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RAPID AND SPECIFIC METHOD FOR THE DETERMINATION OF VANCOMYCIN IN PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY ON AN AMINOPROPYL COLUMN

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SUMMARY

A high-performance liquid chromatographic method has been developed for the quantitative analysis of vancomycin in plasma. The method involves protein precipitation with acetonitrile, followed by normal-phase chromatography on an aminopropyl column The clear supernatant was injected after centrifugation, and the eluent was monitored at 240 nm. No interference was found either with endogenous substances or with many currently used drugs, indicating a good selectivity for the procedure The standard curve was linear between 0.1 and 100 μg/ml, and the detection limit was 0.01 μg/ml of plasma. The mean intra- and inter-assay coefficients of variation were 2.4 and 4 0%, respectively, in the 10–50 μg/ml range. Application of the method to the study of vancomycin pharmacokinetics in a rabbit after a single intravenous dose is also reported.

INTRODUCTION

Vancomycin (Fig. 1) is an amphoteric glycopeptide antibiotic, which was isolated in 1956 from both *Streptomyces orientalis* and *Nocardia lurida* [1]. It is active against a variety of gram-positive bacteria and some gram-negative cocci [2], including methicillin-resistant staphylococci.

Vancomycin is excreted almost entirely via the kidneys: 90–100% of the administered drug activity appears in the urine [3]. Impaired renal function can cause drug accumulation if the dose is not adjusted. The clinically significant side-effects of vancomycin therapy are nephro- and ototoxicity, reported when blood levels reached the 80 μg/ml level [4,5]. Monitoring of vancomycin plasma levels is therefore necessary in order to avoid these side-effects in therapy [6].

Approaches reported for the determination of vancomycin in biological fluids include microbiological assay [7,8], radioimmunoassay (RIA) [9] and fluores-

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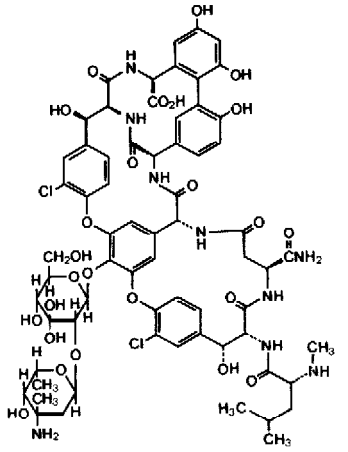

Fig. 1. Structure of vancomycin.

cence polarization immunoassay (FPIA) [10]. These methods suffered from insufficient specificity [7–10] and sensitivity [7,8], and were time-consuming [7–9] or expensive [9,10].

Various high-performance liquid chromatographic (HPLC) methods [11–16] for determination of vancomycin in biological fluids have been described. Some use liquid–liquid [11–13] or solid-phase [14–16] extraction followed by reversed-phase chromatography. However, the appearance of many peaks indicates that these methods lack sufficient specificity.

This report describes a rapid, selective and sensitive method for the determination of vancomycin in plasma. The sample preparation involves only protein precipitation and centrifugation; no internal standardization, extraction or evaporation steps are required. The method involves normal-phase HPLC performed on a polar aminopropyl bonded-phase column (NH_2) with UV detection at 240 nm.

EXPERIMENTAL

Reagents

Vancomycin hydrochloride was supplied by Eli Lilly (Tokyo, Japan). Reagent-grade sodium dihydrogenphosphate and phosphoric acid were purchased from Katayama (Osaka, Japan). HPLC-grade acetonitrile was obtained from Wako (Osaka, Japan).

Chromatography

The isocratic liquid chromatograph consisted of the following units: a Model LC-6A solvent-delivery system (Shimadzu, Kyoto, Japan), a Model 7125 injection valve equipped with a 20-μl loop (Rheodyne, Berkeley, CA, U.S.A.), a Model SPD-6A variable-wavelength UV detector (Shimadzu) and a Model C-R4A Chromatopack chromatographic data processor (Shimadzu). Separation was performed on a 150 mm$\times$6.0 mm I.D. Shim-pack CLC-NH_2 normal-phase ana-

lytical column, particle size 5 μm (Shimadzu). The mobile phase was 0.07 *M* phosphate buffer (pH 2.5)–acetonitrile (40:70, v/v). The flow-rate was 1.5 ml/min, and chromatography was performed at ambient temperature. The detection wavelength was set at 240 nm with the sensitivity at 0.002 and 0.01 a.u.f.s.

Standard and calibration curve

A vancomycin stock solution (5.0 mg/ml) was prepared by dissolving the drug in water. The stock solution was stored at −70°C and maintained at 4°C during use. For plasma the standard solution was diluted 10- and 100-fold with water. These diluted solutions were used to make a calibration curve for drug-free plasma with final concentrations ranging from 0.1 to 100 μg/ml.

Quality control samples for assessing precision were prepared in pooled drug-free human plasma at 0, 10, 30 and 50 μg/ml. Plasma standard and control samples were dispensed in 0.5-ml aliquots and stored at −70°C.

Procedure

A 100-μl volume of plasma standard, quality control or unknown plasma sample was mixed vigorously with 100 μl of acetonitrile in a 1.5-ml plastic microcentrifuge tube. The mixture was then centrifuged at 12 000 *g* for 2 min, after which 20 μl of the clear supernatant were injected into the chromatographic system.

Kinetic study

In a preliminary pharmacokinetic study, a dose of 10 mg/kg vancomycin (total dose 35 mg) was administered with a 30-min intravenous infusion to a rabbit. Plasma concentrations were analysed as described above, and pharmacokinetic parameters were calculated using a two-compartment open model analysis.

RESULTS

Chromatographic separation and specificity

Typical chromatograms resulting from the analysis of various plasma samples are shown in Fig. 2. Vancomycin appears as a well resolved peak with a retention time of 8.3 min. The excellent separation of the vancomycin peak allowed for quantification by simply measuring the peak area. Because the intra- and inter-assay coefficients of variation (C.V.) were satisfactory, an internal standard was unnecessary.

No interference by endogenous substances or coadministered drugs could be detected. The clinical specimens known to contain one or more of the drugs are shown in Table I.

Linearity

The linearity study was carried out with concentrations ranging from 0.1 to 100 μg/ml. Two ranges of the detector sensitivity were employed: in the first step, the plasma concentrations ranged from 0.1 to 10 μg/ml and these samples were chromatographed at 0.002 a.u.f.s.; in the second step, concentrations ranged from 10 to 100 μg/ml and the detector was set at 0.01 a.u.f.s. The correlation coefficient

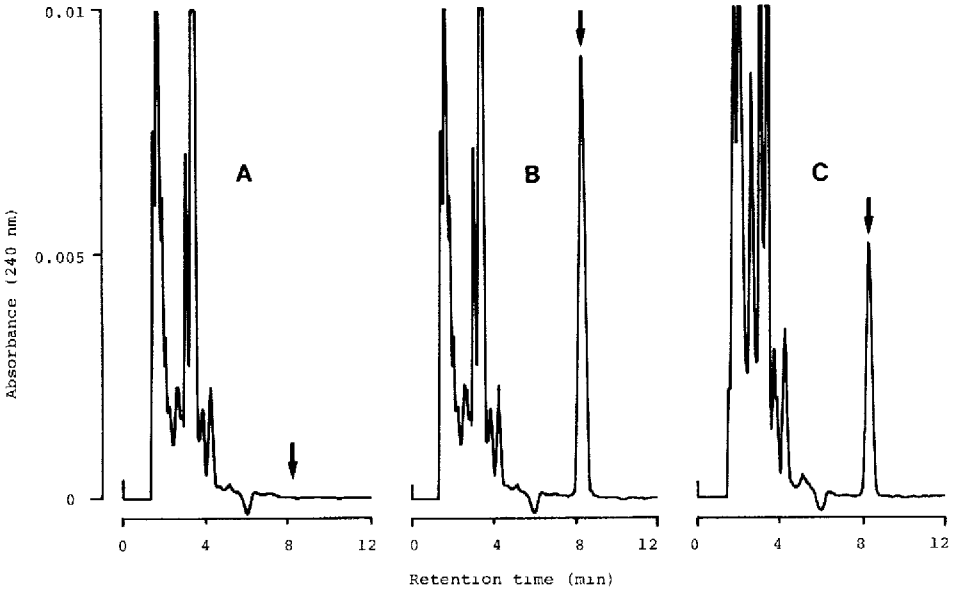

Fig. 2. Chromatograms of (A) human plasma blank, (B) plasma blank spiked with vancomycin (50 μg/ml) and (C) plasma obtained from a rabbit 90 min after intravenous dosing (10 mg/kg) with the drug (calculated concentration: 28.4 μg/ml). The retention time for vancomycin was 8.3 min.

TABLE I

DRUGS FOUND NOT TO INTERFERE WITH DETECTION OF VANCOMYCIN BY HPLC

Antibiotics	Antineoplastics	Other drugs
Amikacin	Adriamycin	Acetaminophen
Amphotericin B	Allopurinol	Acetazolamide
Benzylpenicillin	Cisplatin	Aspirin
Carbenicillin	Cyclophosphamide	Carbamazepine
Cefoxitin	Cytarabine	Chlorpromazine
Cefuroxime	Dactinomycin	Diazepam
Chloramphenicol	5-Fluorouracil	Ethosuximide
5-Fluorocystosine	Methotrexate	Furosemide
Gentamicin	Thioguanine	Phenobarbital
Kanamycin		Phenytoin
Ketoconazole		Primidone
Miconazole		Procainamide
Penicillin G		Quinidine
Tobramycin		Theophylline

between the peak areas and the vancomycin concentrations for the two steps combined was $r=0.999$. The detection limit was 0.01 μg/ml for plasma levels, resulting in a signal-to-noise ratio of 3:1.

TABLE II

RECOVERY DATA FOR THE DETERMINATION OF VANCOMYCIN IN PLASMA

Amount added (μg/ml)	Amount measured ($n=3$) (μg/ml)	Recovery (%)
10	9.84	98.4
20	20.26	101.3
40	38 96	97.4
60	60.36	100.6
80	78 24	97.8
100	101.50	101 5
	Mean	99.5

Recovery

Known amounts of vancomycin were added to drug-free plasma to provide concentrations ranging from 10 to 100 μg/ml. The peak areas obtained after triplicate extractions of each concentrations were compared with the peak areas obtained for plasma standards. The absolute recovery was calculated as (amount of drug measured/amount of drug added) $\times$ 100. The results are summarized in Table II, which shows that 97.4–101.5% absolute recovery of vancomycin is obtained in the concentration range 10–100 μg/ml.

Precision and accuracy

Precision and accuracy data are presented in Table III. Intra-assay coefficients of variation ranged from 1.9 to 3.2% and inter-assay coefficients of variation were slightly higher (3.2–4.8%).

Application of the method to experimental samples

Fig. 3 shows the log (concentration)–time curve determined after a 10 mg/kg infusion for 30 min to a rabbit. The plot indicates that the method permits anal-

TABLE III

PRECISION AND ACCURACY DATA FOR THE DETERMINATION OF VANCOMYCIN IN PLASMA

Expected concentration (μg/ml)	Observed concentration (μg/ml)					
	Intra-assay ($n=10$)			Inter-assay ($n=5$)		
	Mean	S.D.	C.V. (%)	Mean	S.D.	C.V (%)
10	10.1	0.3	3.2	9.8	0.5	4.8
30	29.8	0.6	2.1	30.4	1 2	4.1
50	51.3	1.0	1.9	50.8	1.6	3 2
			Mean 2.4			Mean 4.0

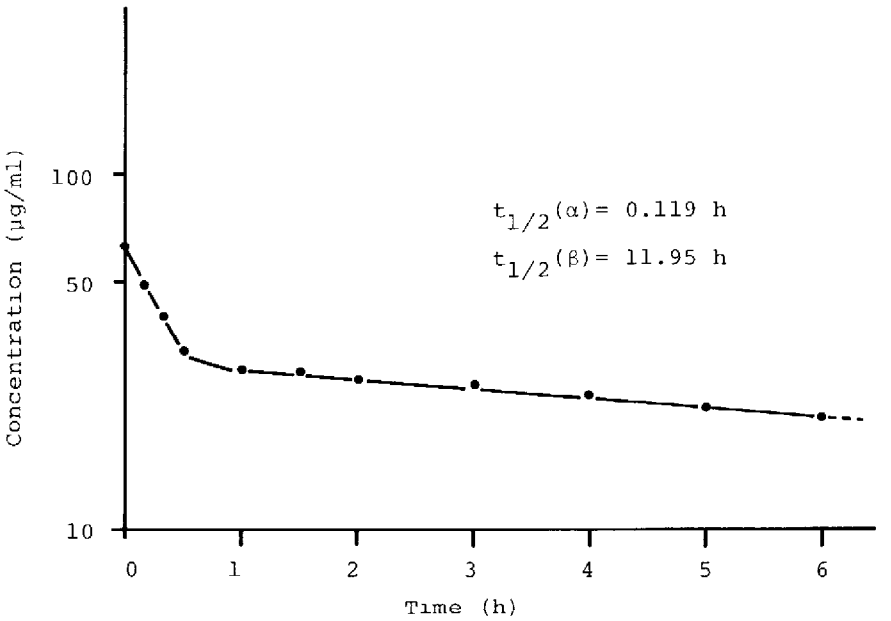

Fig. 3. Plasma concentration–time curve of vancomycin following a 30-min intravenous administration of a single dose of 35 mg to a rabbit.

ysis of samples during at least a 6-h period after this intravenous dose: a short distribution phase (half-life 0.12 h) was observed, followed by a longer elimination phase half-life (12.0 h).

DISCUSSION

The use of vancomycin is complicated by the almost universal development of toxic reactions, particularly those affecting aural and renal functions. Nevertheless, in vitro data as well as animal studies suggest that vancomycin may be useful in the treatment of infections due to gram-positive pathogens, such as methicillin-resistant staphylococci or enterococci [17,18].

Various microbiological assays have been described [7,8]. In comparison with the HPLC assay, bioassays are somewhat less precise, are difficult to standardize, pose problems in interpretation and require 24–48 h of incubation. Furthermore, natural antibiotic activity in the blood of normal individuals as well as other drugs given concomitantly could interfere with the specificity of the assay.

Although RIA [9] and FPIA [10] assays for vancomycin seem more sensitive, accurate and precise than bioassays, they require expensive equipment or facilities for handling radioactive compounds. Furthermore, the reagents are costly and have a relatively short shelf-life.

Clinical trials are now being conducted to establish the potential indications as well as the adverse effects of the antibiotic. For this purpose, a reliable method to assay the drug in plasma is needed. The method described here routinely uses a sample size of 100 µl, but sample volumes as small as 50 µl are adequate. The

use of small sample sizes is important for defining the pharmacokinetic properties of vancomycin in pediatric populations.

The absorbance of vancomycin at 280 nm is low [11], but increases greatly at wavelengths lower than 210 nm [13]. However, interference from endogenous substances and other drugs is greater below 240 nm [12–15] than at 240 nm. The sensitivity at 240 nm is five- to six-fold greater than at 280 nm, the wavelength used in a previously published procedure.

The analytical column and the mobile phase seem to be the best combination for several liquid chromatographic procedures [19]. The present method adjusts the acetonitrile concentration to optimize the capacity factor and assay time for vancomycin. The assay is simple and rapid as well as precise, accurate, sensitive, specific and reproducible.

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REFERENCES

1 H.R. Perkins, Pharmacol. Ther., 16 (1982) 181.
2 H.M. McCormick, W M Stark, G.E Pittenger, R.C. Pittenger and J.M. McGuire (Editors), Vancomycin, a New Antibiotic. I. Chemical and Biologic Properties. Antibiotics Anual 1955–1956, Medical Encyclopedia, New York, 1956, pp. 606–611.
3 W.M.M. Kirby and C.L. Divelbiss (Editors), Vancomycin, Clinical and Laboratory Studies. Antibiotics Annual 1956–1957, Medical Encyclopedia, New York, 1957, pp. 107–117.
4 R.A. Blouin, L A. Bauer, D.D. Miller, K.E. Record and W.O. Griffen, Jr., Antimicrob. Agents Chemother., 21 (1982) 575.
5 N. Brown, D.H.W Ho, K.L. Fong, L. Bogerd, A. Maksymiuk, R. Boliver, V. Fainstern and G.P. Bodey, Antimicrob. Agents Chemother., 23 (1983) 603.
6 J.J. Garaud, B. Regnier, F. Inglebert, F. Faurisson, J. Bauchet and F. Vachon, J Antimicrob Chemother., 14 (1984) 53.
7 K.B. Crossley, J.C. Rotschaeffer, M.M. Cherm, K.E. Mead and D.E. Zaske, Antimicrob. Agents Chemother., 17 (1980) 654.
8 T.R. Beam, J Antimicrob. Chemother., 7 (1981) 89.
9 B.H. Ackerman, H.G. Berg, R.G. Strate and J.C Rotschafer, J. Clin. Microbiol., 18 (1983) 994
10 K.S Schwenzer, C H.J Wang and J.P Anhalt, Ther. Drug Monit., 5 (1983) 341.
11 J.B.L. McClain, R. Bongiovanni and S. Brown, J Chromatogr., 231 (1982) 463.
12 F. Jehl, C. Gallion, R.C Thierry and H. Onteil, Antimicrob. Agents Chemother., 27 (1985) 503.
13 A.F. Rosenthal, I. Sarfati and E. A'Zary, Clin. Chem , 32 (1986) 1016.
14 J R. Uhl and J P. Anhalt, Ther Drug Monit., 1 (1979) 75.
15 J. Bauchet, E. Pussard and J.J. Garaud, J. Chromatogr., 414 (1987) 472.
16 S.V Greene, T. Abdalla, S.L. Morgan and C.S. Bryan, J. Chromatogr., 417 (1987) 121
17 F.V. Cook and W.E. Farrar, Jr., Ann. Intern. Med., 88 (1978) 813
18 J.E. Geraci and P.E. Hermans, Mayo Clin. Proc., 58 (1983) 88.
19 H Hosotsubo, S. Takahara and N. Taenaka, J. Chromatogr., 432 (1988) 340 .