ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 1991, p. 1586–1590
0066-4804/91/081586-05$02.00/0

Vol. 35, No. 8

Clinical Evaluation of Teicoplanin Fluorescence Polarization Immunoassay

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Received 15 June 1990/Accepted 6 June 1991

A teicoplanin fluorescence polarization immunoassay (FPIA) developed by International BioClinical (IBC) was evaluated by using serum samples from patients who had been receiving teicoplanin at Detroit Receiving Hospital (DRH) as part of a clinical investigation. Patient samples collected over a 1-year span were assayed at DRH and at IBC, and the results were compared with those of a standard microbiological assay performed at Merrell Dow Research Institute, Indianapolis, Ind. The FPIA has a rapid turnaround time (circa 20 min), utilizes small sample volumes (less than 100 μl), and is sensitive and accurate in determining concentrations in the range of 5 to 100 μg/ml. The intra-assay and interassay coefficient of variation for controls (7, 35, and 75 μg/ml) was ≤13%. Concentrations greater than 100 μg/ml must be diluted prior to the assay, which may introduce additional error in determination. The FPIA compared well with the bioassay (r = 0.901) for 193 clinical samples. The results obtained utilizing the FPIA system were reproducible at two different sites, as illustrated by the high degree of correlation between the results at DRH and IBC (r = 0.92). There was less than 7% interference noted when teicoplanin was assayed in the presence of other antibiotics. Patient samples stored for up to 1 year retained their potency: the mean recovery rate in these samples was 107%. The FPIA should be useful for monitoring and adjusting teicoplanin dosage regimens in patients.

The glycopeptide teicoplanin is an investigational antibiotic with a spectrum of activity which is similar to that of vancomycin (15). In clinical studies, teicoplanin has proven to be effective against infections by gram-positive organisms such as *Staphylococcus aureus* and *Staphylococcus epidermidis*, including methicillin-resistant strains. Efficacy has been demonstrated in infections including bacteremia, skin and soft tissue infections, bone and joint infections, pneumonia, and endocarditis (1, 6). Advantages over vancomycin include a lower incidence of nephrotoxicity (11), a longer half-life (requiring less-frequent dosing), and the capacity for intramuscular injection (14). Preliminary studies with teicoplanin indicate that population pharmacokinetic parameters are variable and unpredictable in some patients and that therefore, monitoring of levels in serum for efficacy and toxicity may be necessary (13).

Teicoplanin concentrations in serum have been accurately measured by bioassay (4). The major disadvantage of using bioassays for patient monitoring in a clinical setting is the long turnaround time (approximately 24 h, including incubation period). Teicoplanin is composed of a complex of six analogs which have been identified by high-pressure liquid chromatography (HPLC) (7, 9). Although HPLC quantification of teicoplanin is fairly accurate, it suffers from a long analysis time and is too complicated to utilize in the clinical setting to monitor patients.

International Bioclinical (IBC) has recently filed a New Device Application with the Federal Food and Drug Administration supporting the use of their fluorescence polarization immunoassay (FPIA) reagent system for quantifying teicoplanin in serum samples. The purpose of the present study was to evaluate the ability of a this newly developed FPIA to quantitate teicoplanin in clinically obtained serum samples and to compare FPIA with a standard teicoplanin bioassay.

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MATERIALS AND METHODS

FPIA. Conventional FPIA (8) was performed utilizing the IBC reagent system with the American Bioclinical FP Instrument system. The FPIA utilizes flourescein-labeled teicoplanin which competes with unlabeled teicoplanin for antibody. Because the polarization of fluorescent light increases as the flourescein-labeled teicoplanin is bound to antibody, this assay provides a measure of bound and free labeled teicoplanin in a competitive binding assay. Calibration standards and control samples prepared in human serum containing 0.1% azide preservative were obtained from IBC. Calibration standards containing 0, 5.0, 10.0, 25.0, 50.0, and 100.0 μg of teicoplanin per ml were used for the generation of the calibration curve. The FP analyzer calculates the millipolarization value and extrapolates the teicoplanin concentration for each replicate by comparing the millipolarization value of the sample with the millipolarization values from a calibration curve. Calibration curves were performed a minimum of once per week, and reference samples containing high (75-μg/ml), medium (35-μg/ml), and low (7-μg/ml) teicoplanin concentrations were run concomitantly with each calibration curve. All controls and samples (see below) were tested in triplicate. The mean of three derived concentrations corresponds to the reported concentration.

The lower limit of detection by FPIA was determined by examining the variability of the zero calibrator (n = 12). Two standard deviations were subtracted from the resulting mean millipolarization value. The corresponding concentration was derived from the calibration curve.

Bioassay. The bioassay was performed at Merrell Dow Research Institute (MDRI) by the method of Erickson et al. (4). Mueller-Hinton agar was used as the basal medium to which NaCl and $CaCl_2$ were added. Citric acid was utilized to adjust the medium to the desired pH, resulting in an acidic environment. *Bacillus subtilis* ATCC 6633 was used as the indicator organism. Serum containing β-lactam antibiotics was pretreated with an appropriate β-lactamase (10). Samples anticipated to contain more than 96 μg/ml (i.e., all peak samples) were prediluted with antibiotic-free pooled human serum. The assay has a within- and between-run coefficient of variation of less than or equal to 10% over the concentration range of 1.5 to 96 μg/ml. The lower limit of detection for this assay is 0.19 μg/ml.

Samples. Thirty-two serum teicoplanin concentrations ranging from 0 to 260 μg/ml were prepared by IBC. The samples were made with the teicoplanin analytical reference substance (batch ANG-Jan87) provided by MDRI. A stock teicoplanin solution (5,248 μg/ml) was prepared by diluting the analytical reference substance with 1% methanol in water. This stock solution was used to spike pooled human serum to the various concentrations. Quantitation of these samples was performed in a blinded fashion by bioassay and FPIA at MDRI and Detroit Receiving Hospital (DRH), respectively.

One-hundred ninety-seven serum samples collected from 33 patients participating in clinical trials of teicoplanin at DRH over a 1-year period (March 1989 to March 1990) were utilized in this study. These patients were being treated for infections with gram-positive organisms, and none of the 33 patients received any additional antibiotics. Patient serum samples were divided into two aliquots: one portion was retained at DRH, and an equal portion was sent to IBC for assay via FPIA. There was a sufficient quantity of sample available in 193 cases to also send an aliquot of the sample to MDRI for quantification by bioassay.

Serum samples were stored at −20°C until assayed. Patient samples were run at DRH within 24 h from the time of collection. For purposes of assessing the stability of teicoplanin in serum under frozen conditions, eight of these samples were stored for 1 year and assayed for teicoplanin content by FPIA. Serum samples which were anticipated to contain more than 100 μg of teicoplanin per ml (i.e., peak serum samples) were diluted 5:1 with teicoplanin-free human serum.

Pooled human serum was spiked with teicoplanin at a concentration of approximately 30 μg/ml, and the concentration was verified by FPIA. Various antibiotics (vancomycin, daptomycin, ciprofloxacin, gentamicin, erythromycin, nafcillin, cefazolin, rifampin, and piperacillin) were then added in concentrations to simulate in vivo peak concentrations in serum. The samples were tested for specificity of the assay for teicoplanin.

Statistics. The comparison of FPIA results with spiked teicoplanin concentrations was performed by multiple linear regression analysis. The comparison of FPIA with the microbiological assay and of FPIA results at the two sites was performed by orthogonal least-squares regression analysis (3). Stability and specificity were assessed by calculating the mean and standard deviation of the recovery rates.

RESULTS

Teicoplanin quantitation in serum. Linearity was assessed by comparing the values obtained for 32 serum samples to which teicoplanin had been added with their theoretical concentrations by multiple linear regression analysis. The equation of the regression line for the FPIA was $y = 0.93x + 2.60$, with a correlation coefficient of 0.997 (Fig. 1). For all concentrations in the range from 0 to 260 μg/ml, the percent recovered was 92.4. Recovery was highest for concentrations in the range from 10 to 100 μg/ml (96%). However, concentrations less than 5 μg/ml routinely read higher than targeted, and their recovery rate was lowest (71%). The lower limit of detection for FPIA was determined to be 0.5 μg/ml.

The equation of the regression line for the bioassay was $y = 0.99x - 2.11$, with a correlation coefficient of 0.998. For 95% of the samples, the bioassay results read lower than the known concentration.

Comparative accuracy. In a direct comparative study, 193 serum specimens obtained from 33 patient receiving teicoplanin were assayed by FPIA and bioassay. The concentrations determined by these two methods showed good agreement (Fig. 2). The equation of the orthogonal regression line for FPIA versus bioassay was $y = 1.40x - 9.78$, with a correlation coefficient of 0.901.

The reproducibility of our data is illustrated by comparing the results of FPIAs performed at DRH and IBC. One hundred ninety-seven samples obtained from 33 patients at DRH were analyzed at both sites for teicoplanin content (Fig. 3). Patient sample teicoplanin concentrations ranged from approximately 15 μg/ml to 500 μg/ml. The equation of the orthogonal regression line comparing results from the two sites was $y = 1.22x - 5.33$, with a correlation coefficient of 0.92.

Precision. Interassay precision is illustrated by evaluating results obtained from running control samples (Fig. 4). High-, medium-, and low-concentration controls corresponding to 75, 35, and 7 μg/ml, respectively, were supplied by IBC and were run with each calibration curve and each day that patient samples were run. The mean (± one standard deviation) for the controls run over a 1-year period were as follows: high, 70.7 μg/ml (±6.64); medium, 34.7 μg/ml (±3.12); and low, 6.5 μg/ml (±0.83), and the coefficients of variation were 12.7, 9.00, and 9.38, respectively.

Specificity. In the presence of other antibiotics, the FPIA consistently reported teicoplanin concentrations higher than those reported in the absence of additional antibiotics (Table 1). Interference of these agents was ≤6.3%. The addition of vancomycin, which is structurally similar to teicoplanin, resulted in a reading only 2.8% higher than that made prior to the addition of vancomycin. According to IBC, other potentially interfering substances, such as ticarcillin, amikacin, netilmicin, kanamycin, tobramycin, streptomycin, and cyclosporine, have less than 5% interference with teicoplanin FPIA (12).

Stability. Teicoplanin stability under frozen conditions (−20°C) was determined from eight patient clinical serum samples stored for a minimum of 1 year. The samples were originally determined to contain 27.4 to 57.7 μg of teicoplanin per ml. The mean percent recovery (± 1 standard deviation) was 107% (±7.1%).

The stability of teicoplanin in human serum was also assessed by monitoring the variability of teicoplanin low-, medium-, and high-concentration control samples which were prepared by IBC and stored at −20°C for a 1-year period. The overall coefficient of variation, as stated above, was <13%.

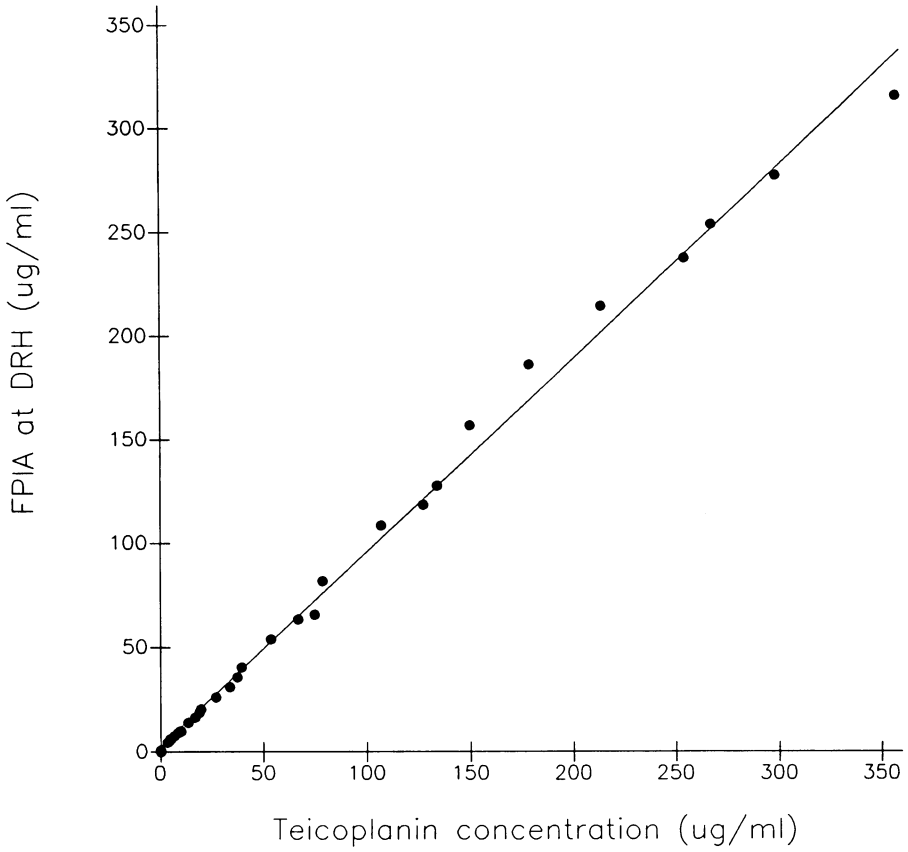

FIG. 1. Linear regression analysis comparing teicoplanin concentrations in 32 samples assayed by FPIA at DRH versus known (spiked) concentrations ($y = 0.93x + 2.60$; $r = 0.997$).

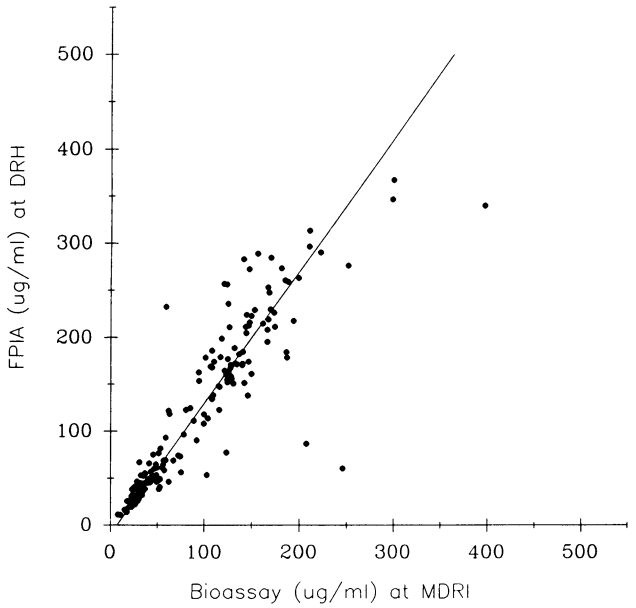

FIG. 2. Orthogonal regression analysis comparing teicoplanin concentrations in 193 patient serum samples assayed at DRH with the results of the bioassay performed at MDRI ($y = 1.40x - 9.78$; $r = 0.901$).

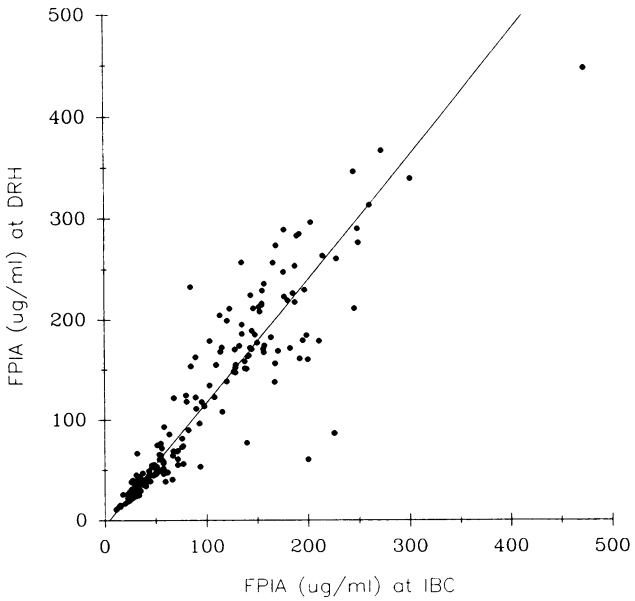

FIG. 3. Orthogonal regression analysis comparing teicoplanin concentrations in 197 patient serum samples assayed by FPIA at DRH and IBC ($y = 1.22x - 5.33$; $r = 0.92$).

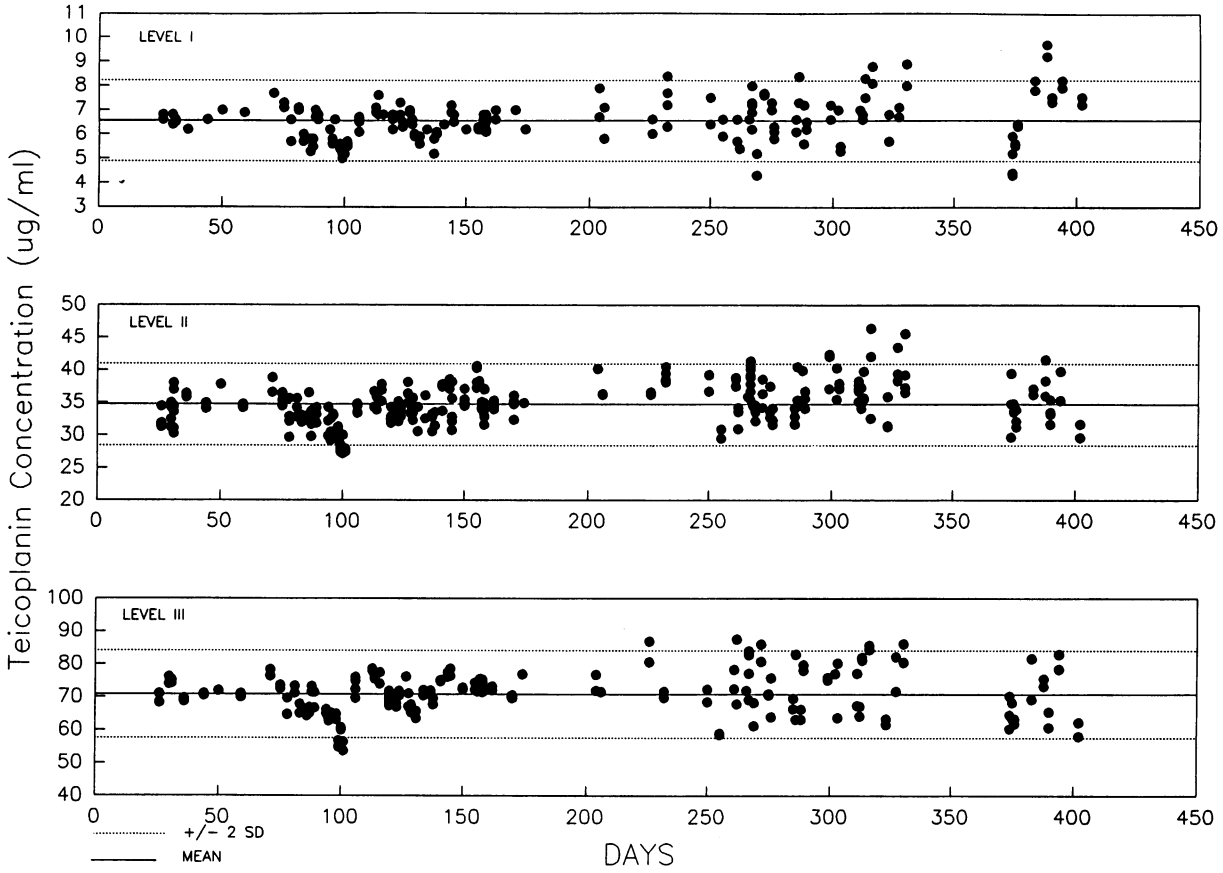

FIG. 4. Teicoplanin controls run during 1-year study period at DRH. Level I, 7 μg/ml; level II, 35 μg/ml; level III, 75 μg/ml.

DISCUSSION

Bioassay has been the method of choice for the quantitation of teicoplanin in serum. Although this method is sensitive and accurate, it is impractical for routine clinical monitoring of patients because of its sample volume requirement and extensive turnaround time. A procedure for removal of interfering β-lactams and aminoglycosides from serum to be assayed by bioassay has been described previously (10). However, other interfering agents (macrolides, lincosamides, and quinolones, etc.) would limit the assay's specificity, since there is no method for their removal.

TABLE 1. Specificity of teicoplanin FPIA assay

Drug (concn added [μg/ml])	Mean teicoplanin concn (μg/ml)		% Difference
	With added drug	Without added drug	
Vancomycin (50)	29.80	28.96	2.8
Ciprofloxacin (3.75)	34.68	33.15	4.4
Gentamicin (4)	32.47	32.40	0.2
Erythromycin (5)	27.86	27.46	1.4
Nafcillin (50)	28.50	27.46	3.6
Cefazolin (100)	28.34	27.46	3.1
Rifampin (3)	29.32	27.46	6.3
Piperacillin (100)	30.85	29.97	2.8
Daptomycin (30)	32.04	31.08	3.0

We evaluated a FPIA reagent system to be used to quantify teicoplanin in serum. By adding known quantities (10 to 260 μg/ml) of teicoplanin to human serum, the ability of FPIA to quantify teicoplanin in serum was determined. Acceptable recovery was obtained, particularly over the concentration range from 0 to 100 μg/ml. Dilution of concentrations greater than 100 μg/ml is required, and this adds error to the determination. A larger degree of variability would be expected for concentrations of less than 5 μg/ml, since this was the lowest calibrator utilized. There was no pattern observed such that the FPIA results were routinely higher or lower than the known concentrations. The bioassay also performed well; however, in most instances the measured concentration was read lower than the known concentration. The reason for this observation is unknown.

The FPIA also performed well in quantifying teicoplanin in patient samples. The results obtained by FPIA were comparable to the results of a previously published microbiological assay (4). A few outliers were noted which are unexplainable but may be related to technical error.

We found that teicoplanin determination by FPIA is reproducible by evaluating multiple clinical specimens assayed at two separate sites. The percent coefficient of variation between replicates of the same sample was found to be less than 10%, indicating minimal intra-assay variability.

When various antibiotics were added to serum which already contained a known quantity of teicoplanin, the ability to quantify teicoplanin remained high. Therefore, this

assay can be used to monitor teicoplanin concentrations in patients receiving the antibiotics which we tested. IBC has also tested the assay's specificity in the presence of various agents; however, additional work is necessary in this area, and caution should be exerted in extrapolating these data to other agents.

Both teicoplanin patient samples and spiked human serum stored frozen over a 1-year period showed minimal change in quantitation. The majority of patient values tended to increase from initial determinations, which may be secondary to sublimation causing the concentration of teicoplanin to rise, since the stored volumes were less than 1 ml.

The teicoplanin FPIA reagent system developed by IBC is sensitive and accurate in the concentration range from 5 to 100 μg/ml. The assay can be performed with reasonable reproducibility and should be useful for monitoring patients receiving teicoplanin. Dosages of 6 to 30 mg/kg/day in patients have typically resulted in concentrations in serum ranging from 5 to 150 μg/ml, which are within the capability of this assay (5, 13).

Compared with the microbiological assay, FPIA is easier to operate and has a faster turnaround time. In regard to the overall performance of the IBC reagents, with the American Bioclinical FPIA analyzer the procedure is semiautomated and the actual polarization is performed by an automated fluorescence detection device which would limit the contribution of error by technician variability. The reagents will also be available to be utilize with an automated analyzer (Abbott TD_x). Although routine monitoring of teicoplanin concentrations in serum is not advocated at this time, there are select populations of patients (i.e., patients being treated for *S. aureus* endocarditis) who may require close monitoring to ensure therapeutic efficacy (13).

ACKNOWLEDGMENTS

We thank Paul Mueggler, International Bioclinical, Portland, Oreg., for providing FPIA technical support and assisting in the data analysis. The biological assay was performed under the direction of Michael T. Kenny by Jacqueline Dulworth, Marcia Brackman, and Michael Graham (all of Merrell Dow Research Institute, Indianapolis, Ind.).

This work was supported by a grant from Merrell Dow Research Institute.

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