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Note

Determination of denopamine in human and dog plasma by high-performance liquid chromatography with electrochemical detection

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Denopamine, (−)-*R*-(*p*-hydroxyphenyl)-2-[3,4-dimethoxyphenethylamino]ethanol, is a new, orally active and selectively positive inotropic agent, which has been developed by Tanabe Seiyaku [1,2]. The pharmacological action of denopamine was found to be closely related to the plasma concentration of denopamine by Nagao et al. [3]. Suzuki et al. [4–6] developed a gas chromatographic–mass spectrometric (GC–MS) assay to determine denopamine and its metabolites in biological specimens. They found that glucuronide conjugates of five denopamine metabolites were excreted in human urine after oral administration of denopamine, but none of the metabolites appeared in the plasma [4]. This indicates that the metabolites do not contribute to the pharmacological actions in humans [7].

Though the GC–MS method has adequate sensitivity and precision for the investigation of the metabolism and disposition of denopamine, the necessary instrumentation is too expensive to be widely available in clinical laboratories. Consequently, a simple and sensitive assay method is required to determine denopamine for clinical monitoring and bioavailability studies.

This paper describes an assay using high-performance liquid chromatography (HPLC) with electrochemical detection (ED). Since denopamine has

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an electrochemically detectable hydroxy group (Fig. 1), the method was expected to be appropriate for the determination of denopamine in human plasma.

EXPERIMENTAL

Materials

Denopamine was synthesized by Tanabe Seiyaku. Phenolphthalin (internal standard) was purchased from Wako (Osaka, Japan). Disposable reversed-phase extraction columns, Bond Elut C_{18} (100 mg), were purchased from Analytichem International (Harbor City, CA, U.S.A.). All other solvents and chemicals were of analytical-reagent grade.

Chromatography

The measurements were carried out with a Yanagimoto Model LC-4000S liquid chromatograph equipped with a Waters Model 710B WISP automated sample injector, a Yanagimoto Model LA-100A column oven, a Yanagimoto Model VMD501 electrochemical detector and a Shimadzu Model C-R2A data processor. The analytical column was a Nucleosil $5C_{18}$ (150 mm × 4.6 mm I.D.; 5 μm particle size; Macherey-Nagel, F.R.G.). The mobile phase was acetonitrile–0.1 *M* dipotassium hydrogenphosphate (27:100, v/v) and the pH was adjusted to 5.5 with orthophosphoric acid. The flow-rate of the mobile phase was 0.9 ml/min. The chromatography was carried out at 25 °C. The potential of the detector was set at +750 mV versus an Ag/AgCl reference electrode.

Assay procedure

Heparinized blood was withdrawn from healthy volunteers and centrifuged (2000 *g*, 10 min). To 1 ml of the plasma sample, 1 ml of 1 *M* dipotassium hydrogenphosphate solution and 10 ml of chloroform were added. The mixture was mechanically shaken for 15 min and centrifuged (2000 *g*, 10 min) and then 8 ml of chloroform were taken into another tube. Next, 9 ml of 0.1 *M* hydrochloric acid were added to the organic layer. This was shaken for 15 min and centrifuged (2000 *g*, 15 min), then 8 ml of aqueous phase were taken and weakly

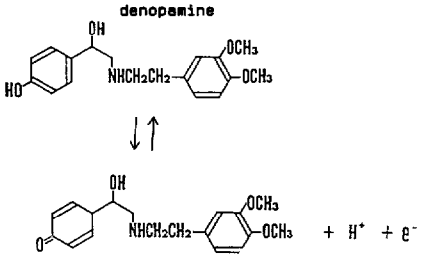

Fig. 1. Oxidation–reduction equilibrium of denopamine.

alkalinized with 1.5 ml of 0.5 *M* sodium hydrogencarbonate solution containing 0.05 *M* sodium hydroxide. All of the solution was loaded on to a Bond Elut C_{18} solid-phase extraction column. The column was washed with 0.3 ml of 25% (v/v) methanol in 0.1 *M* hydrochloric acid. Denopamine was eluted from the column with 0.3 ml of ethanol containing 0.09% (v/v) of concentrated hydrochloric acid. The whole eluate was collected and evaporated to dryness, and the residue was dissolved in 0.3 ml of the mobile phase containing phenolphthalin (125 ng/ml) as the internal standard. Finally 100 μl of this sample were injected into the chromatographic system. For the "standard plasma", 1 ml of plasma collected before dosing was spiked with an aliquot of denopamine and treated in the same manner.

RESULTS AND DISCUSSION

Electrochemical characteristics of denopamine

Denopamine exhibits the oxidation–reduction equilibrium shown in Fig. 1. The current–voltage curve of denopamine is shown in Fig. 2. In view of the magnitude of the response for denopamine and the height of noise peaks, the potential of the detector was set at +750 mV versus an Ag/AgCl reference electrode.

Chromatography

Fig. 3 shows typical chromatograms of the unspiked and spiked plasma after oral administration of denopamine in humans. Denopamine and the internal standard were both eluted as single symmetric peaks at 10 and 17 min, respectively, without any interference from plasma component peaks. It was apparent that the chromatograms of plasma after dosing also gave at the same retention time as that of denopamine in the chromatograms of the spiked plasma.

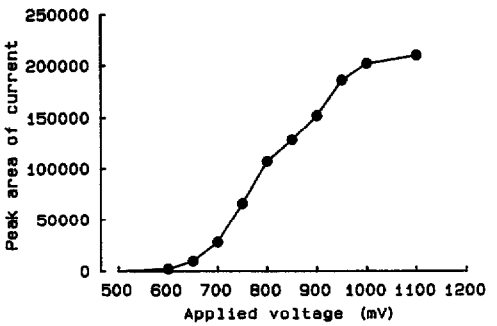

Fig. 2. Current–voltage curve of denopamine: 10 ng of denopamine were injected into the HPLC-ED system.

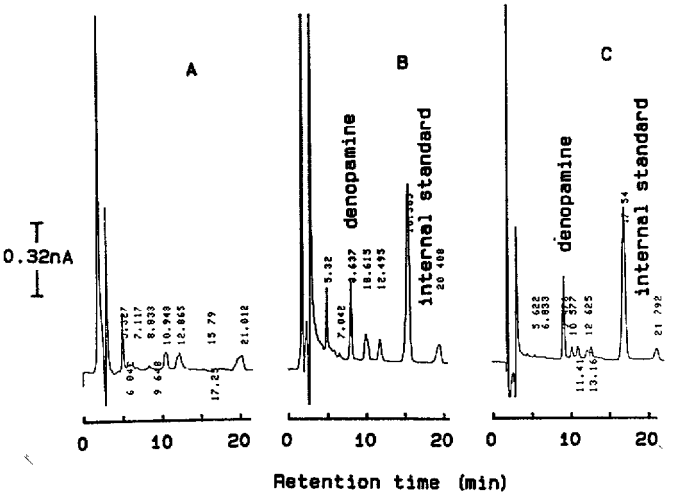

Fig. 3. Typical chromatograms of denopamine in human plasma. (A) Unspiked plasma; (B) plasma spiked with 20 ng of denopamine; (C) plasma after oral administration of 10 mg of denopamine.

Reproducibility and specificity

This method gave a good linearity between the peak-area ratio of denopamine to internal standard and the spiked amount. The linear regression of this curve was $y=0.0241x+0.0124$ ($r=0.999$) for denopamine, where y and x are the peak-area ratio and the concentration (ng/ml) of denopamine. The detection limit of denopamine in plasma (at a signal-to-noise ratio of 3) was 2 ng/ml. The recovery of denopamine was $90\pm3\%$ ($n=3$) at plasma samples spiked with 20 ng/ml denopamine.

Table I shows the coefficients of variation (C.V.) obtained from the repeated determination for spiked plasma samples. The results indicated that reproducibility of the method was sufficient for it to be used to evaluate the bioavailability of the drug.

The plasma concentrations of denopamine after oral administration of 5 mg of denopamine to a beagle dog were determined by this method and separately by a GC–MS method [8]. It was found that the two analytical methods provided similar plasma concentration–time profiles (Fig. 4).

Denopamine may be co-administered with other drugs, such as digoxin, enalapril, captopril, furosemide, nifedipine, diltiazem and nitroglycerine, in clinical practice. However, these potential contaminants were confirmed to be separated from denopamine in the HPLC analysis. Although the metabolites and their glucuronides do not appear in plasma [4], these compounds were also found to be separated from denopamine.

TABLE I

INTRA-ASSAY REPRODUCIBILITY FOR DENOPAMINE IN HUMAN PLASMA

Repetition	Peak-area ratio				
	5 ng/ml	10 ng/ml	15 ng/ml	20 ng/ml	30 ng/ml
1	0.157	0.253	0.298	0.491	0.734
2	0.149	0.258	0.374	0.485	0.700
3	0.122	0.255	0.377	0.487	0.715
4	0.136	0.234	0.390	0.518	0.762
5	0.130	0.260	0.379	N.A.[a]	0.778
Mean	0.139	0.252	0.364	0.495	0.738
S.D.	0.014	0.010	0.037	0.015	0.032
C.V. (%)	10.1	4.0	10.2	3.0	4.3

[a]Sample not analysed.

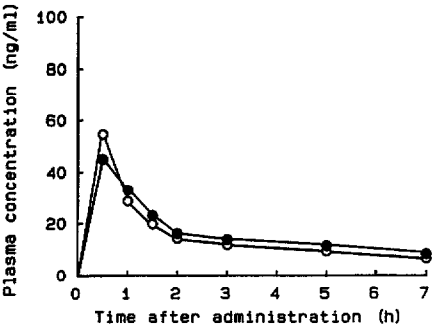

Fig. 4. Typical example of comparative plasma concentrations of denopamine measured with HPLC-ED (●) and GC-MS (○): 5 mg of denopamine were orally given to a dog.

Application

The HPLC-ED method was applied to the evaluation of the bioavailability of three different denopamine preparations in healthy humans. Each volunteer received 10 mg of denopamine in a three-way crossover design. Fig. 5 shows a comparison of the mean plasma concentration–time profiles of denopamine among three preparations. Table II gives the pharmacokinetic parameters of denopamine in each preparation. The measurements revealed that the plasma concentration of denopamine increased rapidly, then reached the maximum at 0.5–2 h and declined with an elimination half-life of ca. 4 h in human subjects. The three oral preparations of denopamine were shown to be bioequivalent to each other by statistical analysis of the parameters.

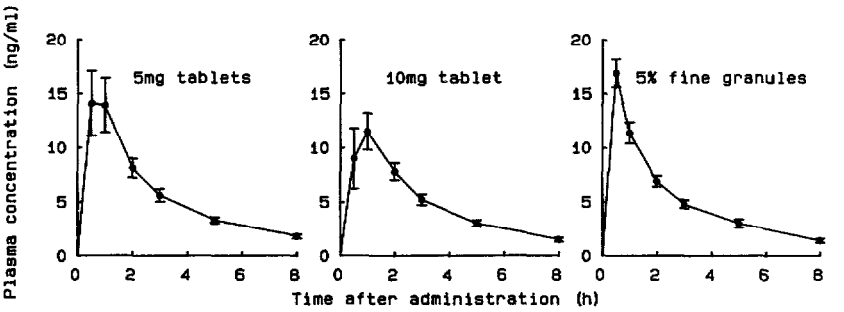

Fig. 5. Mean plasma concentration of denopamine versus time curve after oral administration of 10 mg of denopamine in three preparations to nine healthy volunteers. Each point represents the mean ± standard error.

TABLE II

PHARMACOKINETIC PARAMETERS OF DENOPAMINE AFTER ORAL ADMINISTRATION

Each value represents the mean ± standard error for nine healthy volunteers.

Preparation	C_{max} (ng/ml)	T_{max} (h)	AUC_{24} (ng h/ml)	AUC_{∞} (ng h/ml)	$t_{1/2}$ (h)
5-mg Tablets (two tablets)	19.2 ± 2.4	0.83 ± 0.17	64.4 ± 5.5	68.3 ± 6.4	4.02 ± 0.88
10-mg Tablets (one tablet)	15.5 ± 1.6	0.89 ± 0.16	54.3 ± 3.3	57.2 ± 3.5	4.00 ± 0.93
5% Fine granules (200 mg)	16.9 ± 1.3	0.50 ± 0.00	56.7 ± 4.5	59.3 ± 4.8	3.67 ± 0.61

CONCLUSION

We have established an HPLC–ED method that is simple, but sensitive, selective and reproducible for determination of denopamine in human and dog plasma. The method was applied to the determination of denopamine in human plasma after oral administration of a 10-mg dose. Because of its accuracy and simplicity, the method is expected to be useful for the clinical monitoring of this drug.

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