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Note

Determination of rifampicin and its main metabolites in human plasma by high-performance liquid chromatography

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Rifampicin is a first-choice antituberculosis agent, and it has also been used for other infections recently. It is metabolized extensively by the liver, especially during its first passage through the hepatoportal system. It is also one of the strongest known microsomal enzyme-inducing agents, so it causes a decrease of the plasma levels of oxidative-metabolized drugs: rifampicin itself [1], β-blockers such as propranolol and metoprolol [2,3], theophylline [4,5] and other many drugs [1]. The pharmacokinetics of rifampicin varies with the age of the patient [6], and is affected by impaired liver and kidney function [7,8]. In such circumstances therapeutic drug monitoring of rifampicin might be of value in optimizing the dose.

The usual microbiological assay of rifampicin does not allow separate determination of rifampicin and its active metabolite, 25-desacetylrifampicin. For precise pharmacokinetic studies rifampicin and its metabolites should be analysed separately. Recently, high-performance liquid chromatography (HPLC) has been used for determination of rifampicin and its metabolites [9–13], and this permits separate determination of rifampicin and its metabolites. However, neither complete chromatographic separation from plasma components nor a sharp and symmetrical peak pattern has yet been achieved, which may lead to analytical error and subsequent inaccurate analysis of rifampicin pharmacokinetics.

In an effort to improve this HPLC method, we used one extraction step and a wavelength of 340 nm for UV detection. The revised method permits separation of rifampicin, 25-desacetylrifampicin, 3-formyl-25-desacetylrifamycin SV, 3-formylrifamycin SV and papaverine hydrochloride as an internal standard, but quantitative studies were carried out only for rifampicin and its main metabolite,

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25-desacetylrifampicin, because 3-formyl-25-desacetylrifamycin SV and 3-formylrifamycin SV were negligible in plasma of a healthy volunteer and a patient treated with rifampicin.

EXPERIMENTAL

Materials

Rifampicin, 25-desacetylrifampicin, 3-formyl-25-desacetylrifamycin SV and 3-formylrifamycin SV were kindly offered by Kanebo (Osaka, Japan) and Ciba-Geigy (Basle, Switzerland). Papaverine hydrochloride (Yoshida Seiyaku, Tokyo, Japan) was used as an internal standard. Ascorbic acid (Wako, Osaka, Japan) was required to protect rifampicin from oxidative degradation. Other solvents and reagents were of analytical grade.

Analytical instruments

The HPLC system used consisted of a pump (Shimadzu LC-6A; Shimadzu, Kyoto, Japan), a syringe-loading injector (Model 7125 Rheodyne), a column packed with Nucleosil C_{18} (7 μm, 250×4.0 mm I.D.), a column oven (Shimadzu CTO-2A) operated at 40°C, a spectrophotometric detector (Shimadzu SPD-6AV) operated at 0.01 a.u.f.s. and a wavelength of 340 nm, and an integrator (Shimadzu CR-3A Chromatopac).

Mobile phase

The mobile phase, acetonitrile–0.1 *M* potassium dihydrogenphosphate, adjusted to pH 4.0 with 0.2 *M* phosphoric acid (38:62, v/v), was pumped at a constant flow-rate of 1.2 ml/min at 40°C.

Sample preparation

Standard solutions. A stock solution, prepared by dissolving rifampicin and 25-desacetylrifampicin in dimethylformamide to 1 mg/ml and stored at 4°C, was stable for at least one month. It was diluted to an appropriate concentration with acetonitrile–2-propanol (1:1, v/v) when needed. The diluted standard solution was used within one day. Plasma standards were prepared by spiking standard solutions to pooled human plasma.

Samples from healthy volunteers and patients. Blood (3 ml) was sampled to a heparinized dry tube with 10 mg of ascorbic acid to prevent oxidation of rifampicin and its metabolites. The samples were obtained from a healthy volunteer (male, 23 years, 75 kg) and a patient (male, 20 years, 65 kg) treated for tuberculosis by oral administration of 450 mg of rifampicin. The samples were centrifuged for 5 min and the plasma was stored at −20°C and shielded from light until analysed.

Extraction procedure. To a 0.5-ml aliquot of plasma were added 2 ml of 0.5 *M* sodium dihydrogenphosphate with the pH adjusted to 7.2 by 0.5 *M* potassium dihydrogenphosphate and 100 μl of a solution of papaverine hydrochloride (20 μg/ml) as an internal standard. The mixture was shaken mechanically with 7 ml of chloroform for 10 min. After centrifugation at 1800 *g* for 10 min, the upper

aqueous phase was aspirated off and 5 ml of the lower organic phase were taken to dryness under a stream of nitrogen gas at 50°C. The residue was dissolved in 300 μl of acetonitrile–2-propanol (1:1, v/v), and a 20-μl aliquot of the solution was injected into the HPLC system.

RESULTS AND DISCUSSION

Fig. 1 shows a chromatogram of rifampicin, its three metabolites and papaverine hydrochloride. In therapeutic treatment, isoniazid, ethambutol and streptomycin are often co-administered with rifampicin as antituberculosis drugs. It was shown that these drugs and their metabolites did not interfere the chromatogram (Fig. 1) after analysis of plasma samples treated with the drugs except for rifampicin. Fig. 2A and B show that plasma components did not interfere with rifampicin, its metabolite of the internal standard, and Fig. 2C shows a chromatogram of a plasma extract from a tuberculosis patient treated with rifampicin, isoniazid and ethambutol, in which 3-formyl-25-desacetylrifamycin SV (<0.19 μg/ml) and 3-formylrifamycin SV (<0.37 μg/ml) were not detected as metabolites of rifampicin.

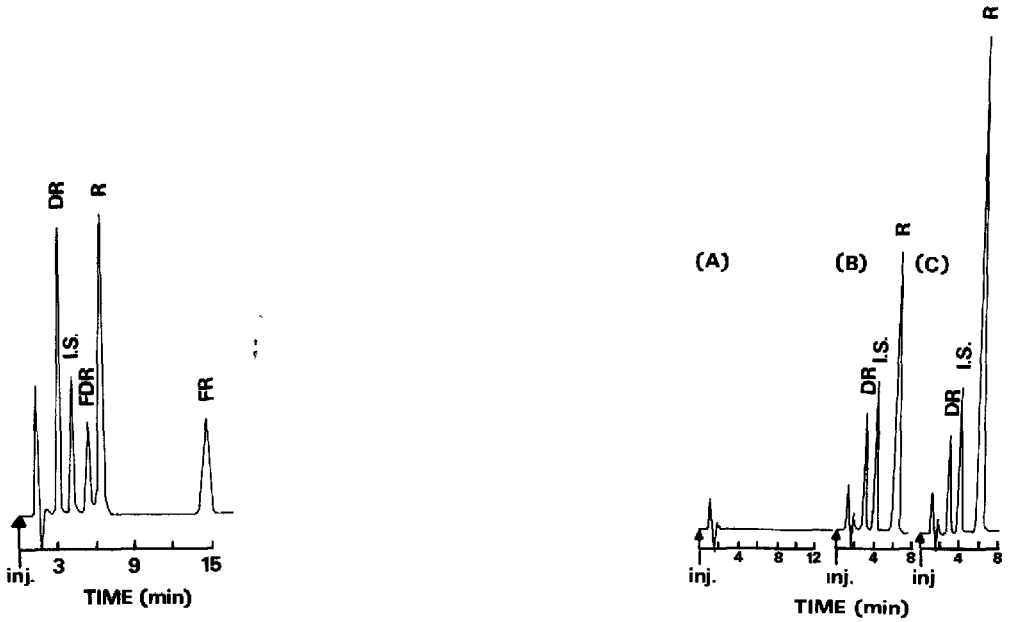

Fig. 1. Chromatogram of rifampicin (R), 25-desacetylrifampicin (DR), 3-formyl-25-desacetylrifamycin SV (FDR), 3-formylrifamycin SV (FR) and papaverine as internal standard (I.S.).

Fig. 2. (A) Chromatogram of a blank plasma extract. (B) Chromatogram of rifampicin (R), 25-desacetylrifampicin (DR) and papaverine as internal standard (I.S.), spiked to a blank plasma. The spiked concentrations of R and DR were 3 and 1 μg/ml in plasma, respectively. (C) Chromatogram of plasma extracted from a tuberculosis patient treated chronically with 450 mg of rifampicin, 300 mg of isoniazid and 1.0 g of ethambutol. The concentrations of R and DR were estimated to be 5.47 and 0.85 μg/ml, respectively.

TABLE I

REPRODUCIBILITY AND ACCURACY OF THE ASSAY IN HUMAN PLASMA

Rifampicin			25-Desacetylrifampicin		
Added (μg/ml)	Found (mean ± S.D., $n=5$) (μg/ml)	C.V. (%)	Added (μg/ml)	Found (mean ± S.D., $n=5$) (μg/ml)	C.V. (%)
0.5	0.50 ± 0.01	2.0	0.5	0.49 ± 0.01	2.0
3.0	2.99 ± 0.03	1.0	1.0	1.01 ± 0.02	2.0
10.0	10.07 ± 0.13	1.3	3.0	2.99 ± 0.03	1.0

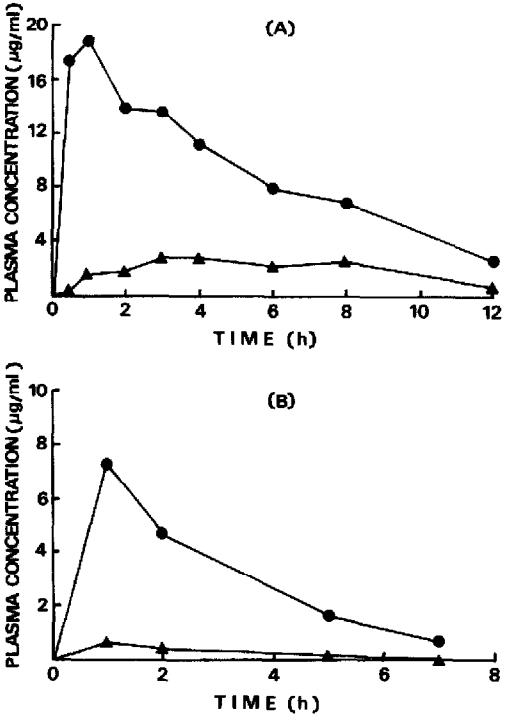

Fig. 3. Plasma levels obtained in one healthy volunteer (A) and in one tuberculosis patient (B) following an oral dose of 450 mg of rifampicin. Data points: ● = rifampicin; ▲ = 25-desacetylrifampicin.

Quantification was achieved using the peak-height ratio of rifampicin or 25-desacetylrifampicin to internal standard. Plots of peak-height ratios against concentrations were linear. Typical standard curves for plasma extract had correlation coefficients of 0.9999 (rifampicin) and 0.9998 (25-desacetylrifampicin). The mean recoveries of rifampicin and 25-desacetylrifampicin were 88 and 93%, respectively, and the mean recovery of papaverine hydrochloride was 90%.

The lower detection limits (three times the baseline) of rifampicin and 25-desacetylrifampicin in plasma were 0.1 μg/ml and 0.06 μg/ml, respectively. Table I shows the good reproducibility and accuracy of the assay method.

The method described was applied to the determination of rifampicin and 25-desacetylrifampicin in the plasma of a healthy volunteer and a tuberculosis patient following oral administration of 450 mg of rifampicin subsequent to fasting. Fig. 3 shows the plasma levels determined.

The use of a wavelength of 340 nm for detection and a single extraction step eliminated the chromatographic peaks that would otherwise have interfered the rifampicin and its metabolites or the internal standard (Fig. 2A). This ensures the correct determination of the concentration in plasma with less analytical error. Furthermore, the method shortens the retention times: rifampicin, 6.7 min; 25-desacetylrifampicin, 3.5 min (Fig. 2B and C). An HPLC method with an internal standard is necessary to compensate for potential loss of analysis at each stage. Papaverine hydrochloride is a suitable internal standard because of the high recovery, ca. 90%, and the short retention time, 4.5 min. The method is suitable for pharmacokinetic studies of rifampicin and 25-desacetylrifampicin.

REFERENCES

1 W. Zilly, D.D. Breimer and E. Richter, Clin. Pharmacokin., 2 (1977) 61.
2 R.J. Herman, K. Nakamura, G.R. Wilkinson and A.J.J. Wood, Pharmacologist, 24 (1982) 181.
3 W. Kirch, I. Rose, I. Klingmann, J. Pabst and E.E. Ohnhaus, Eur. J. Clin. Pharmacol., 31 (1986) 59.
4 A.B. Straughn, R.P. Henderson, P.L. Lieberman and T.H. Self, Ther. Drug Monit., 6 (1984) 153.
5 E.G. Boyce, G.E. Dukes, D.E. Rollins and T.W. Sudds, J. Clin. Pharmacol., 26 (1986) 696.
6 C. Advenier, C. Gobert, G. Houin, D. Bidet, S. Richelet and J.P. Tillement, Ther. Drug Monit., 5 (1983) 61.
7 G. Acocella, Clin. Pharmacokin., 3 (1978) 108.
8 M.T. Kenny and B. Strates, Drug Metab. Rev., 12 (1981) 159.
9 J.B. Lecaillon, N. Febvre, J.P. Metayer and C. Souppart, J. Chromatogr., 145 (1978) 319.
10 M. Gidoh and S. Tsutsumi, J. Chromatogr., 223 (1981) 379.
11 B. Ratti, R.R. Parenti, A. Toselli and L.F. Zerilli, J. Chromatogr., 225 (1981) 526.
12 M. Guillaumont, M. Leclercq, Y. Frobert, B. Guise and R. Harf, J. Chromatogr., 232 (1982) 369.
13 S. Oldfield, J.D. Berg, H.J. Stiles and B.M. Buckley, J. Chromatogr., 377 (1986) 423.