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Short Communication

Simultaneous determination of a new dihydropyridine calcium antagonist (MPC-1304) and its metabolite in dog plasma by high-performance liquid chromatography with electrochemical detection

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ABSTRACT

A rapid and sensitive high-performance liquid chromatographic analysis, with electrochemical detection, has been developed for the simultaneous determination of a new calcium-channel antagonist, (±)-methyl 2-oxopropyl-1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylate (I, MPC-1304), and its active metabolite in dog plasma. The plasma extract with toluene was chromatographed on a reversed-phase column and detected by an electrochemical detector at +0.92 V. Calibration curves were linear from 2.0 to 100.0 ng/ml, and the detection limit was *ca.* 0.25 ng/ml. This method is applicable to the simultaneous determination of I and its metabolite in dog plasma following the oral administration of I.

INTRODUCTION

(±)-Methyl 2-oxopropyl-1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylate (I, MPC-1304) is a 1,4-dihydropyridine derivative, and was developed as a calcium-channel antagonist [1]. Compound I is mainly metabolized to (±)-methyl 2-hydroxypropyl-1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylate (I-keto-H_2) in humans and other animals. The structures of compound I and its active metabolite I-keto-H_2 are illustrated in Fig. 1.

For the determination of 1,4-dihydropyridine derivatives and their metabolites in plasma, various methods have been reported [2]. Gas chromatography with mass spectrometry (GC–MS) [3–6] and high-performance liquid chromato-

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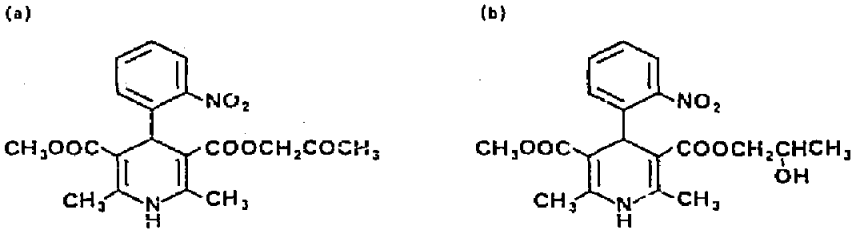

Fig. 1. Structures of (a) I and (b) I-keto-H_2

graphy (HPLC) [7–11] have been extensively developed for this purpose. However, GC–MS determinations suffer from the serious problem of oxidation of the dihydropyridines to the respective pyridines prior to analysis. Moreover, the instruments are expensive and the operation is tedious. Most of the HPLC determinations with UV detection are of low sensitivity, but sensitive detection with an electrochemical detector has been recently reported [10,11]. This paper presents a procedure for the sensitive and simultaneous determination of compounds I and I-keto-H_2 in dog plasma by HPLC with electrochemical detection (ED).

EXPERIMENTAL

Materials

Compounds I and I-keto-H_2 were supplied by Maruko (Nagoya, Japan). Nifedipine was obtained from Yodogawa (Tokyo, Japan). Sodium perchlorate was purchased from Kanto Kagaku Kogyo (Tokyo, Japan). HPLC-grade methanol and toluene were purchased from Wako (Osaka, Japan). All other reagents were of analytical-reagent grade.

Standard solutions

Standard solutions were prepared in 50% methanol solution at concentrations of 2, 5, 10, 50 and 100 ng/ml. Calibration curves were constructed by plotting ratios of the peak areas of compound I or I-keto-H_2 and the internal standard *versus* concentration. Both standard curves showed good linearity. The equation of the regression line of compound I was $y = 30.106x + 0.039$, and that of I-keto-H_2, $y = 29.916x - 0.005$.

Sample preparation

Sample preparation was according to the method of Suzuki *et al.* [10]. To 1.0 ml of plasma, 20 ng of nifedipine as an internal standard and 0.5 ml of 0.1 *M* borate buffer (pH 9.0) were added, and the mixture was vortexed for 10 s. Following the addition of 6.0 ml of toluene, the sample was shaken mechanically for 10 min and centrifuged at 1000 *g* for 15 min. The organic layer was transferred to a test-tube and evaporated to dryness at 40°C under a flow of nitrogen. The residue was dissolved in 200 μl of the HPLC mobile phase, and 50-μl aliquots were

injected into the HPLC column. All steps were carried out under yellow fluorescent lighting to prevent photodegradation of compounds I and I-keto-H_2.

Chromatography

An HPLC system consisting of a Shimadzu LC-6AD pump, a SIL-6B autoinjector, a C-R4A Chromatopac (Kyoto, Japan), and a BAS LC-4B/17AT amperometric detector (Tokyo, Japan) was used. The mobile phase, degassed by bubbling of helium, was prepared by mixing 0.36 *M* sodium perchlorate solution and methanol (55:45, v/v). The flow-rate was set at 0.8 ml/min. Chromatography was carried out on an Inertsil ODS-2 column (5 μm particle size, 150 mm × 4.6 mm I.D.) (Gasukuro Kogyo, Tokyo, Japan) at 40°C. The applied potential of the detector was set at +0.92 V against an Ag/AgCl reference electrode.

Pharmacokinetic study

A suspension of compound I (1.5 mg/kg) in 5% gum arabic was administered orally with a catheter to each of three male beagle dogs, which were fasted for a day before administration. Blood samples were drawn with a heparinized syringe at 0, 0.25, 0.5, 1, 2, 4 and 7 h after administration. Following immediate centrifugation, the plasma was stored at −80°C until analysis. The plasma levels of compounds I and I-keto-H_2 were measured as described above.

RESULTS AND DISCUSSION

Fig. 2 shows typical chromatograms of blank plasma and plasma spiked with 5.0 ng/ml I and I-keto-H_2. The retention times for I, I-keto-H_2 and the internal standard were *ca.* 16, 17 and 26 min, respectively. No interfering endogenous peaks were observed in the chromatograms. The sensitivities of both compounds were highest at a potential of +1.15 V. However, at potentials greater than

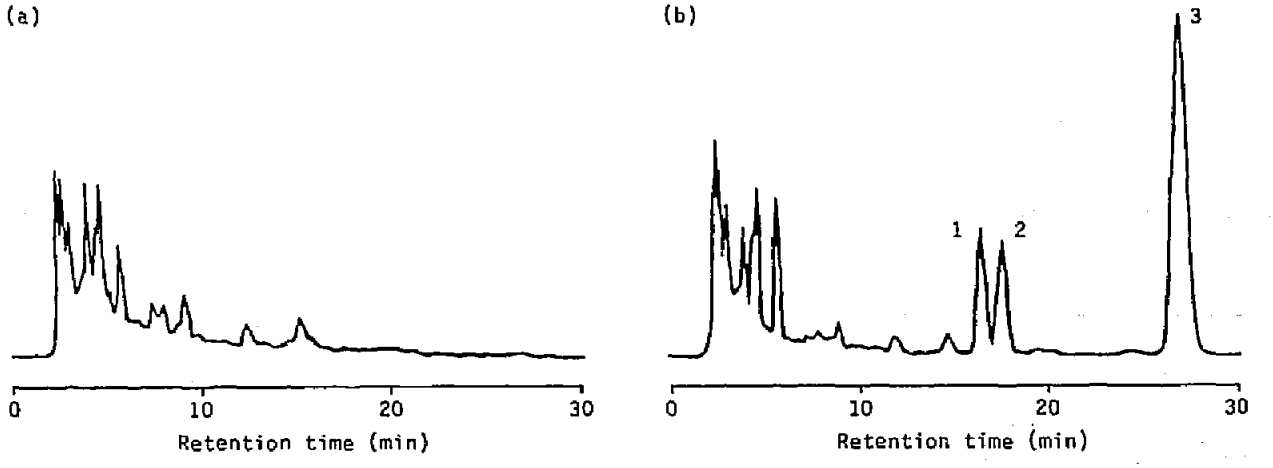

Fig. 2. Chromatograms of extracts from (a) blank plasma and (b) plasma spiked with 5.0 ng /ml each of compounds I and I-keto-H_2. Peaks: 1 = I; 2 = I-keto-H_2; 3 = I.S.

TABLE I

EXTRACTION RECOVERIES OF COMPOUNDS I AND I-KETO-H_2 FROM DOG PLASMA

Compound	Added (ng/ml)	Recovery (mean ± S.D., n = 5) (%)
I	2.5	98.3 ± 7.7
	5.0	101.9 ± 2.3
	10.0	101.7 ± 3.6
	50.0	103.2 ± 2.0
	100.0	102.1 ± 1.4
I-keto-H_2	2.5	97.6 ± 10.8
	5.0	96.5 ± 5.7
	10.0	95.7 ± 4.9
	50.0	95.8 ± 1.6
	100.0	95.5 ± 1.3

+0.95 V, the background current became high and the baseline drifted owing to oxidation of water, oxygen and other mobile phase compounds. Hence, an applied potential of +0.92 V was chosen. The detection limit of both compounds was *ca.* 0.25 ng/ml, at a signal-to-noise ratio of 2.

The recoveries of compounds I and I-keto-H_2 from dog plasma are summarized in Table I. Spiked plasma samples in the concentration range 2.5–100 ng/ml of both compounds were treated as described previously. The recoveries of compound I were 98.3–103.2% and those of I-keto-H_2 95.5–97.6%. The average recovery of compound I was 101.4% and that of I-keto-H_2 96.2%. The recovery was inadequate at a concentration below 2.5 ng/ml (data not shown). Thus, the lower practical limit of quantitation was set at 2.5 ng/ml for both compounds.

The within-day reproducibility was assessed for five spiked plasma samples at each concetration on the same day. The day-to-day reproducibility was assessed for three spiked samples at each concentration on three different days. As shown in Table II, the within-day coefficients of variation (C.V.) of compound I were 1.9–3.6% and those of I-keto-H_2, 1.6–5.9%. The day-to-day C.V. of compound I were 2.2–3.9% and those of I-keto-H_2, 3.5–6.9%.

Fig. 3 shows the mean plasma levels of compounds I and I-keto-H_2 following the oral administration of 1.5 mg/kg I to dogs. After rapid absorption, the maximum plasma concentrations (C_{max}) were 84.5 ng/ml for I and 21.0 ng/ml for I-keto-H_2. The area under the curve (AUC) of the active metabolite I-keto-H_2 was one fourth that of compound I.

TABLE II

REPRODUCIBILITY FOR COMPOUNDS I AND I-KETO-H_2 IN DOG PLASMA

Compound	Added (ng/ml)	Within-day		Day-to-day	
		Found[a] (ng/ml)	C.V. (%)	Found[b] (ng/ml)	C.V. (%)
I	5.0	5.1 ± 0.1	2.2	5.1 ± 0.2	3.7
	10.0	10.2 ± 0.4	3.6	10.1 ± 0.4	3.9
	50.0	51.6 ± 1.0	1.9	51.6 ± 1.1	2.2
I-keto-H_2	5.0	4.8 ± 0.3	5.9	4.8 ± 0.2	3.5
	10.0	9.6 ± 0.5	5.2	9.5 ± 0.7	6.9
	50.0	47.9 ± 0.8	1.6	47.8 ± 1.7	3.6

[a] Mean ± S.D. (n = 5).
[b] Mean ± S.D. (n = 9).

CONCLUSION

A rapid and sensitive HPLC–ED method was established for the simultaneous determination of compounds I and I-keto-H_2 in dog plasma. The selectivity and precision of this method are sufficient for pharmacokinetic study. The sensitivity was *ca.* twenty times higher than that of UV detection. The assay method has been successfully used for bioavailability and bioequivalent tests of I in animals.

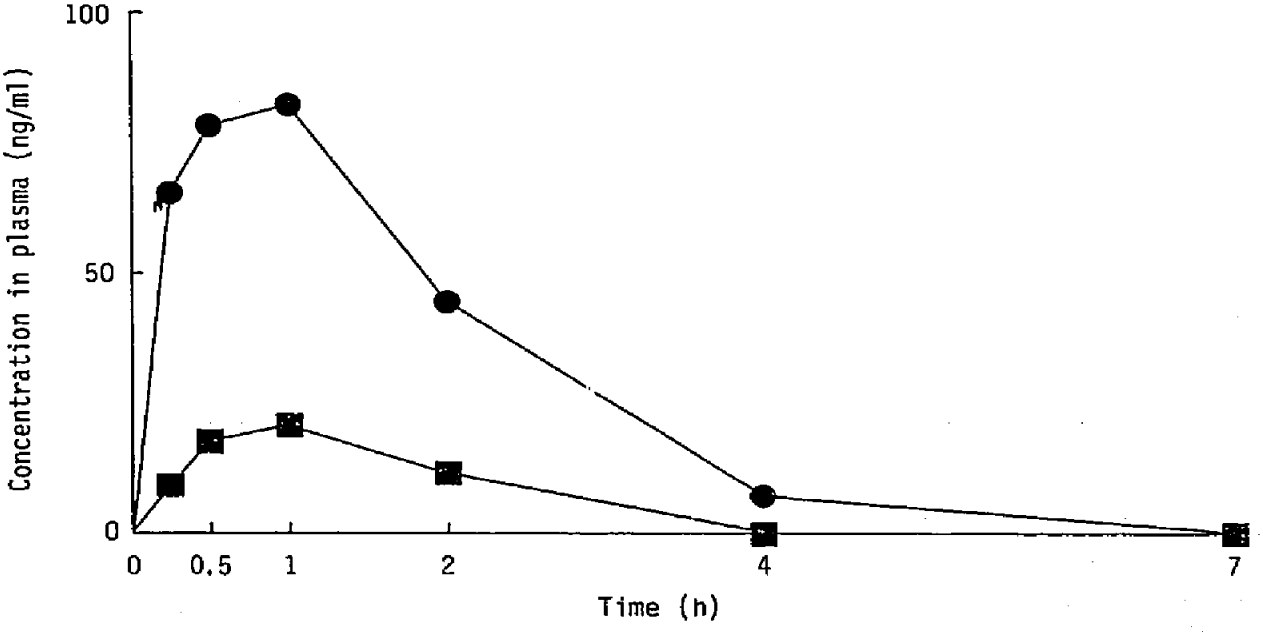

Fig. 3. Mean plasma levels of compounds I and I-keto-H_2 after oral administration of suspension of I at a dose of 1.5 mg/kg to three beagle dogs: (●) I; (■) I-keto-H_2.

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