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Note

Determination of vancomycin in serum and tissues by column liquid chromatography using solid-phase extraction

J. BAUCHET, E. PUSSARD* and J.J. GARAUD

Clinical Pharmacology Department, INSERM U.13, Hôpital Claude-Bernard, 10, Avenue de la Porte d'Aubervilliers, 75019 Paris (France)

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Vancomycin is a cell-wall active glycopeptide antibiotic that has bactericidal activity against Gram-positive bacteria, including methicillin-resistant staphylococci. Monitoring of vancomycin serum levels is needed in order to avoid both oto and nephro potentially toxic levels [1] in patients under treatment. The available assays to quantitate vancomycin in serum included microbiological procedure [2–5], radioimmunoassay [6], fluorescent polarization immunoassay [7,8] and high-performance liquid chromatography (HPLC) [9–13]. However, all these methods are expensive and/or time-consuming for determination of vancomycin levels in a large number of samples.

The purpose of this study was to develop a cheaper, easier and more rapid and sensitive method suitable for routine use in hospitals and/or for the determination of low levels in tissues. The method, based on a clean-up on a Sep-Pak column and quantification by HPLC procedure, was set up to assess vancomycin levels in serum and heart valves during cardiac surgery (aortic valve replacement).

EXPERIMENTAL

Chemicals

Vancomycin hydrochloride was obtained from Eli-Lilly (Strasbourg, France) and the internal standard, trimethoprim (TMP), from Sigma (St. Louis, MO, U.S.A.). Stock solutions of vancomycin and TMP (1000 μg/ml) were prepared in distilled water and methanol, respectively. Working standards were prepared from these stock solutions. Methanol and acetonitrile (chromatographic purity; Merck, Darmstadt, F.R.G.) were used. Sep-Pak C_{18} cartridges (ca. 1 cm × 1 cm),

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containing octadecylsilane bonded phase retained between two filters, were purchased from Waters Assoc. (Milford, MA, U.S.A.). All other chemicals were of analytical-reagent grade.

Instrumentation

The method was developed on a Waters liquid chromatograph consisting of a WISP 710B sample processor, a 6000 A high-pressure pump, a Model 440 absorbance detector with a 229-nm filter and an Omniscribe recorder. Chromatography was carried out on a C_{18} Nucleosil column (15 cm$\times$0.46 cm I.D.) with a particle size of 5 μm (Société Française de Chromatographie Colonne).

Chromatography

The mobile phase consisted of 5 m*M* potassium dihydrogen phosphate (adjusted to pH 2.8 with orthophosphoric acid) containing 22% of methanol. The solvent was degassed prior to use by applying a vacuum. Drug elution was done at room temperature using a flow-rate of 1.5 ml/min.

Clinical study

Seven patients (average weight 59 $\pm$ 11 kg) were admitted for aortic heart valve replacement. Before surgery, each subject was given a 30-min infusion of 10 mg/kg vancomycin. In addition, 5 mg/kg vancomycin was added to the priming of the extra-corporal circuit. Serum samples were collected after vancomycin infusion at the peak (S_1), at aortic clamping (S_2), and at the end of cardio-pulmonary bypass (S_3). Valves were removed 3–47 min after S_2. Serum and valves were stored at -20°C until assayed.

Sample treatment procedure

Sep-Pak C_{18} cartridges were pre-wetted with ca. 2 ml of methanol and then with ca 5 ml of distilled water. To 200 μl of serum were added 50 μl of 100 μg/ml TMP solution, and the mixture was injected through the cartridge. The column was washed successively with 8 ml of distilled water and 5 ml of methanol–distilled water (5:95, v/v). After elimination of all the washing solution, the column was eluted with 800 μl of an acetonitrile–50 m*M* potassium dihydrogen phosphate (pH 4.0) solution (30:70, v/v) and 40–200 μl of the eluate phase were injected into the column.

Heart valves were homogenized in 2 ml of 50 m*M* potassium dihydrogen phosphate (pH 4.0) buffer containing 50 μl of 10 μg/ml TMP solution with a glass tissue homogenizer Ultraturax. The homogenate was centrifuged for 10 min at 1500 *g* and the supernatant was injected through the cartridge.

Calibration, recovery and precision

Evaluation of the assay was carried out using seven calibration points in the concentration range 0.5–70 μg/ml vancomycin in drug-free plasma. The calibration curves were obtained by linear regression of the peak-height ratio of vancomycin/TMP versus the concentration of vancomycin.

Recovery was calculated by comparing measured values of spiked samples with

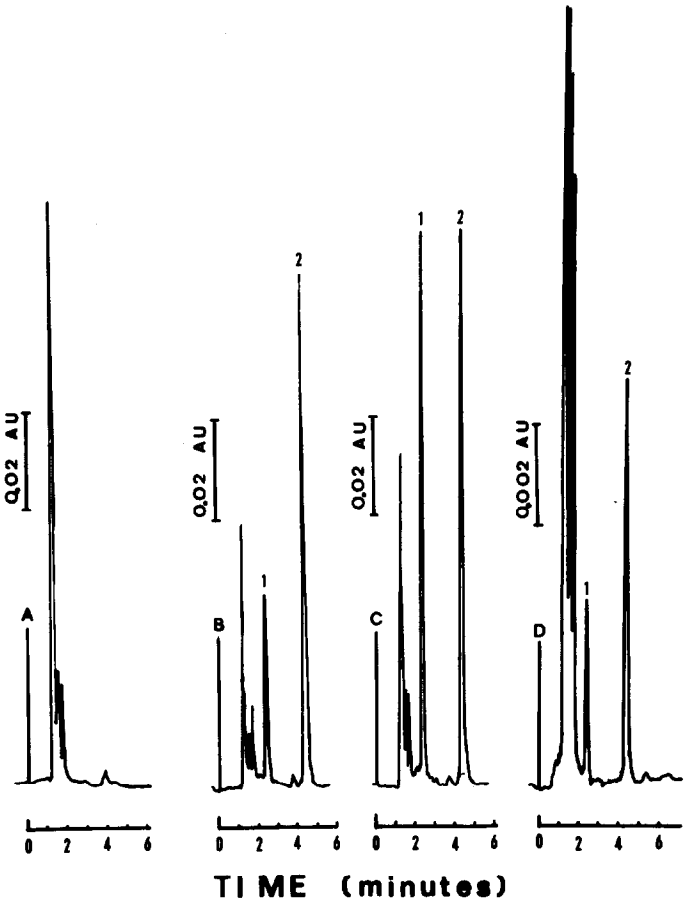

Fig. 1. Chromatograms of (A) a drug-free blank serum sample, (B) a calibration standard containing 20 μg/ml vancomycin and 5 μg/ml trimethoprim, (C) a serum sample from subject 2 drawn at the end of cardiopulmonary bypass (S_3) and containing 45.2 μg/ml vancomycin and (D) an aortic valve sample from subject 2 containing 7.6 μg/g vancomycin. Peaks: 1 = vancomycin; 2 = trimethoprim.

those of standard aqueous solution at two concentrations (10 and 40 μg/ml). Inter- and intra-assay precision was determined at two concentrations (10 and 40 μg/ml) for ten analyses.

RESULTS AND DISCUSSION

Representative chromatograms are shown in Fig. 1. In the chromatogram obtained from a blank plasma sample (Fig. 1A), no peak is present that might interfere with the determination of vancomycin. Fig. 1B shows a chromatogram of plasma spiked with 20 μg/ml vancomycin. The retention times of vancomycin and TMP are 2.5 and 4.5 min, respectively.

Extraction recoveries of vancomycin are shown in Table I, and all values were greater than 70% at the two concentrations. The recovery of TMP averaged $81 \pm 5.3\%$ ($n = 10$) for a concentration of 10 μg/ml. Replicate samples ($n = 10$)

TABLE I

RECOVERY OF VANCOMYCIN FROM PLASMA

Amount added (μg/ml)	Number of determinations	Recovery (mean ± S.D.) (%)	Coefficient of variation (%)
10	5	72 ± 9.3	12.9
40	6	76 ± 6.4	8.4

of standards containing 10 and 40 μg/ml vancomycin yielded within-day coefficients of variation of 6.7 and 3.1%, respectively. The day-to-day coefficients of variation ($n=10$) for the same concentrations were 7.8 and 5.2%, respectively.

The accuracy (mean percentage differences between added and measured amounts) for the values of the recovery standards, when calculated as unknown values against the linear regression line, were 8.2 and 3.4% for the concentrations of 10 and 40 μg/ml vancomycin, respectively ($n=10$).

The equations describing the standard curves, determined by linear least-squares regression analysis, were $y=0.0017+0.2169x$ and $y=0.0032+0.0176x$ for the ranges 0.5–5 and 5–70 μg/ml, respectively. The corresponding correlation coefficients (r) were always better than 0.994 and 0.997 ($n=6$).

The minimal detectable concentration was 0.1 μg/ml at a signal-to-noise ratio greater than 5:1 at 0.01 a.u.f.s. Above this concentration, the plasma components do not interfere significantly with internal standard and vancomycin peaks.

Selectivity was studied with drugs that could be coadministered with vancomycin to patients. None of the following substances interfered with the determination of vancomycin at 229 nm: furosemide, gentamicin, tobramycin, netilmicin, ampicillin, cefotaxim, penicillin.

Typical chromatograms of serum (S_3) and heart valves obtained from subject 2 are shown in Fig. 1C and D, respectively. The mean (±S.D.) serum levels at S_1, S_2 and S_3 are 55.5 ± 16.2, 63.4 ± 22.3 and 31.1 ± 15.5 μg/ml, respectively.

The mean (± S.D.) heart valve level (S_v) is 12.7 ± 7.2 μg/g (Table II). These data suggest a good penetration of vancomycin into the valves at a concentration well above the minimal inhibitory concentration for *Staphylococci* and *Streptococci*. This supports the use of vancomycin for prophylaxis of endocarditis [14].

In conclusion, the Sep-Pak procedure is rapid and requires minimal laboratory

TABLE II

VANCOMYCIN CONCENTRATIONS IN SERUM (S_1, S_2, S_3) AND AORTIC VALVE (S_v) ($n=7$)

	Valve weight (mg)	Vancomycin concentration			
		S_v (μg/g)	S_1 (μg/ml)	S_2 (μg/ml)	S_3 (μg/ml)
Mean	465.7	12.7	55.5	63.4	31.1
S.D.	303.8	7.2	16.2	22.3	15.5
Range	124–882	4.7–23.1	26.7–69.7	34.9–90.4	12.0–56.3

equipment. It offers appreciable accuracy and precision, and uses only 200 μl of serum concentration during therapy. Furthermore it is sensitive enough to monitor pharmacokinetic studies or to determine tissue levels.

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