Rapid, Reproducible Enzyme Immunoassay for Gentamicin

DENIS PHANEUF, ELLIOT FRANKE, AND HAROLD C. NEU*
Division of Infectious Diseases, Department of Medicine, College of Physicians and Surgeons, Columbia University, New York, New York

An enzyme immunoassay for gentamicin utilizing glucose-6-phosphate dehydrogenase was compared to the radioimmunoassay. The enzyme immunoassay for gentamicin was accurate, specific, and easily performed. It offers an alternative method to assay aminoglycosides and could be used in institutions using enzyme immunoassay to assay other drugs.

Aminoglycoside antibiotics—gentamicin, tobramycin, amikacin—have proved to be extremely useful agents in the chemotherapy of serious infections due to gram-negative aerobic bacteria (1). However, initial enthusiasm over these agents has been muted by a realization that the toxic-to-therapeutic ratio is narrow (1, 2). Furthermore, a number of studies have shown that failure of these agents to cure certain infections may be related to inadequate serum levels of the agents (1). Although serum levels have proved to be extremely predictable in the healthy, male volunteers used to evaluate the initial clinical pharmacology of these drugs, patient serum levels have proved to be less predictable and the necessity of monitoring serum levels is generally accepted (2, 8).

Bioassays of serum levels of aminoglycosides are reliable if careful attention is given to the problems inherent in these assays (10). However, the results are not available as rapidly as needed to make initial dosage adjustments. The use of adenylylating enzymes as an assay method utilizing \[^{14}C\]adenosine triphosphate has been limited primarily to research laboratories, although the results are obtained rapidly and accuracy is good (6, 11). Radioimmunoassay (RIA) has been the most common method used to assay levels. This technique also has problems attendant with its use.

Homogenous enzyme immunoassay (EIA) was introduced in 1972 (9). The technique has been utilized widely to monitor serum levels of anti-epileptic agents, asthma medications, and cardiac drugs, and to monitor thyroid function (4, 5). Since the equipment to perform the aforementioned tests is available in many hospitals, we wished to evaluate the EIA technique to assay gentamicin serum levels and to compare the technique to the RIA methods.

MATERIALS AND METHODS

Serum samples were collected from patients hospitalized at The Columbia-Presbyterian Medical Center who were receiving gentamicin. The dose, route of administration, and time of administration were obtained in all cases. Other medications were noted. Sera were separated by centrifugation and assayed immediately by both this technique and the RIA method. Samples were also stored frozen at \(-20^\circ\text{C}\) for later assay to determine changes. Samples were also prepared from gentamicin provided by Schering Corp.

Enzyme Assay. Reagents for the EIA study were obtained from Syva Corp., Palo Alto, Calif. The method employs a bacterial glucose-6-phosphate dehydrogenase enzyme to which gentamicin has been linked. The active site of the enzyme is adjacent to the bound aminoglycoside. Antigentamicin antibody will bind to the gentamicin adjacent to the enzyme, thereby decreasing enzymatic activity. The assay follows the conversion of nicotinamide adenine dinucleotide to reduced nicotinamide adenine dinucleotide which occurs when the enzyme is able to act on a substrate. Free gentamicin in standards or from a sample competes for antibodies with drug bound to the enzyme. When less antibody blocks the active site of the glucose-6-phosphate dehydrogenase, the activity of the enzyme is increased.

With a pipetter-diluter, 50 \(\mu\)l of a standard was diluted with 250 \(\mu\)l of tris(hydroxymethyl)amino-methane-hydrochloride buffer, pH 8, 0.055 M. Fifty microliters of the first dilution was diluted again with 250 \(\mu\)l of buffer. To this was added 50 \(\mu\)l of substrate and antibody (reagent A) and 250 \(\mu\)l of buffer. Finally, 50 \(\mu\)l of gentamicin-enzyme preparation plus 250 \(\mu\)l of buffer were added. A sample was taken into the cuvette.

Absorbance of the reaction mixture was measured at 340 nm with a Gilford 300-N microsample spectrophotometer equipped with a thermally regulated flow cell set at 30°C. Readings were taken at two points in the reaction, 15 and 45 s, and recorded with a CP-1000 EMIT timer printer. A standard curve was prepared by using gentamicin standards of 1, 2, 4, 8, and 16 \(\mu\)g/ml. Data was applied to specially designed modified log-function graph paper to yield a straight line within the range of the assay.

RESULTS

A standard curve produced by assay of standards containing 1, 2, 4, 8, and 16 \(\mu\)g/ml of gentamicin is shown in Fig. 1. The data yielded a sigmoid shaped curve but, with the use of probit paper, this is converted to a straight line from
which unknown concentrations can be calculated. The precision of the EIA method was determined by assaying samples 20 times on the same day and subsequent assaying 20 times for 25 successive days. Utilizing a 4 μg/ml gentamicin standard for the EIA method, an assay mean of 3.88 μg/ml with a standard deviation of 0.16 μg/ml was obtained. The coefficient of variation was 4.1%. Utilizing a 6 μg/ml gentamicin standard in the RIA, the standard deviation was 0.44 μg/ml with a coefficient of variation of 5.7%. Over a 25-day period the standard deviation for the EIA was 0.21 μg/ml with a coefficient of variation of 3.6%.

The specificity of the assay was determined by assaying prepared serum samples containing amikacin, sisomicin, netilmicin, kanamycin, and tobramycin. Only with sisomicin and netilmicin did the test show cross-reaction. This could be expected since sisomicin is structurally a dehydrogenated C₁₉₄ gentamicin and netilmicin is an ethyl derivative of C₁₅₈ gentamicin in which the ethyl addition is at position 1 of the deoxystreptamine nucleus. Samples which contained both gentamicin and amikacin yielded a result only for the gentamicin present. A serum specimen from a patient receiving amikacin showed the presence of gentamicin by both the EIA and RIA. It was discovered that the patient, who had no renal function, had received gentamicin earlier by error. A number of β-lactam compounds—ampicillin, azlocillin, methicillin, carbenicillin, ticarcillin, cephalothin, mecillinam, and chloramphenicol—added to the serum at concentrations up to 200 μg/ml did not affect the assay. The coefficient of correlation was 0.98 for the results of the assay by EIA versus RIA for the presence of gentamicin when mixed with the foregoing agents.

The effect of heparin upon the EIA and RIA tests was determined. Gentamicin serum levels were lower by 0.43 μg/ml for the RIA and 0.45 μg/ml for the EIA when heparin-containing tubes were used to collect blood in comparison to the levels obtained from serum drawn at the same time. To see if the same serum level was obtained by RIA and EIA when the serum contained both gentamicin and β-lactam compounds, assays were made of mixtures stored at room temperature for 72 h. There was no statistical difference between the first value and the 72-h value for either method.

Sera (111 samples) from 51 patients were analyzed by both methods. The coefficient of correlation for the methods was 0.97 (Fig. 2). The level of gentamicin was less than 1 μg/ml for 17...
sera by both methods. Four sera less than 1 \( \mu g \) by EIA were above 1 \( \mu g \) by RIA, and six sera less than 1 \( \mu g \) by RIA were above 1 \( \mu g \) by EIA.

**DISCUSSION**

Clinically the measurement of gentamicin is of value if the result is returned to the physician rapidly (1, 2, 6). Many hospitals using the RIA method set up the test once a day. We found a setup time for the first sample by RIA is 1 h and 35 min, and 5 min for each additional test. Most hospitals we have contacted in the New York area run aminoglycoside serum levels once each day due to the technician time involved. With the EIA system, the setup time for the first sample was 25 min, and 2 min for each additional test. In our experience, the EIA standard curve was reproducible for a working day and samples could be run at any time during the day. This should be confirmed in the laboratory using the test.

With the EIA, the delay time between obtaining the sample and the return of an assay result to the physician may be 30 min, a delay acceptable in a decision-making situation.

Compared with RIA, the EIA for gentamicin was simpler and had no radioactive waste. Also, EIA requires only 50 \( \mu l \) of serum, a useful characteristic in pediatric situations. The precision and specificity of the EIA and the RIA were similar, but in this study the EIA accuracy was better. For laboratories already using the same instrumentation to measure other drugs, the EIA certainly would be a preferable method for determining serum gentamicin levels. For other laboratories considering which system to use, the EIA offers an alternative to the currently available methods.

**LITERATURE CITED**


