Analysis of Serum for Gentamicin by Radioimmunoassay

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ABSTRACT

A radioimmunoassay method for the determination of combined gentamicin isomers in serum has been adapted and tested. The procedure uses tritiated gentamicin, rabbit antiserum against a gentamicin-human albumin conjugate, and dextran-charcoal separation. The day-to-day coefficient of variation is 6 percent, and frozen samples are stable for at least one month. There was no cross reactivity of the antiserum with any of several tested antibiotics or with any of some tested commonly used non-antibiotic hospital pharmacy preparations. The results from the procedure correlated well with those of an enzymatic radioacetylation technique and, except for a significant incidence of "outliers", with a microbiological assay. As expected, patient serum values, both maximum and minimum, showed no correlation with dose size.

Introduction

Gentamicin is a mixture of three aminoglycoside structural isomers used in the treatment of severe gram negative infections. The drug is not well absorbed from the gastrointestinal tract and is usually administered as an intramuscular or intravenous injection. The usefulness of gentamicin is somewhat limited by potential side effects. For example, nephrotoxicity is a significant possibility, and severe oto-toxicity has been known to occur, particularly in patients with renal insufficiency. As with other drugs of low therapeutic index, serial serum concentration determinations is probably helpful in the maintenance of a safe regimen. The lack of correlation between dosage and serum level is a well established phenomenon. The method of analysis of serum for gentamicin has been microbioassay, enzymatic radioacetylation or radioimmunoassay. The radioimmunoassay method of Mahon has been adapted and tested for accuracy, precision and specificity. Results of this assay have been compared
with those of a microbiassay, an acetylating enzyme assay, and a radioimmunoassay performed by an independent laboratory.

Materials and Methods

GENTAMICIN ANTISERUM

Gentamicin sulfate* was coupled to human serum albumin† using the carbodiimide method described by Spector and Parker.§ The conjugate was purified on a column of Sephadex G-25, adjusted to an albumin concentration of 20 g per liter and sterilized by filtration using a 0.20 micrometer disposable filter. This sterile conjugate solution was then diluted to an albumin concentration of 5 g per liter. An equal volume of Freund's adjuvant was added, and 0.1 ml of this mixture was injected into both hind leg muscles of albino New Zealand rabbits. Initially, the injections were given weekly for three weeks. Following these immunizing doses, the antibody titer was checked using blood drawn from an ear vein. Subsequently, a single booster dose followed by a bleeding one week later yielded adequate antiserum. The working dilution of antiserum was that which would bind from 35 to 50 percent of a trace amount of gentamicin against charcoal separation. This usually was a 1:100 dilution of the original serum.

3H-GENTAMICIN

Gentamicin sulfate was randomly labeled with tritium. The labeled material was returned rich in impurities as shown by thin layer chromatography and was purified with a column of Sephadex G-10. Thin layer chromatography of the column effluent with peak radioactivity showed only the three isomers of gentamicin. The dilution of this 3H-gentamicin as used in the assay was that which gave approximately 5,000 counts per minute (CPM) for the "O" standard. This was a trace amount of gentamicin and was 50 percent bound by the 1:100 dilution of antiserum.

STANDARDS

Garamycin® powder, 175 mg, was dissolved in 100 ml of water to yield a stock solution containing 1.0 mg per ml gentamicin. Dilutions were made to 1 μg per ml with assay buffer. From this, working solution standards containing 2.5, 5.0, 10, 25, 50, 75, 100, 250 and 500 ng per ml were prepared by additional dilution with buffer.

Standards were prepared in human serum for a colinearity check on antiserum specificity.

PROCEDURE

Reference sera or patient's sera were diluted 1:100 with phosphate buffered saline (PBS) (0.05M, pH 7.4) containing one percent bovine serum albumin.

Exactly 0.2 ml of 3H-gentamicin solution was added to each of the appropriately labeled sample and standard tubes containing 0.1 ml of either the gentamicin standard or diluted serum sample.

To two additional tubes are added, respectively, 0.2 ml of 3H-gentamicin solution and 0.3 ml of phosphate buffered saline. These two tubes are carried through the procedure without addition of antiserum. One is used to determine total counts per minute per tube, and so does not undergo the charcoal separation step. The other does undergo the charcoal step and is used as a measure of the ability of the separation to remove all unbound radioactive material.

To each of the samples and standard tubes is added 0.2 ml of diluted antiserum. The samples are incubated at
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room temperature for one hour, after which separation of free gentamicin from bound is carried out with 0.5 ml of a dextran-charcoal slurry.

After centrifugation, the bound supernatant $^3$H-gentamicin is decanted into liquid scintillation vials, and is counted.* The percent bound is calculated, and a standard curve of percent bound vs gentamicin is plotted on three-cycle semi-log paper.

SPECIFICITY

Synthetic samples were prepared by addition of 10 $\mu$g per ml of each of tobramycin sulfate, streptomycin sulfate, sodium ampicillin, kanamycin sulfate, lincomycin hydrochloride and neomycin sulfate to drug-free human serum. Each of these samples were assayed.

Similar sera were prepared containing dextroamphetamine, phenobarbital, secobarbital, amobarbital, pentobarbital, dextropropoxyphene, diphenylhydantoin, glutethimide, chlordiazepoxide, meprobamate, etchlorvynol, procainamide, salicylic acid, sulfanilamide and diazepam at concentrations ranging from 2 $\mu$g per ml to 50 $\mu$g per ml. These samples were assayed for gentamicin.

Aliquots of samples assayed by the aforementioned procedure were sent to Clin Chem Laboratories, the aliquot parts were assayed by a radioimmunoassay procedure using a commercially prepared antiserum and an $^{125}$I labeled gentamicin conjugate. At the hospital, they were assayed by a radioenzymatic procedure. Each of the three comparative sets of assays were performed without knowledge of the results of the other two. Since the specimens were sent serially from one laboratory to another, the study was also a test of the ability of the specimens to withstand travel without alteration.

* Nuclear Chicago.

A second series of samples assayed by the described procedure was also assayed by a microbioassay after treatment with a penicillinase preparation.4

Patient sera for gentamicin assay in our institution were routinely collected as paired samples, one immediately before and one 60 minutes after intramuscular injection. However, some samples were collected for analysis at random times.

Results and Discussion

In figure 1 is shown a typical curve for gentamicin standard prepared in an aqueous solution and in serum. Serum
concentration in micrograms per ml were obtained by dividing the results from the curve by 10 to compensate simultaneously for the pre-dilution of the sample and the difference in concentration units. The colinearity of the normal serum curve with the aqueous curve provide necessary (though not sufficient) evidence for specificity of the antiserum for gentamicin with respect to normal human serum components. Charcoal separation of a trace amount of labeled gentamicin from normal serum demonstrated that the shift between the curves was entirely attributable to the nonspecific binding of the undiluted serum used as a matrix for the serum standards.

A synthetic human control serum prepared at a concentration of 10 μg per ml of gentamicin was frozen and assayed with each set of determinations over three months. The standard deviation for the 10 μg per ml control was 0.45 μg per ml, and no deterioration of the sample was noted during this period.

No interference by the other antibiotics or other drugs tested in the gentamicin assay procedure was observed.

In figure 2, correlation is shown between 3H and 125I radioimmunoassay procedures. In figure 3, correlation is shown between the 3H radioimmunoassay and the radioenzymatic assay. Correlation coefficients were 0.988 for the 3H/I comparison and 0.921 for the 3H/enzyme comparison. There is somewhat more scatter about the regression line in figure 3.
A comparison between the radioimmunoassay (RIA) and the microbiological assay can be seen in figure 4. More significant, however, is the number of outliers seen when comparing these two techniques. In a total of 30 samples cross-assayed, five were varied by more than four RIA standard deviations. This result is in contrast to Stevens et al. In figure 5 are shown serum gentamicin concentrations in randomly selected patients receiving the drug by either the intramuscular or intravenous route. The dosage was either 80 mg or 40 mg every eight hours. Blood for peak gentamicin concentration was taken five to ten minutes after I.V. injection. For those receiving gentamicin by the I.M. route, the peak samples were taken one hour after administration. Thorough samples were taken immediately before administration of the drug. None of the patients examined for this study was in renal failure. Each patient had been receiving the drug for at least two days before the serum level was obtained, so that each was at least approaching a steady state. Inspection of figure 5 shows the variation to be expected in serum concentration among patients receiving gentamicin. No attempt was made to normalize these serum levels for body mass, nor was any consideration given to other drugs being administered. Nevertheless, the lack of correlation between dose and serum concentration is obvious.

References


