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Note

High-performance liquid chromatographic procedure for the determination of a new antithrombotic and vasodilating agent, cilostazol, in human plasma

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Cilostazol (OPC-13013), 6-[4-(1-cyclohexyl-1*H*-tetrazol-5-yl)butoxy]-3,4-dihydro-2(1*H*)-quinolinone, is a newly synthesized compound by Nishi et al. [1] and a potential antithrombotic and vasodilating drug. Its effectiveness and mechanism of action have been described previously [2—5]. It has been reported that cilostazol has both antithrombotic and cerebral vasodilating effects [2], and one of the mechanisms is the selective inhibition of platelet cyclic AMP phosphodiesterase [3—5]. Consequently, it was suggested that cilostazol has clinical potential in the management of post-stroke syndrome and the prevention of recurrent stroke, and cilostazol is currently being evaluated in clinical studies. We were interested in the determination of plasma cilostazol levels in man for the routine therapeutic monitoring of this compound, which is valuable for the elucidation of its therapeutic effect and mechanism of action. Therefore, the establishment of a simple assay technique for routine analysis was studied.

In this paper, we describe a simple, highly sensitive and selective method for the determination of plasma cilostazol levels in man using reversed-phase high-performance liquid chromatography (HPLC). This paper also describes how plasma cilostazol levels after a single oral administration of 100 mg to healthy male volunteers were determined by this method.

MATERIALS AND METHODS

Chemicals

Cilostazol (Fig. 1A) and the internal standard (OPC-13012, Fig. 1B) were

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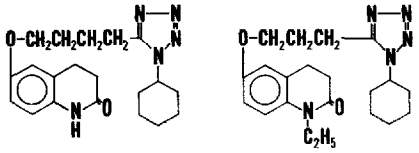

Fig. 1. Chemical structure of cilostazol (A) and internal standard (B).

supplied by the Laboratories of Medicinal Chemistry, Otsuka Pharmaceutical (Tokushima, Japan). All another reagents and solvents were of analytical grade (Wako, Tokyo, Japan).

Chromatography

The HPLC system consisted of a Waters Assoc. ALC/GPC 204 compact system (Model 6000A pump, Model 440 UV detector equipped with a 254-nm filter kit), Waters 710B autosample processor (Waters Japan, Tokyo, Japan) and Shimadzu Chromatopack C-R1B (Shimadzu, Kyoto, Japan). A μBondapak C_{18} reversed-phase column (30 cm × 3.9 mm I.D., particle size 10 μm, Waters) was used with the mixture of acetonitrile—water (42:58, v/v) as the mobile phase at a flow-rate of 1.7 ml/min.

Sample preparation

The internal standard (600 ng per 10 μl of methanol) and acetonitrile (4.0 ml) were added to human plasma (1.0 ml). The contents were stirred on a vortex mixer and centrifuged at 1700 *g* for 10 min. The supernatant was transferred to another centrifuge tube, and the acetonitrile was evaporated under a stream of air. To the residue, 0.2 *M* sodium hydroxide (1.0 ml) and chloroform (5.0 ml) were added and the mixture was shaken and centrifuged at 1700 *g* for 10 min. The chloroform layer was transferred to another centrifuge tube, and the chloroform evaporated to dryness under a stream of air. To the residue, 0.2 *M* sodium hydroxide (1.0 ml) and diethyl ether (5.0 ml) were added. The mixture was again shaken and centrifuged, and the diethyl ether layer was transferred to another centrifuge tube and evaporated to dryness. The residue was redissolved in methanol (100 μl), and an aliquot (40 μl) of the methanol solution was injected into the HPLC system. The calibration curve was constructed at cilostazol levels of 25—2000 ng/ml of plasma.

Healthy male volunteers received a single oral dose of 100 mg of cilostazol. Blood samples were collected at scheduled intervals and centrifuged at 1700 *g* for 10 min to obtain plasma samples.

RESULTS AND DISCUSSION

Typical chromatograms of the extracts from plasma containing cilostazol (250 ng/ml), blank plasma and sample plasma after a single oral dose of 100 mg of cilostazol obtained in the above procedure are shown in Fig. 2. No significant interference was observed in the regions for cilostazol and the internal standard on the chromatogram. The retention time was 7.5 min for cilostazol and 12.0 min for the internal standard.

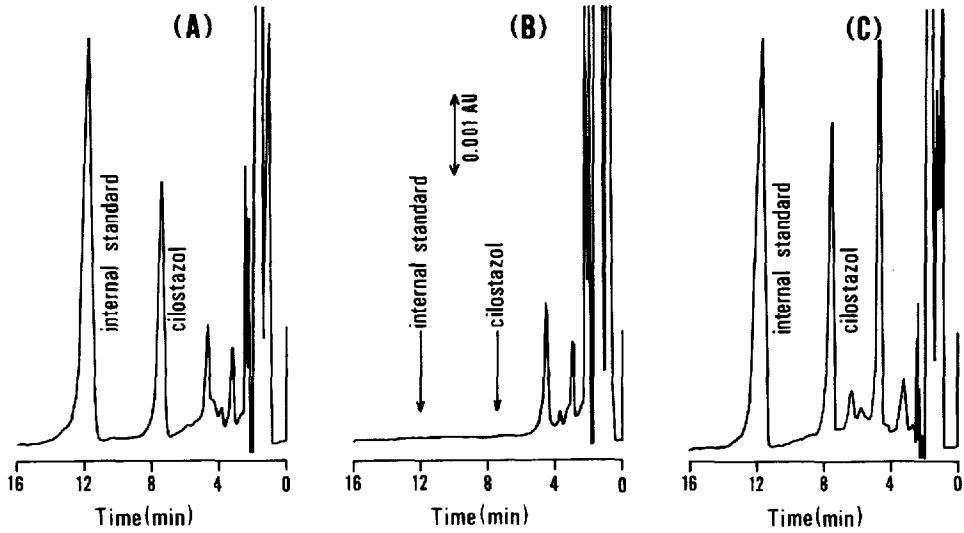

Fig. 2. (A) Chromatogram of human plasma containing cilostazol (250 ng/ml) and internal standard (600 ng/ml). (B) Chromatogram of blank human plasma. (C) Chromatogram of sample human plasma after a single oral dose of 100 mg of cilostazol.

The linearity of the calibration curve constructed for the determination of cilostazol at levels of 25—2000 ng/ml is shown in Table I. The precision of the HPLC assay was determined by calculating a mean peak height ratio ± standard deviation (S.D.) for each of the five standards. The peak height ratio was 0.059 ± 0.002 with a coefficient of variation (C.V.) of 3.6% at 25 ng/ml, and correspondingly 0.122 ± 0.006 and 4.6% at 50 ng/ml. At levels higher than 50 ng/ml the C.V. values were smaller than 1.6%, which were very much smaller than at 25 and 50 ng/ml, showing very little deviation of the peak height ratio. The equation for the resulting line was $y = 0.00259x - 0.01371$ with a correlation coefficient of 0.999. Plasma cilostazol levels calculated from the calibration curve were comparable to corresponding amounts of cilostazol

TABLE I

LINEARITY AND PRECISION OF HPLC PROCEDURE FOR CILOSTAZOL IN HUMAN PLASMA BY INTERNAL STANDARD METHOD

Concentration of cilostazol added to plasma (ng/ml)	Peak height ratio (mean ± S.D.)*	C.V. (%)	Recalculated concentration (ng/ml)	Percentage of theory	Recovery (%)
25	0.059 ± 0.002	3.6	27.9	112	69
50	0.122 ± 0.006	4.6	52.3	105	74
100	0.246 ± 0.004	1.6	100.2	100	79
250	0.628 ± 0.005	0.8	247.2	99	77
500	1.280 ± 0.018	1.4	498.6	100	77
1000	2.571 ± 0.022	0.9	996.4	100	73
2000	5.180 ± 0.039	0.8	2002.4	100	70

*Results from five replicate samples were used.

added to the plasma. They agreed well, being 99—112% of the amounts added to the plasma.

The recovery of cilostazol in this assay procedure was determined by comparing peak heights of the compound from processed samples to heights of other prepared reference solutions. Recoveries were greater than 69% at any level.

This assay system was applied to the study of the pharmacokinetics of cilostazol in man. A plasma cilostazol level versus time curve after a single dose of 100 mg orally is shown in Fig. 3. Cilostazol was rapidly absorbed, and reached peak plasma levels of 764 ng/ml at 3 h. The plasma elimination half-life was 2.2 h (α-phase) and 18.0 h (β-phase). The quantifiable limit (25 ng/ml) of this procedure was considered sufficient to determine plasma cilostazol levels, since the dose of 100 mg used in this study will be equal or lower than intended daily clinical doses of cilostazol [6].

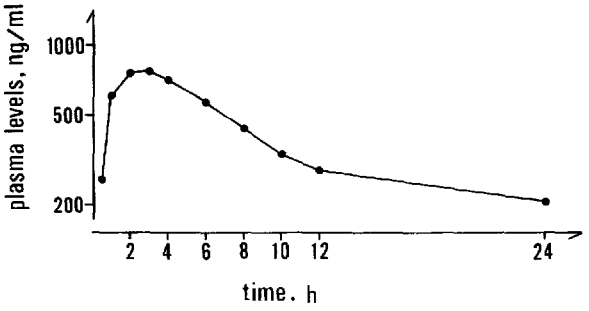

Fig. 3. Plasma cilostazol levels after a single oral dose of cilostazol (100 mg per body) in healthy male volunteers (n = 12).

In view of these results, it was concluded that HPLC is a simple, sensitive and reproducible procedure for the determination of plasma cilostazol levels and, therefore, a suitable and valuable tool in the investigation of the clinical pharmacokinetics and bioavailability of cilostazol.

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