Evaluation of a Novel Fluorescence Polarization Immunoassay for Teicoplanin

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A fluorescence polarization immunoassay (FPI) for teicoplanin that uses the TDx Instrument System (Abbott, Irving, Tex.) as an automated analyzer has been developed by Innotron of Oregon Inc. and was evaluated in patients with staphylococcal infections enrolled in a clinical trial of the antibiotic. The assay proved accurate in estimating concentrations of between 5 and 100 mg/liter. The intraassay coefficient of variation was <7.3%, while the interassay variance was <11.6% against three commercially prepared standards at known concentrations of approximately 5, 35, and 75 mg/liter. Against routinely prepared standards at 10 concentrations between 5 and 100 mg/liter analyzed in a single run, the coefficient of variance did not exceed 4.3%. Compared with bioassay, the FPI demonstrated good correlation in terms of reliability (r = 0.909) in samples containing teicoplanin only and specificity (r = 0.916) in samples containing both teicoplanin and gentamicin. With a turnaround time of 20 min and with only 50 μl of serum needed for estimation of the amount of drug in a sample, the FPI described here should provide a useful method of teicoplanin measurement in routine diagnostic laboratories.

Teicoplanin is a new glycopeptide antibiotic with a spectrum of activity similar to that of vancomycin, showing both in vitro and clinical efficacy against most gram-positive organisms including Staphylococcus aureus (methylcin-resistant strains), Staphylococcus epidermidis, and Corynebacterium spp., in patients with a wide variety of moderate and severe infections (1, 9). The need for universal testing in patients receiving this antibiotic is yet unproven. However, although teicoplanin is reportedly less nephrotoxic than vancomycin, at least within certain subpopulations with serious infections, individual variation in pharmacokinetic parameters will require that monitoring of levels in serum, within the range of 5 to 150 mg/liter, for both efficacy and toxicity will be advisable (6).

Bioassay is established as an accurate method of estimation of teicoplanin levels (2, 4), albeit with a slow turnaround time and with potential interference by concomitantly administered antibiotics. Recently, a fluorescent polarization immunoassay (FPI) for teicoplanin, which was developed by International Biochemical and which uses the semiautomatic American Biochemical FP Instrument System, has been reported to be both sensitive and specific (5). A similar system has been developed by Innotron of Oregon Inc. (Portland, Oreg.), which uses the automated TDx Instrument System (Abbott, Irving, Tex.). Here we report an evaluation of the accuracy and precision of the Innotron assay kit.

MATERIALS AND METHODS

Bioassay. The bioassay was performed by the method of Patton et al. (4) by using an agar diffusion technique. Antibiotic medium 1 (Oxoid, Basingstoke, England) containing sodium chloride to a final concentration of 3% and adjusted to pH 5.5 to 5.7 by the addition of 1 N hydrochloric acid (0.125 N HCl to 100 ml of medium) was used, and Bacillus subtilis ATCC 6633 was used as the indicator organism. All samples were assayed in triplicate, and the reported concentrations are the arithmetic means of these three results. Serum containing aminoglycosides was adsorbed and separated by using Cellul-ion phosphate (100 mg/100 ml of serum; Sigma Chemical Co., Sydney, Australia) by the published technique of Stevens and Young (8). Samples anticipated to contain more than 100 mg of teicoplanin per liter were prediluted with antibiotic-free pooled human serum. The assay has intra- and interassay coefficients of variance of <10% over the concentration range of 1.5 to 100 mg/liter.

FPI. The Innotron FPI is based on the competitive binding principle, by which the teicoplanin in the sample competes with fluorescent-labelled teicoplanin for a fixed number of antibody sites. The assay exploits the fact that when a fluorescent molecule is excited with polarized light, the polarization of the emission is related to molecular size. An increased amount of unlabelled teicoplanin in the sample will result in decreased binding of fluorescent-labelled teicoplanin by antibody and, thus, decreased polarization of emitted light from the sample. The concentration of teicoplanin in a sample can be determined by comparing the polarization values of the unknown specimen with polarization values of a known calibration curve.

Innotron of Oregon Inc. supplied standards containing 0, 5, 10, 25, 50, and 100 mg of teicoplanin per liter for preparation of the calibration curve by using the kanamycin channel of a TDx instrument. Calibration curves were prepared at least once a week, and reference samples containing known concentrations of approximately 75 mg (high), 35 mg (medium), and 7 mg (low) of teicoplanin per liter were run concomitantly with each calibration curve and with each assay run. All controls and samples were tested in duplicate by using 50-μl aliquots, with the reported result being the arithmetic mean of the duplicate concentrations. The test carousel contains 20 wells, so that 10 samples can be tested in duplicate together in a single run.

Prepared (spiked) samples. Ten specific concentrations (54 samples) ranging from 5 to 100 mg/liter were independently prepared from a teicoplanin analytical reference substance.
batch 0024; Marion Merrell Dow) by dilution with 1% methanol in water. Quantitation of these samples was determined by FPI in a single day.

**Patient samples.** One hundred forty-five serum samples collected from 76 patients participating in a clinical trial of teicoplanin between 1988 and 1990 were assayed. Of these, 63 contained teicoplanin only and 82 contained teicoplanin and gentamicin. These samples were divided and assayed for teicoplanin concentrations by both FPI and bioassay.

**Statistical methods.** Results obtained by FPI for standard (control) and prepared (spiked) samples were analyzed by linear regression. Because a heterogeneity of variance was demonstrated, i.e., increasing measurement variance with an increasing value being measured, the linear regressions were performed by using a logarithmic scale. Results obtained from patients samples by both FPI and bioassay were also analyzed on a logarithmic scale by using an errors-in-variables regression model to allow for method error in both assay systems (3). All calculations were performed by using the SAS Statistical Package (7).

**RESULTS**

**Prepared (spiked) serum samples.** Comparison of values obtained by FPI in the 54 samples in which teicoplanin was added with theoretical concentrations is illustrated in Table 1. By using least-squares linear regression on a logarithmic scale, the equation of the regression line for FPI was \( y = 1.14x^{0.952} \), with a correlation coefficient of 0.999. The coefficient of variation at each concentration was equal to or less than 4.3%.

**Comparison with bioassay.** Sixty-three samples containing teicoplanin only were assayed by FPI and bioassay. Figure 1 illustrates the degree of agreement between the two methods. An errors-in-variables model on a logarithmic scale was used to analyze the correlation between techniques and yielded an equation of \( y = 0.09x^{0.84} \), where \( y \) is FPI and \( x \) is the bioassay parameter. A correlation coefficient of 0.909 was established.

**Precision.** Intraassay and interassay variabilities were determined by using supplied standards (controls) of three different concentrations (approximately 75, 35, and 7 mg/liter). These assays were undertaken on each occasion that the calibration curve was confirmed and on each day that patient samples were assayed. Results are presented in Table 2. These data were assessed by linear regression by using the method of least squares on a logarithmic scale and yielded an equation of \( y = 1.21x^{1.23} \), with a correlation coefficient of 0.993. Intraassay variation was small, being between 0.7 and 7.3%, while interassay variability was slightly greater, at 8.6 to 11.6%.

**Specificity.** Results for samples containing teicoplanin and gentamicin are illustrated in Fig. 2. By using an errors-in-variables method of linear regression on a logarithmic scale,
the equation determined was \( y = 1.96x^{0.81} \), where \( y \) is the FPI and \( x \) is the bioassay parameter. A correlation coefficient of 0.916 was established, suggesting that the presence of gentamicin does not interfere with accurate estimation of teicoplanin within a sample.

**DISCUSSION**

As with many new antimicrobial agents, the initial assay of concentrations of teicoplanin in serum was achieved by an agar diffusion bioassay, which has become the standard methodology (2, 4). Such a bioassay also has an added advantage of detecting all microbiologically active molecules of both the parent compound and its metabolites. However, such a system may be significantly affected by the concomitant clinical administration of other antibiotics, for which adequate removal or neutralization preassay is not always possible. Biological assays lack short turnaround times, which may be clinically important. Assay by high-pressure liquid chromatography is often the next development, but because teicoplanin is a complex of six analogs, such a method would be time-consuming and difficult in a routine laboratory.

FPI would be clinically useful, provided that it demonstrated acceptable correlation with the results of bioassay and showed high intraassay and interassay precision which were unaffected by the rapidity of the method. Such a technique has previously been described by using a semiautomated analyzer (American Biochemical FP Instrument Systems), with a reported turnaround time of 20 min, a sample volume of 100 µl, and a high accuracy and sensitivity when detecting concentrations within the range of 5 to 100 mg/liter (5).

We evaluated a similar assay developed by Innotron of Oregon Inc. which uses the fully automated Abbott TDx instrument to determine teicoplanin concentrations. This system also provides a turnaround time of 20 min or less and uses specimen aliquots of 50 µl. The system appears to measure all microbiologically active antibiotics in that the correlation coefficient, when compared with bioassay results, is estimated to be 0.909. The method is not affected by the presence of gentamicin (\( r = 0.916 \); 92 samples); this compound is one of a class of antibiotics which is frequently coadministered with teicoplanin to potentially broaden the spectrum of activity of therapy in patients with serious undiagnosed infections.

The Innotron FPI system results, when assaying known standards over the range of 5 to 100 mg/liter in a single day, demonstrated a correlation coefficient of 0.999. Coefficient of variance was 4.3%, although dilution of samples with expected concentrations of greater than 100 mg/liter could be anticipated to further add to the error. Comparison of estimated concentrations of a set of three standards presenting the high, medium, and low end of the anticipated therapeutic range showed excellent correlation with expected results (\( r = 0.993 \)), with an intraassay variability of 0.7 to 7.3% and interassay variability of 8.6 to 11.6%.

Because concentrations of 5 to 150 mg of teicoplanin per liter in serum would be expected in the majority of patients after administration of a dosage of between 2 and 30 mg/kg of body weight (6), this degree of variability would not lead to clinically misleading results.

The Innotron FPI with the Abbott TDx instrument can be described as sensitive and precise; it uses technology familiar to workers in many routine diagnostic laboratories and has advantages over bioassay in terms of turnaround time and specificity. It should prove to be a useful method of measuring teicoplanin.

**REFERENCES**