Journal of Chromatography, 277 (1983) 419—422
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 1806

Note

High-performance liquid chromatographic procedure for the determination of probucol in human plasma

S. KUDO, H. AKIYAMA, M. ODOMI and G. MIYAMOTO*

Laboratories of Drug Metabolism and Analytical Research, Tokushima Research Institute, Otsuka Pharmaceutical Co., Ltd., 463-10 Kagasuno, Kawauchi-cho, Tokushima 771-01 (Japan)

(First received March 1st, 1983; revised manuscript received May 25th, 1983)

Probucol, 2,2-bis(3,5-di-*tert.*-butyl-4-hydroxyphenylmercapto)propane, has been reported to have a potent cholesterol-lowering activity. Its effectiveness has been demonstrated in normal animals, experimental hypercholesterolaemic animals [1—3], healthy volunteers [4, 5] and lipaemic patients [6]. The compound has been reported to be a unique drug whose nature is different from that of clofibrate and colestipol, which are reference drugs [7], in that it produced a significant decrease in serum low density lipoprotein cholesterol level and exerted little influence on the serum triglyceride level [4].

However, little work has been done with respect to the determination of its plasma levels which are supposed to be indexes for the evaluation of its effectiveness and safety in man. The only quantitative analysis of probucol in plasma has been performed by mass fragmentography [8], and few reports are available on its pharmacokinetic profile [9].

Therefore, an attempt was made to develop a method for the quantitative determination of plasma probucol concentrations that would be simple, suitable for routine analysis and highly sensitive. In this paper, we describe a simple method for the determination of probucol in human plasma using high-performance liquid chromatography (HPLC). The results of the HPLC assay for plasma probucol concentrations after a single oral dose of 250 mg to healthy male subjects are also described.

EXPERIMENTAL

Probucol (Fig. 1a) and the internal standard, 2,2-bis(3,5-di-*tert.*-butyl-4-

0378-4347/83/$03.00 © 1983 Elsevier Science Publishers B.V.

Fig. 1. Chemical structure of probucol (a) and internal standard (b).

hyroxyphenylmercapto)pentane (Fig. 1b), were supplied by the Dow Chemical Company (Indianapolis, IN, U.S.A.). Acetonitrile, methanol and ethyl ether were purchased from Wako Pure Chemical Industries (Tokyo, Japan).

The HPLC separations were carried out using a Waters Assoc. ALP/GPC 244 Compact System equipped with 254 nm filter kit. A μBondapak C_{18} reversed-phase column (30 cm × 3.9 mm I.D., particle size 10 μm, Waters Assoc.) was used with the mobile phase acetonitrile—water (85:15, v/v) at a flow-rate of 2.0 ml/min.

Sample preparation

To 1.0 ml of human plasma the internal standard and ethanol were added. The contents were stirred on a Vortex mixer and centrifuged for 10 min at 1700 *g*. A portion of the supernatant was transferred to a centrifuge tube and the ethanol was evaporated under a stream of air. To the residue 0.2 *N* NaOH and diethyl ether were added and the mixture was shaken and centrifuged for 10 min at 1700 *g*. The ether layer was evaporated to dryness under a stream of air. The residue was redissolved in methanol (100 μl) and an aliquot (40 μl) was injected into the HPLC system and analyzed.

The calibration curve was constructed at probucol concentrations of 0.25—25 μg/ml plasma.

Healthy volunteers received a single 250-mg oral dose of probucol. Blood samples were collected at scheduled intervals and were centrifuged at 1700 *g* for 10 min to obtain plasma samples.

RESULTS AND DISCUSSION

Chromatograms obtained by the above procedures using the plasma samples with and without probucol and the internal standards are given in Fig. 2. No significant interference was observed in the regions for probucol and the internal standard on the chromatogram. A good chromatographic separation was obtained with apparent retention times for probucol and the internal standard of 7.0 and 10.4 min, respectively, a separation factor (α) of 1.055 and a resolution (R_s) of 1.36.

The linearity of the calibration curve constructed for the determination of probucol at concentrations of 0.25—25 μg/ml is demonstrated in Table I. At a concentration of 0.25 μg/ml the peak height ratio was 0.084 ± 0.003 with a coefficient of variation (C.V.) of 3.9%. At concentrations higher than 0.25 μg/ml the C.V. was smaller than 3.9% showing very little deviation of the peak height ratio. A higher extraction ratio for probucol was obtained with a value greater than 89%. The equation for the resulting line was $Y = 0.31795X -$

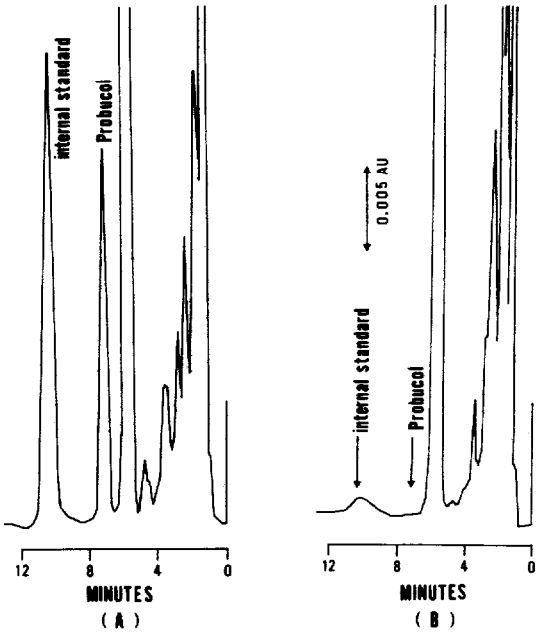

Fig. 2. (A) Representative chromatogram of probucol and internal standard in human plasma. Concentrations in plasma: probucol 2.5 μg/ml, internal standard 4.5 μg/ml. (B) Chromatogram of a blank human plasma.

TABLE I

LINEARITY OF HPLC PROCEDURE FOR PROBUCOL IN HUMAN PLASMA BY INTERNAL STANDARD METHOD

Theoretical plasma probucol conc. (μg/ml)	Mean peak height ratio ± S.D. (± C.V.%)*	Recalculated conc. (μg/ml)	Percentage of theory	Extractability (%)
0.25	0.084 ± 0.003 (± 3.9)	0.270	108	90
0.50	0.163 ± 0.005 (± 3.3)	0.518	104	93
1.00	0.319 ± 0.003 (± 0.8)	1.008	101	94
2.50	0.789 ± 0.012 (± 1.5)	2.486	99	89
5.00	1.593 ± 0.013 (± 0.8)	5.015	100	94
10.00	3.170 ± 0.019 (± 0.6)	9.976	100	94
250.00	7.949 ± 0.103 (± 1.3)	25.007	100	99

*Results from four (0.25 μg/ml) and five (0.50, 1.00, 2.50, 5.00, 10.00, 25.00 μg/ml) replicate samples were used.

0.00186 with a correlation coefficient of 0.999. Plasma concentrations of probucol calculated from the calibration curve were comparable to the corresponding theoretical values, being 99—108% of the theoretical values.

Plasma concentrations of probucol were determined as described above. Fig. 3 shows the time-course of plasma probucol concentrations after a single dose of 250 mg. The plasma levels at 4 h were 1.17 μg/ml and reached a peak level of 5.04 μg/ml at 18 h, after which they declined to a level of 1.13 μg/ml

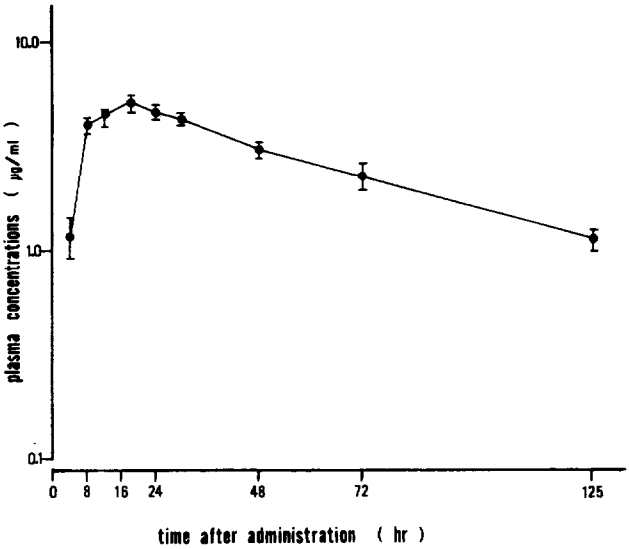

Fig. 3. Plasma concentrations of probucol after oral administration of probucol (250 mg/body) in healthy volunteers.

at 125 h with an apparent biological half-life of 49.7 h. The area under the curve (AUC) from time 0 to 125 h was 331.3 $\mu g\ ml^{-1}$ h.

The detection limit (0.25 μg/ml) of this procedure was considered sufficient to determine plasma probucol concentrations, since the dose of 250 mg used in the present study was about half its intended clinical dose [7] whose plasma concentrations were within the range (0.25—25 μg/ml) of the calibration curve constructed here.

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

REFERENCES

1 J.W. Barnhart, J.A. Sefranka and D.D. McIntosh, Amer. J. Clin. Nutr., 23 (1970) 1229.
2 D. Kritchevsky, Fed. Proc., Fed. Amer. Soc. Exp. Biol., 30 (1971) 692.
3 J.W. Barnhart, D.J. Rytter and J.A. Molello, Lipids, 12 (1977) 29.
4 J. Colmore, The American Society for Clinical Pharmacology and Therapeutics Meetings, Atlantic City, NJ, 1969.
5 Z. Kalams, M. Dacquisto and G.S. Kornett, Curr. Ther. Res., 13 (1971) 692.
6 J.W. Drake, R.H. Bradford, M. McDearmon and R.H. Furman, Metabolism, 18 (1969) 916.
7 R.C. Heel, R.N. Brogden, T.M. Speight and G.S. Avery, Drugs, 15 (1978) 409.
8 H. Tachizawa, K. Igarashi and M. Sano, personal communication.
9 J.F. Heeg and H. Tachizawa, Nouv. Presse Med., 9 (1980) 2290.