Rapid, Reproducible Enzyme Immunoassay for Gentamicin

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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 adenylating enzymes as an assay method utilizing [1’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°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 μl of a sample or standard was diluted with 250 μl of tris(hydroxymethyl)aminomethane-hydrochloride buffer, pH 8, 0.055 M. Fifty microliters of the first dilution was diluted again with 250 μl of buffer. To this was added 50 μl of substrate and antibody (reagent A) and 250 μl of buffer. Finally, 50 μl of gentamicin-enzyme preparation plus 250 μl 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 μg/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 μg/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 \( \mu g/ml \) gentamicin
standard for the EIA method, an assay mean
of 3.88 \( \mu g/ml \) with a standard deviation of 0.16
\( \mu g/ml \) was obtained. The coefficient of variation
was 4.1\%. Utilizing a 6 \( \mu g/ml \) gentamicin
standard in the RIA, the standard deviation was 0.44
\( \mu g/ml \) with a coefficient of variation of 5.7\%.
Over a 25-day period the standard deviation for
the EIA was 0.21 \( \mu g/ml \) 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 dehy-
drogenated \( C_9 \) gentamicin and netilmicin is an
ethyl derivative of \( C_9 \) gentamicin in which the
ethyl addition is at position 1 of the deoxystrep-
tamine 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 \( \beta \)-lactam com-
 pounds—ampicillin, azlocillin, methicillin, car-
benicillin, ticarcillin, cephalothin, mecillinam,
and chloramphenicol—added to the serum at
concentrations up to 200 \( \mu g/ml \) 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 aforesaid agents.

The effect of heparin upon the EIA and RIA
tests was determined. Gentamicin serum levels
were lower by 0.43 \( \mu g/ml \) for the RIA and 0.45
\( \mu g/ml \) 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 con-
tained both gentamicin and \( \beta \)-lactam com-
 pounds, assays were made of mixtures stored at
room temperature for 72 h. There was no statis-
tical difference between the first value and the
72-h value for either method.

Sera (111 samples) from 51 patients were an-
yzed by both methods. The coefficient of cor-
relation for the methods was 0.97 (Fig. 2). The
level of gentamicin was less than 1 \( \mu g/ml \) for 17
Concentration by EIA, µg/ml

Concentration by RIA, µg/ml

Fig. 2. Assays of gentamicin-containing sera by using the EIA and RIA methods.

sera by both methods. Four sera less than 1 µg by EIA were above 1 µg by RIA, and six sera less than 1 µg by RIA were above 1 µg 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 µl 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


