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Note

High-performance liquid chromatographic procedure for the determination of a new anti-gastric ulcer agent, 2-(4-chlorobenzoylamino)-3-[2(1H)-quinolinon-4-yl]propionic acid, in human plasma and urine

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2-(4-Chlorobenzoylamino)-3-[2(1H)-quinolinon-4-yl]propionic acid (OPC-12 759, Ia, Fig. 1) is a new anti-gastric ulcer agent synthesized by Uchida et al. [1,2]. It was reported that Ia has an inhibitory effect for an acetic acid-induced ulcer, which is an experimental chronic ulcer model in rats. The mechanism is considered to increase the prostaglandin E_2 (PGE_2) content in the gastric mucosa [3]. Compound Ia was found to have very low toxicity in acute and subacute toxicity studies. Consequently, it was suggested that Ia has clinical potential in the therapy of gastric ulcers. Ia is currently being evaluated in clinical studies.

In this study, we established a simple, highly sensitive and selective method for the determination of plasma Ia levels in humans, using reversed-phase high-performance liquid chromatography (RP-HPLC). The plasma concentrations of Ia

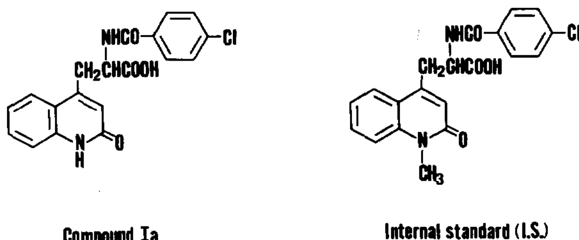

Fig. 1. Chemical structures of Ia and the internal standard (I.S.).

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were determined by this method after a single oral administration of Ia to healthy male volunteers.

EXPERIMENTAL

Chemicals

Compound Ia and its internal standard (OPC-12 823, I.S., Fig. 1) were supplied by the Second Tokushima Factory and the Laboratories of Medicinal Chemistry of Otsuka Pharmaceutical (Tokushima, Japan), respectively. All reagents and solvents used were of analytical grade or of liquid chromatographic grade (Wako, Tokyo, Japan). Standard solutions of Ia and I.S. were prepared by dissolving Ia and I.S. in methanol (0.5 mg/ml), respectively, and were appropriately diluted with methanol before use.

Chromatography

The HPLC system consisted of a Waters Assoc. ALC/GPC 204 compact system (Model 6000A pump, Model 440 UV detector equipped with a 280-nm filter kit and WISP 710B sample processor) (Milford, MA, U.S.A.), Model RF-530 fluorescence spectromonitor (λ_{ex} 330 nm, λ_{3m} 375 nm) and Chromatopack C-R1B (Shimadzu, Kyoto, Japan). A YMC Pack A-303 ODS reversed-phase column (25 cm×4.6 mm I.D., particle size 10 μm, Yamamura Chemicals, Kyoto, Japan) was used, with acetonitrile–tetrahydrofuran–acetic acid–water (32:3:1:64) as mobile phase at a flow-rate of 1.2 ml/min. The determination was performed at room temperature.

Sample preparation

Human plasma. To 0.5 ml of human plasma, 10% metaphosphoric acid aqueous solution (0.1 ml) and toluene (3.0 ml) were added. The mixture was shaken for 5 min and centrifuged at 1800 *g* for 5 min. After the toluene layer was discarded, I.S. (400 ng per 10 μl of methanol), 50% metaphosphoric acid aqueous solution (0.1 ml) and ethyl acetate (0.5 ml) were added to the remaining aqueous solution and shaken for extraction. The organic layer was transferred to another centrifuge tube and evaporated to dryness under a stream of air. The residue was redissolved in methanol (100 μl), and an aliquot (40 μl) of the solution was injected into the HPLC system. The calibration curve was constructed at Ia levels of 10–1000 ng/ml in plasma.

Human urine. To 0.5 ml of human urine, 0.5 mol/l sodium hydroxide aqueous solution (0.5 ml) and toluene (3.0 ml) were added. The mixture was shaken for 5 min and centrifuged at 1800 *g* for 5 min. After the toluene layer was discarded, I.S. (1 μg per 10 μl of methanol), 10% metaphosphoric acid aqueous solution (1.0 ml) and ethyl acetate (0.5 ml) were added to the remaining aqueous solution and shaken for extraction. The organic layer was transferred to another centrifuge tube and evaporated to dryness under a stream of air. The residue was redissolved in methanol (200 μl), and an aliquot (10 μl) of the solution was injected into the HPLC system. The calibration curve was constructed at Ia levels of 0.5–500.0 μg/ml in urine.

RESULTS AND DISCUSSION

Chromatograms of the extracts from the plasma and urine containing Ia (plasma, 0.01 and 0.1 μg/ml; urine, 0.5 and 5 μg/ml), blank plasma and urine, and human plasma and urine samples after oral dosing of Ia are presented in Figs. 2 and 3, respectively. No significant interference peaks were observed in the regions for Ia and I.S. on the chromatograms. The retention time was 6.2 min for Ia and 10.0 min for I.S., and the two compounds were well separated.

The calibration curve constructed for the determination of Ia exhibited good linearity by transforming concentrations and peak-height ratios into their logarithms at levels of 0.01–10.0 μg/ml in plasma and 0.5–500.0 μg/ml in urine, as

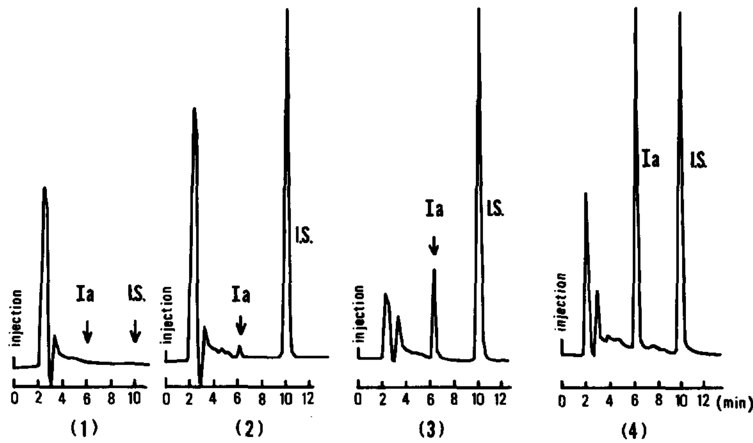

Fig. 2. Chromatograms of human plasma containing Ia and I.S.: 0.5 ml of plasma containing (1) 0 μg/ml Ia, (2) 0.01 μg/ml Ia and (3) 0.1 μg/ml Ia and (4) human samples after oral dosing of Ia were treated as described in the text.

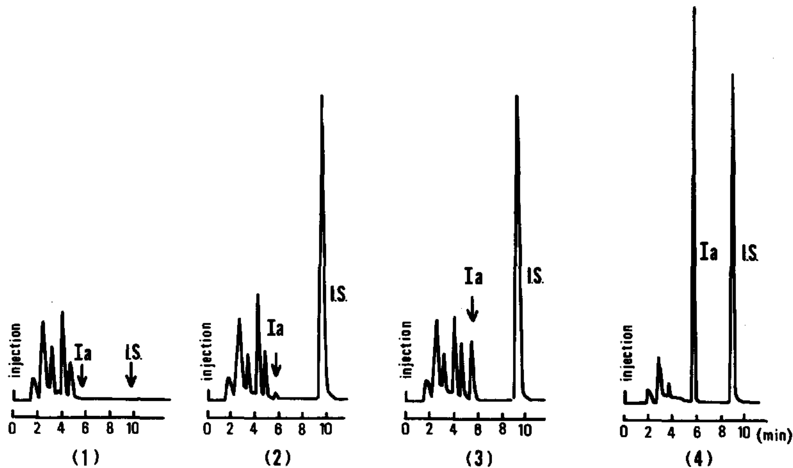

Fig. 3. Chromatograms of human urine containing Ia and I.S.: 0.2 ml of human urine containing (1) 0 μg/ml Ia, (2) 0.5 μg/ml Ia and (3) 5.0 μg/ml Ia and (4) human sample after oral dosing of Ia were treated as described in the text.

shown in Tables I and II, respectively. The accuracy of the HPLC method was determined by calculating a mean (± S.D.) peak-height ratio for each of the three standards, which were prepared for each of various theoretical concentrations of Ia in the plasma and urine.

The peak-height ratios in plasma were 0.029 ± 0.001 with a coefficient of variation (C.V.) of 3.8% at 0.01 μg/ml, 0.055 ± 0.002 with a C.V. of 3.7% at 0.02 μg/ml and 24.869 ± 0.895 with a C.V. of 3.7% at 10.0 μg/ml. At the concentrations of 0.05–5.0 μg/ml, the C.V. values were less than 2.1%. The equation for the calibration curve was $\log y = 0.9870 \log x + 0.4162$, with a correlation coefficient of 0.9999 in plasma.

The peak-height ratios in urine were 0.069 ± 0.002 with a C.V. of 2.6% at 1.0

TABLE I

ACCURACY AND REPRODUCIBILITY OF HPLC PROCEDURE FOR Ia IN HUMAN PLASMA BY INTERNAL STANDARD METHOD

Theoretical concentration (μg/ml)	Peak-height ratio (mean ± S.D., $n=3$)	Recalculated concentration* (mean ± S.D., $n=3$) (μg/ml)	Coefficient of variation (%)	Percentage of theory
0.01	0.029 ± 0.001	0.010 ± 0.001	3.8	102.9
0.02	0.055 ± 0.002	0.020 ± 0.001	3.7	99.3
0.05	0.134 ± 0.001	0.049 ± 0.001	0.7	98.7
0.10	0.260 ± 0.006	0.097 ± 0.002	2.1	96.8
0.50	1.328 ± 0.022	0.505 ± 0.008	1.7	101.0
1.00	2.639 ± 0.017	1.012 ± 0.006	0.6	101.2
5.00	13.035 ± 0.172	5.107 ± 0.068	1.3	103.3
10.00	24.869 ± 0.895	9.826 ± 0.358	3.7	98.3

*$\log y = 0.9870 \log x + 0.4162$, $r = 0.9999$.

TABLE II

ACCURACY AND REPRODUCIBILITY OF HPLC PROCEDURE FOR Ia IN HUMAN URINE BY INTERNAL STANDARD METHOD

Theoretical concentration (μg/ml)	Peak-height ratio (mean ± S.D., $n=3$)	Recalculated concentration* (mean ± S.D., $n=3$) (μg/ml)	Coefficient of variation (%)	Percentage of theory
0.5	0.038 ± 0.0003	0.516 ± 0.004	0.7	103.2
1.0	0.069 ± 0.002	0.953 ± 0.025	2.6	95.3
2.0	0.144 ± 0.004	2.000 ± 0.059	3.0	100.0
10.0	0.708 ± 0.014	9.928 ± 0.202	2.0	99.3
20.0	1.478 ± 0.014	20.825 ± 0.196	0.9	104.1
100.0	6.951 ± 0.119	98.952 ± 1.710	1.7	99.0
200.0	14.043 ± 0.083	200.839 ± 1.191	0.6	100.4
500.0	34.446 ± 0.262	495.564 ± 3.798	0.8	99.1

*$\log y = 0.9934 \log x + 0.0570$, $r = 0.9999$.

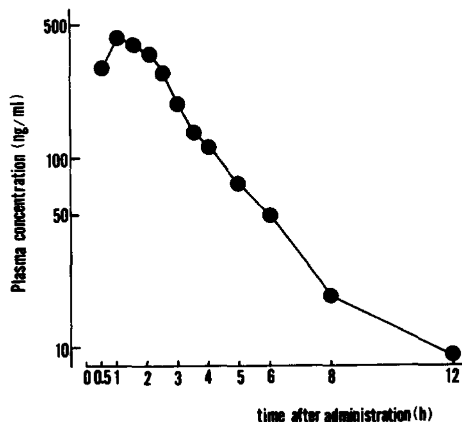

Fig. 4. Plasma concentration of Ia after a single oral administration of Ia (160 mg per subject) in healthy male volunteers ($n=6$).

μg/ml and 0.144 ± 0.004 with a C.V. of 3.0% at 2.0 μg/ml. At the other concentrations, the C.V. values were less than 2.0%. The equation for the calibration curve was $\log y = 0.9934 \log x + 0.0570$, with a correlation coefficient of 0.9999 in urine. Plasma and urine levels of Ia calculated from the calibration curve correlated with the amounts of Ia which were added to plasma and urine, respectively. The values at both levels agreed well, being 97–103 and 95–104% of the amounts added to plasma and urine, respectively. The recovery of Ia in this assay procedure was determined by comparing the peak height of the sample with that of the reference solution. The recovery rate was greater than 82% at 500 μg/ml in plasma and 97% at 10 μg/ml in urine.

The present assay method was applied to pharmacokinetic studies of Ia in healthy male volunteers. A plasma concentration–time curve after a single oral dose of 160 mg is shown in Fig. 4. Ia was rapidly absorbed and reached a peak level of 422 ng/ml at 1.1 h. The plasma elimination half-life was ca. 0.9 h. The detection limit (10 ng/ml at a signal-to-noise ratio of 3) of this HPLC procedure was considered sufficient to determine the concentration of Ia in plasma. On the other hand, Ia was excreted as unchanged drug in the urine to the extent of 9% during 0–120 h after Ia was orally administeed at 160 mg.

From these results, it was concluded that the present HPLC method is sensitive and reproducible for the determination of the plasma and urine concentrations of Ia, and is, therefore, valuable for the investigation of the clinical pharmacokinetics and bioavailability of Ia.

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