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Note

Vancomycin quantitation by high-performance liquid chromatography in human serum

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Since its introduction, vancomycin has found broad application in therapy of gram-positive infections where resistance or allergy has precluded the use of penicillin [1, 2]. Administration of vancomycin is complicated by renal insufficiency since drug is only excreted via glomerular filtration and accumulates to high levels if the dose is not reduced [3]. When the glomerular filtration rate is rapidly changing as in a seriously ill patient with hemodynamic instability, prediction of therapeutic levels is difficult and monitoring of drug levels must be done to prevent subtherapeutic or toxic levels. The major adverse effect of vancomycin is ototoxicity which has been reported when blood levels have reached the 80 μl/ml range [4]. Currently, therapeutic monitoring of vancomycin levels is done using a microbiologic assay which takes from 24 to 48 h. We developed a rapid method of vancomycin assay which would be useful in the therapeutic range needed for most infections which does not require vancomycin extraction or internal standards.

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METHODS

All reagents were of analytical grade and were commercially available. Vancomycin standard 1150 mg of activity per g was a gift of Eli Lilly Research Corp. (Indianapolis, IN, U.S.A.). Vancomycin measurements were determined relative to potency. Water was deionized and filtered. Serum used in assay was from a common donor. Other antibiotic specimens were obtained from commercial sources of antibiotic standards. Chromatography was performed on a Waters Associates Model 6000 solvent pump, Model 440 ultraviolet detector at a wavelength setting of 280 nm, sensitivity of 0.005 a.u.f.s., and a flow-rate of 2 ml/min. We found that vancomycin gave maximal absorption at 280 nm. A Waters reversed-phase μBondapak C_{18} column (30 cm × 3.9 mm I.D., 10 μm particle size) was used with a mobile phase of 12% acetonitrile and 88% 0.01 *M* 1-heptane sulfonic acid (PIC-B7, Waters). Under isocratic conditions quantitation was based on peak height and standardized against a sample with known amount of vancomycin by activity.

Sample preparation

A 500-μl volume of serum was combined with 1 ml of cold trichloroacetic acid (TCA) and mixed in a vortex mixer. The mixture was then spun at 2500 *g* for 15 min in a refrigerated Sorvall DPR 6000 centrifuge. The supernatant was removed to screw-top test tubes. Then 4 ml of diethyl ether were added, mixed, and the organic supernatant was decanted removing the TCA; 100 μl of the aqueous layer were injected on the column.

Microbiologic assay was done using standard methods employing *Bacillus subtilis* ATCC 6633 obtained commercially [5]. Clinical specimens were bioassayed using the Microbiology Laboratory's vancomycin standards.

Initial studies were performed with aqueous and serum specimens with known amounts of the same vancomycin standard added. The samples were split and run simultaneously by microbiologic and high-performance liquid chromatographic (HPLC) methods. Clinical specimens were frozen after bioassay and chromatographed together. Preliminary work indicated no loss of vancomycin activity over a four-week interval in frozen specimens.

RESULTS

Fig. 1 demonstrates the HPLC peaks after TCA precipitation for serum, aqueous vancomycin and vancomycin in serum. Retention time for vancomycin is 16 min. Total sample analysis time is 20 min.

We found vancomycin to be in the aqueous layer of the ether extraction only. No vancomycin was detected in the ether fraction even when levels of 128 μg/ml were tested, which is far above the levels used clinically.

Fig. 2 shows correlation of peak heights before and after precipitation with TCA in aqueous and serum samples. As one may see, the activity loss in aqueous specimen is the same as activity loss in serum specimens; 87% of activity is recovered in the precipitated aqueous and serum samples compared to unprecipitated samples.

Fig. 3 demonstrates comparison between microbiologic assay and HPLC

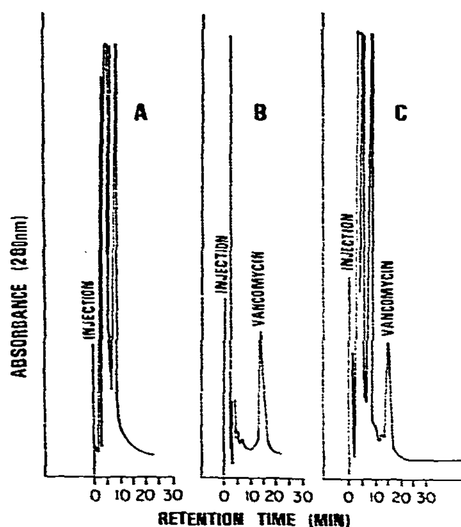

Fig. 1. (A) Chromatogram of blank serum treated with TCA. (B) Chromatogram of an aqueous solution of vancomycin (25 μg/ml) treated with TCA. (C) Chromatogram of serum spiked with vancomycin (25 μg/ml) and treated with TCA. Ultraviolet detection at 280 nm, sensitivity 0.005 a.u.f.s.

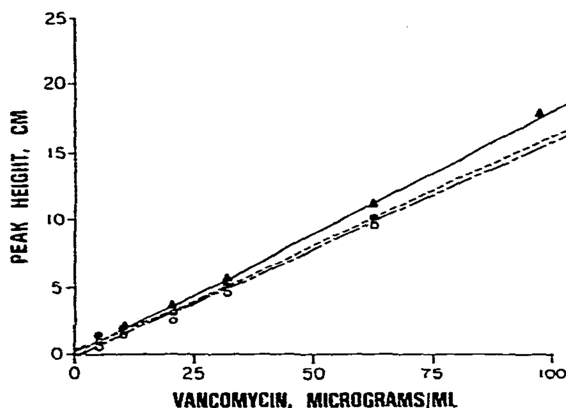

Fig. 2. Correlation of peak heights before and after precipitation with TCA in aqueous and serum samples. ▲, Aqueous vancomycin, $y = 0.18x + 0.03$, correlation coefficient $r = 1.00$; •, TCA-precipitated aqueous vancomycin, $y = 0.16x + 0.20$, $r = 1.00$; o, TCA-precipitated vancomycin-spiked serum, $y = 0.16x + 0.11$, $r = 1.00$. Conditions: 100 μl injected on column; ultraviolet detection at 280 nm, sensitivity 0.005 a.u.f.s.

methods when the same vancomycin standard was used in each method. Standard deviation of the assay at 5 μg/ml was $\pm$ 0.25 μg.

Fifteen clinical specimens from patients who had vancomycin levels determined by bioassay as part of vancomycin therapy were run by HPLC. Our method showed a correlation coefficient $r = 0.87$. Different lots of vancomycin standard were used by the Microbiology Laboratory and the HPLC Laboratory in these clinical comparisons.

The following drugs were found not to interfere with the assay: penicillin G, nafcillin, ampicillin, cephalothin, amphotericin, amikacin, tobramycin, gentamicin, and tetracycline.

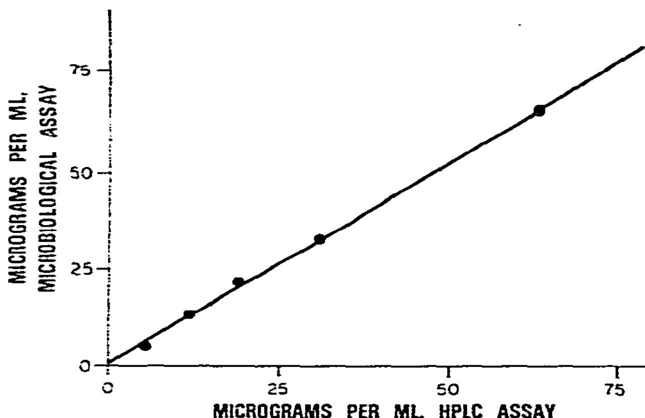

Fig. 3. Comparison between the present method and a microbiological assay for a series of spiked serum samples. $y = 1.05x + 0.64$, $r = 1.00$.

DISCUSSION

Microbiologic assay is the current standard of vancomycin determination. HPLC offers a rapid and accurate alternative vancomycin assay. The advantage of our method is its simplicity, requiring no extractions, internal standards or derivatization and its usefulness in the range of drug concentrations that vancomycin normally achieves in serum. The previously reported method of Uhl and Anhalt [6] differs from our method in several aspects: the different composition of the mobile phase, the use of cold TCA in a refrigerated centrifuge and the absence of an extraction step. The use of cold TCA may be responsible for the increased recovery of vancomycin in our method. Our assay is not useful in as low a range as the method of Uhl and Anhalt, but we decided levels below 5 µg/ml would not be clinically important to measure. Vancomycin is not altered in the body to any extent and, since the body's only method of excretion is via glomerular filtration, renal failure makes the blood levels of the drug very difficult to estimate even with a nomogram. There is no safety factor as with aminoglycosides or penicillin where the drug is altered in the body giving one some cushion in dose determination. Therapeutic monitoring with short turn-around times may greatly improve the quality of patient care in patients treated with vancomycin.

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