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Short communication

Comparison of high-performance liquid chromatography with fluorescence polarization immunoassay for the analysis of vancomycin in patients with chronic renal failure

Tawfeeg A. Najjar*, Abdulatif A. Al-Dhuwailie, Asgedom Tekle

Department of Clinical Pharmacy, College of Pharmacy, P.O. Box 2457, K.S.U, Riyadh 11451, Saudi Arabia

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Abstract

Eighty-two plasma samples from patients with chronic renal failure undergoing vancomycin treatment and hemodialysis (HD) were analyzed with fluorescence polarization immunoassay (FPIA) and high-performance liquid chromatography (HPLC). Vancomycin was infused once and the samples were collected during three subsequent HD sessions at 2 h, 3 days and 5 days post-infusion. The HPLC method, modified from an earlier assay, was simple. There was a wide variation in the estimated concentration between the two assay methods. The results obtained by HPLC were 69% lower than those obtained by FPIA. This difference in vancomycin concentration was independent of the sampling time after vancomycin infusion. HPLC analysis commenced approximately 1.5 year after that of FPIA. To study the effect of in vitro degradation, the vancomycin concentration in ten of the samples was redetermined with FPIA during HPLC analysis. The concentrations of those samples decreased to 78–98% (average 92%) of the original concentration. Because FPIA appears to lack specificity, there is a need of other methods such as HPLC for vancomycin measurements, particularly in samples from patients with end-stage renal failure.

1. Introduction

Vancomycin is the drug of choice for treating many serious gram-positive bacterial infections including methicillin-resistant *Staphylococcus aureus* (MRSA) [1]. Its use has increased dramatically over the past two decades due to the increased clinical significance of these infections. Vancomycin in plasma is measured routinely in many hospitals, and the level measured is used to guide the therapy. Most hospitals use fluorescence polarization immunoassay (FPIA) (Abbott TD_x system) [2]. Over the past 10 years, several investigators questioned the specificity of FPIA, which overestimates the levels of drugs such as methotrexate, theophylline and vancomycin [3–5]. However, there are a limited number of studies comparing the two methods of analysis of vancomycin [5–7]. The kidney function appears to play an important role in the degree of overestimation reported for FPIA [8]. In this paper we aimed to study this phenomenon in patients with end-stage renal disease (ESRD), where the plasma was collected during three

* Corresponding author.

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subsequent HD sessions after vancomycin administration.

2. Experimental

2.1. Sample collection

Seven patients with chronic renal failure undergoing maintenance hemodialysis (HD, thrice weekly) were included in this study. Each received a single dose of vancomycin (1 g) as a 1-h infusion followed by 4-h HD sessions on days 1, 3 and 5 post-infusion. The HD session on day 1 began 2 h after the vancomycin infusion. The samples were collected from the arterial site of the HD system (before and at 0.25, 2 and 4 h after HD) and from the peripheral vein (4 h after HD). Eight of the samples collected from the peripheral vein were missing. All samples were kept at −20°C until the day of analysis. Three patients did not enter the third HD session, resulting in 82 plasma samples assayed with FPIA and HPLC.

2.2. Assay of vancomycin

All samples were initially assayed with the FPIA technique using vancomycin kits on the TD_x system (Abbott). The system is well established and used routinely for the major part of the therapeutic drug monitoring program. Analysis of vancomycin by HPLC commenced approximately 1.5 years later. The long-term stability of vancomycin (>1 year) at −20°C has been reported in previous studies [9]. To insure consistency of vancomycin concentration, ten of the samples which had been assayed with FPIA were assayed during the period of HPLC analysis.

2.3. HPLC assay

The HPLC assay of vancomycin was based on a modification of the method described by McClain et al. [10]. This involved the precipitation of plasma proteins in the sample with cold 40% trichloroacetic acid (TCA) before extracting the drug with diethyl ether. Essentially, to 100 μl plasma sample was added an equal volume of TCA, and the mixture was mixed for 2 min before centrifugation at 1000 *g* for 10 min. The resulting supernatant was transferred to Eppendorf tubes and shaken with 1 ml of diethyl ether. The ether layer was discarded and 50 μl of the remaining aqueous phase were withdrawn and mixed with an internal standard (phenacetin, 10 μl, 10 ppm). After mixing with the internal standard, 20 μl of the final mixture were injected into the chromatograph. The assay instrumentation included a solvent delivery system coupled to an injector (U6K), a variable-wavelength UV detector (M-484) and a μBondapak C_{18} (10 μm, 30 cm × 3.9 mm I.D.) column (Waters Assoc., Milford, MA, USA). The mobile phase consisted of acetonitrile–methanol–0.05 *M* Pic-B7 reagent (Waters Assoc.) (8:17:75) and was run at 1 ml/min; the effluent was monitored at 280 nm. Vancomycin was quantified using the vancomycin/internal standard peak-height ratio in a calibration curve prepared by dilution of vancomycin in drug-free plasma.

2.4. Data analysis

The difference between HPLC and FPIA in quantitating vancomycin was calculated using the following relationship:

$$\text{Difference}(\%) = [\text{vancomycin(FPIA)} - \text{vancomycin(HPLC)}]/\text{vancomycin(FPIA)}$$

3. Results and discussion

Under the chromatographic conditions described above, the relative retention times for vancomycin and the internal standard, phenacetin, were 7.2 and 10.8 min, respectively. As can be seen in Fig. 1, the two drugs were well resolved with no apparent interference from endogenous plasma components. The calibration curve showed a good linearity in the concentration range 0–50 μg/ml. Vancomycin levels measured by HPLC and FPIA (mean ± S.D.) for the three HD sessions in seven patients are shown in Table 1. The difference was marked but

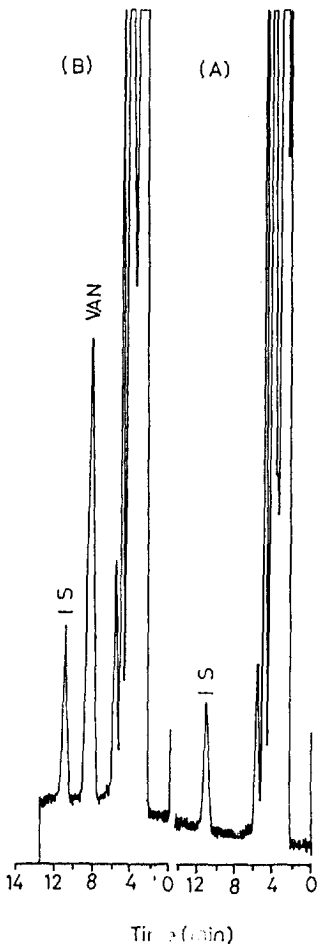

Fig. 1. Chromatogram of (A) a blank and (B) a vancomycin-spiked (10 ppm) human plasma extract. The internal standard (I.S.) (10 ppm) was included in both chromatograms.

remained within a narrow range, between 62 and 75%, regardless of the time of HD. Simple linear regression analysis programs on a personal computer (FX-720 p, Casio) were used for the correlation analysis. The correlation coefficient (r) was in the range 0.686–0.962 for the individual patients and 0.913 for all the samples ($n = 82$) (Fig. 2). Linear regression equations based on the vancomycin concentrations in 82 plasma samples and in the plasma of two selected patients representing the lowest and highest difference are summarized below.

y (FPIA) = 2.93 (HPLC) + 2.81 ($n = 82$)

y (FPIA) = 2.33 (HPLC) + 3.48 ($n = 14$)

Table 1
Mean (±S.D.) vancomycin levels (μg/ml) as measured by HPLC and FPIA in patients

	Vancomycin concentrations (μg/ml)				n
	HPLC	FPIA	Difference.	%	
First HD session.	8.5 ± 3.9	29.5 ± 11.0	21.1	71	33
Second HD session.	3.9 ± 1.7	14.4 ± 5.2	10.5	73	33
Third HD session.	3.5 ± 1.0	10.1 ± 4.1	6.6	65	16

Each patient was infused with vancomycin (1 g) over 1 h followed by three hemodialysis (HD) sessions, on days 1, 3 and 5 post-infusion; n = number of samples collected during each session from seven patients.

y (FPIA) = 3.84 (HPLC) + (−0.5) ($n = 14$)

The mean (±S.D.) vancomycin level for the samples collected 2 h post-infusion (time zero of the first HD session) were 11.3 ± 4.7 and 39.5 ± 10.0 μg/ml, respectively, for HPLC and FPIA.

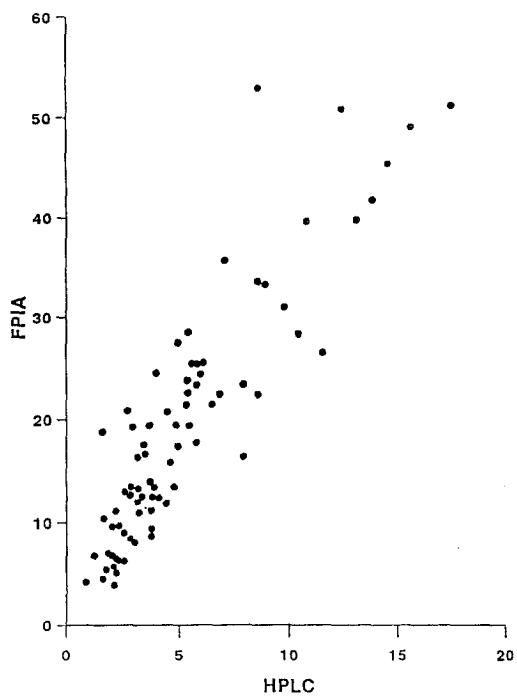

Fig. 2. Comparison of FPIA and HPLC for vancomycin levels in 82 plasma samples collected from seven end-stage renal disease patients during hemodialysis therapy ($r = 0.913$).

The mean (±S.D.) concentrations for the 82 samples were 5.5 ± 3.7 and 18.9 ± 10.0 μg/ml, respectively, for the two methods. Vancomycin levels in ten of the samples which were assayed twice over 1.5 year with FPIA were 41.0 ± 8.7 and 37.4 ± 9.1 μg/ml, respectively, for the first and second time.

This study confirms the presence of wide variations between the two assay methods, the concentration of vancomycin measured by HPLC being 69% lower than that measured by FPIA (range 62–75%). To date, there are only a few studies that have compared FPIA and HPLC measurements in plasma of patients treated with vancomycin [5,6]. One of these studies reported an overestimation by FPIA in the range of 16–56% (mean 39.8%) in patients taking vancomycin (thrice weekly, intraperitoneally) during peritoneal dialysis [5]. In the other study, which included samples of patients with renal failure, the vancomycin concentration reported by HPLC was 17% lower than that of FPIA, according to the reported equation [6]. In the same study the bias (in vitro) between the two methods reached a value of ca. 10%. The investigators explained this difference by the formation of degradation products, mainly CDP-1, which may interfere with the polyclonal antibody of FPIA. The formation of CDP-1 in vivo has been documented in the literature, and in vitro cross-reactivity of this product with FPIA reagents has been estimated to be in the range of 32–44% [6,11]. In addition, this phenomenon was also studied in human plasma spiked with vancomycin (in vitro) and incubated at 37°C for 19 days. The overestimation observed was about 64% after 10 days [5].

This is probably the first time that the two methods have been compared using samples collected from ESRD patients during HD. The difference in vancomycin concentrations observed in this study is high compared to the few reports in the literature. Its reason cannot be precisely determined. The HPLC method used is simple, the chromatograms do not show broader peaks as reported in previous isocratic procedures [12], and vancomycin is measured with precision. Further, the conditions of the assay were stable as indicated by the good correlation between the results of the two methods for the individual patients ($r = 0.686$ to 0.962) as well as for the total number of samples ($r = 0.913$). In this study, HPLC measurements began about 1.5 year after those of FPIA, during which the samples were stored at −20°C. An earlier study confirmed that vancomycin, which was analyzed by bioassay, was stable for more than 1 year [9]. Because the bioassay does not detect degradation products their findings seem to be a good evidence for vancomycin stability. Another study has indicated that the degradation products, mainly CDP-1, were not formed under the routine storage conditions [7]. To investigate the effect of storage intervals, ten of the samples previously assayed with FPIA were re-assayed using the same method. The results showed a minimal decline of about 8% (range 2–22%) over the 1.5 year. Because CDP-1 cross-reacts with FPIA to an extent of 32–44% [6], the decline in concentration could be underestimated by FPIA if CDP-1 was produced during storage. However, such degradation should be minor and does not eliminate the wide variation between the two methods if used for therapeutic vancomycin monitoring in this group of patients.

Morse et al. [5] investigated the influence of the duration of vancomycin therapy on the FPIA and HPLC vancomycin assay results. They found mean overestimations of 13.7, 25.3 and 39.6%, respectively, for the (thrice weekly) intraperitoneal vancomycin doses. This was explained by the presence of excessive degradation products accumulated with time during the vancomycin treatment. In this study the difference was not dependent on the time of sampling after vancomycin infusion.

In conclusion, FPIA overestimates the vancomycin level by as much as 69% in patients undergoing HD. The degree of overestimation remains unaffected by the time of sampling after infusion. Vancomycin in plasma stored below −20°C seems to be stable for 1.5 year. Until this issue has been solved, there is a need for methods which do not cross-react with the degradation products of vancomycin such as HPLC or

enzyme immunoassay, particularly for samples collected from patients with end-stage renal failure.

References

[1] G.R. Matzke, G.G. Zhanel and D.R.P. Guay, Clin. Pharmacokin., 11 (1986) 257.
[2] A.J. Sedman, B.A. Molitoris, L.M. Nakata and G. Gal, Am. J. Nephrol., 6 (1986) 132.
[3] T.A. Najjar, K.M. Mattar and I.M. Alfawaz, Ther. Drug Monit., 14 (1982) 142.
[4] K.E. Opheim and V. Ainardi, Clin. Chem., 29 (1983) 1698.
[5] G.D. Morse, D.K. Nairn, J.S. Bertino, Jr. and J.J. Walshe, Ther. Drug Monit., 9 (1987) 212.
[6] M.W. Hu, L. Anne, T. Forni and K. Gattwald, Ther. Drug Monit., 12 (1990) 562.
[7] L. Anne, M.W. Hu, K. Chan, L. Colin and K. Gattwald, Ther. Drug Monit., 11 (1989) 585.
[8] L.M. Perino and B.A. Mueller, Ann. Pharmacother., 27 (1993) 892.
[9] R.O. Moellering, D.J. Jrogstad and D.J. Greenblatt, Ann. Intern. Med., 94 (1981) 343.
[10] J.B.L. McClain, R. Bongiovanni and S. Brown, J. Chromatogr., 231 (1982) 463.
[11] L.O. White, R. Edwards, H.A. Holt, A.M. Lovering, R.G. Finch and D.S. Reever, J. Antimicrob. Chemother., 22 (1988) 739.
[12] F. Fehl, H. Monteil, C. Gallion and R.C. Thiery, Pathol. Biol. (Paris), 33 (1985) 511.