A.). Bovine serum albumin (BSA, fraction V, 96–99% albumin) was purchased from Sigma (St. Louis, MO, U.S.A.). HPLC-grade acetonitrile and distilled water were obtained from Wako (Osaka, Japan). Other chemicals were of analytical grade.

Cyclic voltammetry

The cyclic voltammograms were obtained in a potentiostat/galvanostat HA301 with a function generator HB105 (Hokuto Denko, Tokyo, Japan) at a scan-rate of 30 mV/s. Glassy carbon served as the working electrode and a stainless-steel rod as the counter electrode. A saturated calomel electrode (SCE), separated from the bulk solution by an agar bridge, was used as the reference electrode. Current–voltage curves were recorded on a Type 3086 X–Y recorder (Yokogawa-hokushin, Tokyo, Japan).

Chromatographic instrumentation

The chromatographic system consisted of an ALG/GPC-204 liquid chromatograph (Waters Assoc., Milford, MA, U.S.A.) and an ECP-1 amperometric detector with a glassy carbon electrode and Ag/AgCl reference electrode (Kotaki, Tokyo, Japan). A Develosil ODS-7 column (7 μm, 25 cm × 4.6 mm I.D., Nomura Chemical, Aichi, Japan) was used at 35°C. Acetonitrile–0.1 *M* KH_2PO_4 containing 0.5 m*M* disodium EDTA (50:65, v/v) was used as the mobile phase at a flow-rate of 1.5 ml/min.

Analytical procedure

To a 10-ml glass test-tube, 0.5 ml of plasma and 1 ml of 0.2 *M* acetate buffer (pH 4.0) containing 100 ng of I.S. and 0.2% (w/v) BSA were added. After pH adjustment to 4.0 with 0.1 ml of 1 *M* hydrochloric acid, the solution was passed through a Bond Elut C_{18} cartridge and washed successively with 10 ml each of distilled water, methanol and chloroform. AJ and I.S. were then eluted with 3 ml of 0.1 *M* citrate buffer (pH 4.0)–methanol (40:60, v/v). The eluate (pH 4) was placed in a glass-stoppered tube, and 3 ml of chloroform were added. The tube was shaken for 10 min with a reciprocal shaker and centrifuged at 1600 *g* The organic layer (3 ml) was transferred to another tube and evaporated to dryness under a stream of nitrogen at 40°C. The residue was dissolved in 25 μl of acetonitrile, and an aliquot of 10 μl was injected into the column.

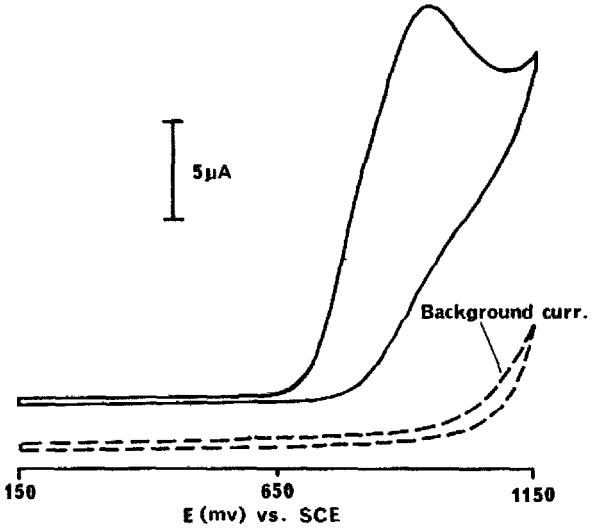

Fig. 2 Cyclic voltammogram of AJ (0.33 mg/ml) in the mobile phase

RESULTS AND DISCUSSION

Electrochemistry

The cyclic voltammogram of AJ exhibited an irreversible oxidation curve with a peak potential (E_p) at +940 mV *versus* SCE as shown in Fig. 2. This suggests that AJ is detectable using HPLC–ED. Fig. 3 shows a hydrodynamic voltam-

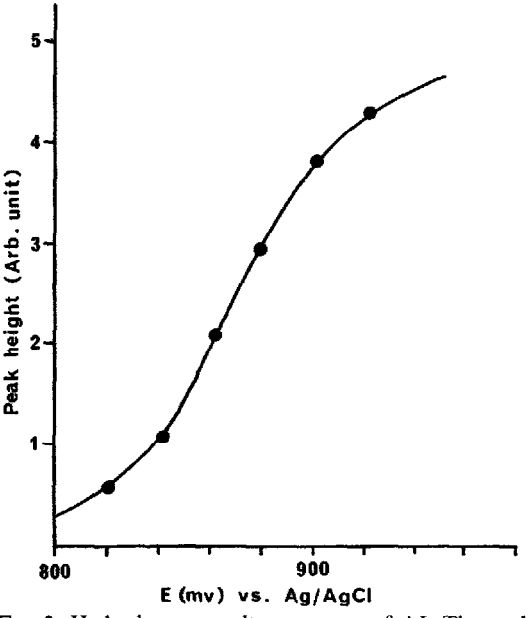

Fig. 3. Hydrodynamic voltammogram of AJ The peak heights of AJ as a function of the electrode potential are shown.

mogram under the conditions chosen for chromatographic separation. Although the detector response for AJ is proportional to the applied potentials in the range from +800 to +920 mV *versus* Ag/AgCl, we chose +900 mV as the applied potential in order to decrease the noise arising from plasma components. Under these conditions the absolute sensitivity of ED was *ca.* twenty times greater than that of UV (254 nm) detection.

The mechanism of the electrochemical oxidation of AJ is different from that of phenothiazine [3] and has not yet been explored.

Extraction and purification

Preliminary examination revealed that AJ and I.S. were lost to some extent by adsorption on the glass surface. To prevent this, BSA was added to the I.S. solution and the AJ standard solution. AJ and I.S. were strongly retained on the Bond Elut C_{18} cartridge because of their lipophilic properties. Despite successive washing with water, methanol and chloroform, neither AJ nor I.S. could be liberated from the cartridge at all. Those compounds were well eluted with 0.1 *M* citrate buffer (pH 4.0)–methanol (40:60). The absolute recovery of AJ was *ca.* 87%, based on the comparison of the HPLC peak areas of the spiked plasma samples with those of each methanolic standard solution, in respect of the volumes handled through the procedure.

Calibration curve

Blank plasma samples spiked with AJ at known amounts were assayed as described above. The peak-height ratios *versus* concentrations yielded a straight-line relationship in the range 4–400 ng/ml with a correlation coefficient of 0.999.

Precision and accuracy

In order to evaluate within-day variation, we prepared two different plasma samples containing 200 and 5 ng of AJ as the presumed higher and lower plasma levels, respectively. By replicate analyses we assessed the precision (coefficient of variation) and accuracy of the present method. These are given in Table I.

TABLE I

PRECISION AND ACCURACY OF DETERMINATION OF AJ IN RAT PLASMA (n = 6)

Concentration added (ng/ml)	Concentration recovered (mean ± S.D.) (ng/ml)	Coefficient of variation[a] (%)	Accuracy[b] (%)
5.00	5.20 ± 0.24	4.6	4.0
200.0	204.7 ± 3.2	1.6	2.3

[a] S.D. × 100/mean.

[b] The deviation of the mean recovered concentration from the added concentration.

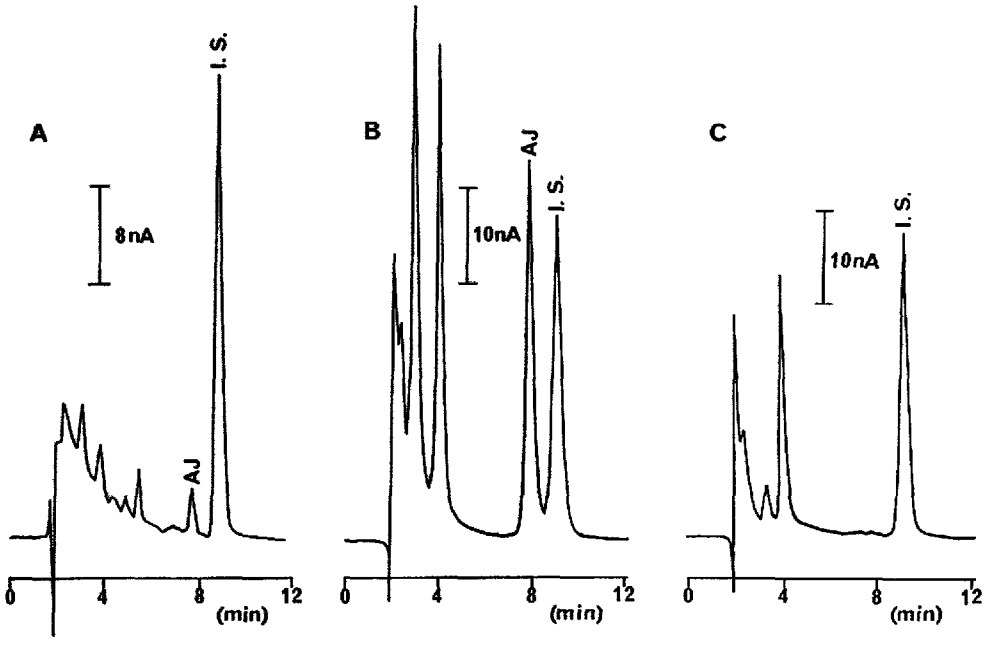

Fig 4. HPLC of extracts from (A) a dog plasma sample at 1 h (AJ 21 ng/ml) after oral administration of 5 mg/kg AJ, (B) a rat plasma sample at 1 h (AJ 256 ng/ml) after oral administration of 30 mg/kg AJ and (C) a rat blank plasma spiked only with I.S

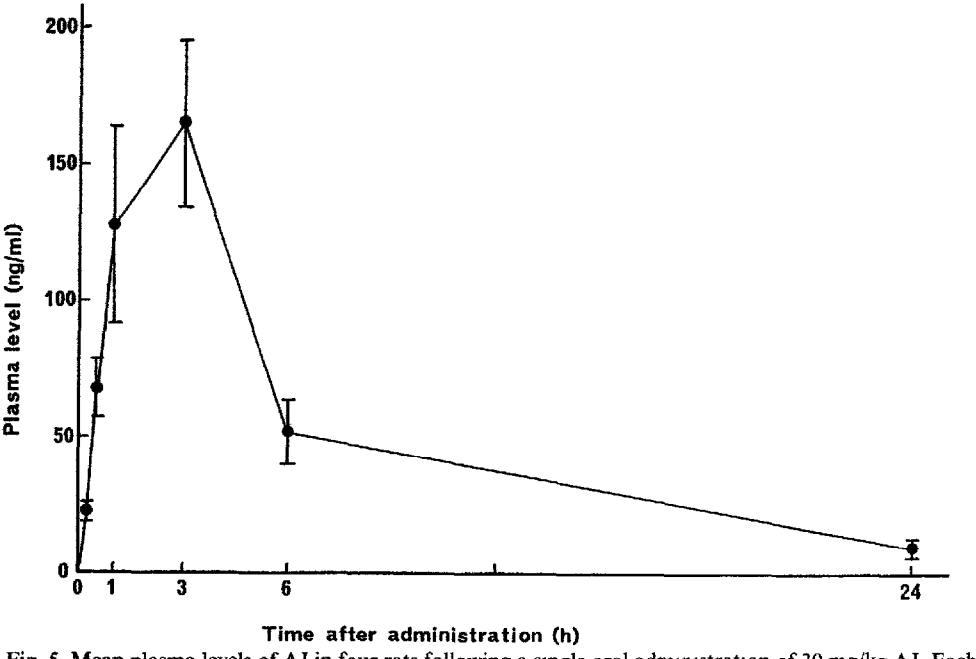

Fig. 5. Mean plasma levels of AJ in four rats following a single oral administration of 30 mg/kg AJ. Each point represents the mean ± standard error for four rats

Chromatography

Typical chromatograms of a dog plasma sample (dose 5 mg/kg), a rat plasma sample (dose 30 mg/kg) and a rat blank plasma spiked only with I.S are shown in Fig. 4. The retention times of AJ and I.S. were *ca.* 8 and 9 min, respectively. No interfering peaks were observed. The determination limit of AJ in plasma (0.5 ml) was *ca.* 1 ng/ml, which is sufficient for the plasma levels expected in biological specimens from rats and dogs.

Application to biological samples

The proposed method was applied to the determination of AJ in plasma samples. Fig. 5 shows the mean plasma levels of AJ in rats after a single oral dose of 30 mg/kg AJ. The maximum plasma level of 165 ng/ml was attained 3 h after administration.

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