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NOTES

Comparison of a Radioimmunoassay with an Enzymatic Assay for Gentamicin

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A radioimmunoassay and an enzymatic assay for gentamicin have been compared. The correlation coefficient for results of gentamicin assays performed by the two methods with 45 serum specimens was 0.90. A similar standard curve for the radioimmunoassay was obtained with gentamicin complex, with gentamicin C1, C1a, or C2, or with sisomicin as ligand, but tobramycin did not compete with [^{3}H]gentamicin for binding to the antiserum.

Within the last few years a number of methods have been developed for measuring the concentration of gentamicin in serum and other biological specimens. These methods include microbiological assays (1, 8, 10, 11), enzymatic assays (4, 5, 12, 13), and radioimmunoassays (7, 9). Each type of assay has its own particular advantages. Enzymatic assays and radioimmunoassays are highly specific and are therefore particularly useful for measuring gentamicin in the presence of other antibiotics. Microbiological assays are inexpensive and can be performed in most diagnostic laboratories without special equipment, but they are limited in specificity by the antibiotic susceptibilities of the test organisms. In the present study, a radioimmunoassay for gentamicin is compared with the enzymatic assay previously used in this laboratory. In addition, published observations concerning the specificity of radioimmunoassays for gentamicin have been extended (7, 9).

Reagents for radioimmunoassay of gentamicin were prepared by the following methods. Gentamicin sulfate was radioisotopically labeled with tritium by Amersham/Searle (Des Plains, Ill.). The [^{3}H]gentamicin was purified by chromatography on Sephadex G-10 as described previously (9). The purified [^{3}H]gentamicin contained 17.8% of the radioactivity in the initial sample and had a specific activity of 0.8 mCi/mg based on determinations of gentamicin concentration by microbiologic (8) and enzymatic (5) assays. Throughout this paper the concentration of each aminoglycoside is expressed as the free base. To prepare the gentamicin antigen, 100 mg of bovine thyroglobulin (Sigma Chemical Co., St. Louis, Mo.) and 200 mg of carbodiimide HCl [1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, Ott Chemical Co., Muskegon, Mich.] were dissolved at room temperature in 7 ml of 0.02 M (pH 7.8) potassium phosphate buffer and incubated at 4 C with stirring (3). After 1 h, 160 mg of gentamicin sulfate was added. After incubation overnight, an additional 200 mg of carbodiimide was added, and stirring was continued for 5 h. The preparation was exhaustively dialyzed for 5 days against several 4-liter volumes of distilled water. Three New Zeland white female rabbits were given six subcutaneous injections at 10-day intervals each of which contained 2 mg of the conjugated gentamicin emulsified in Freund complete adjuvant. A 1-mg booster dose was administered 1 month after the sixth injection, and the animals were bled 1 week later. Two of the three rabbits produced satisfactory antibodies against gentamicin.

The radioimmunoassay for gentamicin was performed in the following manner. Each 500-μliter assay mixture contained 350 μliters of 0.02 M (pH 7.4) phosphate buffer with 0.01 M NaCl, 18 ng of [^{3}H]gentamicin, 50 μliters of antiserum diluted 1:600 in buffer, and 100 μliters of a 10^{-1} dilution in buffer of serum containing gentamicin. The mixtures were incubated for 15 min at 4 C. Three hundred-μliter volumes of dextran-coated charcoal (Dextran 150, Pharmacia Fine Chemicals, Uppsala, Sweden) (6) in buffer were

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added to each reaction mixture to give a final concentration of 0.25% charcoal, and the samples were incubated for 15 min at 4 C. After centrifugation to remove the charcoal, 500-μliter samples of the supernatants were mixed with 4 ml of Aquasol (New England Nuclear, Boston, Mass.) and counted for 5 min. In the absence of added nonradioactive ligand, about 70% of the [^{3}H]gentamicin was bound to the antibody and the measured radioactivity in the sample of supernatant was approximately 2,500 counts/min.

To construct a standard curve, nonradioactive gentamicin was added to control assay mixtures in the amounts indicated in Fig. 1. For convenience in calculating the concentration of gentamicin in clinical specimens, standard curves were transformed to a linear form by plotting 1/b (reciprocal of fraction of [^{3}H]gentamicin bound) versus concentration of gentamicin in the assay as described by Ekins (2). The values for unknown specimens were then determined from the linear regression equations for the standard curves calculated by the least squares method. The sensitivity of the radioimmunoassay for gentamicin is approximately 10 ng. The lowest concentration of gentamicin that can be measured quantitatively in serum is therefore 1 μg/ml with the procedure described above, but if the initial 10-fold dilution of serum is omitted, the limit of the assay is 0.1 μg/ml. The specificity of this radioimmunoassay for gentamicin is also shown by the data in Fig. 1. The binding of gentamicins C1, C1a, and C2, and of sisomicin was similar to that of gentamicin complex. However, tobramycin at concentrations up to 2,000 ng/ml did not inhibit the binding of [^{3}H]gentamicin. Other reports indicate that there is little or no cross-reaction of gentamicin with kanamycin or with other antibiotics unrelated to aminoglycosides (7, 9). Our findings that gentamicins C1, C1a, and C2 cross-react almost completely with gentamicin complex are similar to those of Lewis et al. (7) but differ from the observations of Mahon et al. (9). These discrepancies may reflect differences in the specificities of the antibodies to gentamicin prepared in different laboratories.

The concentrations of gentamicin in 45 specimens of serum obtained from patients were measured both by radioimmunoassay and by the enzymatic assay of Holmes and Sanford (5). The results from these two sets of assays are compared in Fig. 2. The correlation coefficient (r) for values obtained by the two methods is 0.90 and the slope of the linear regression line is 0.99. There is no statistically significant difference between the results obtained by these two methods for measurement of gentamicin.

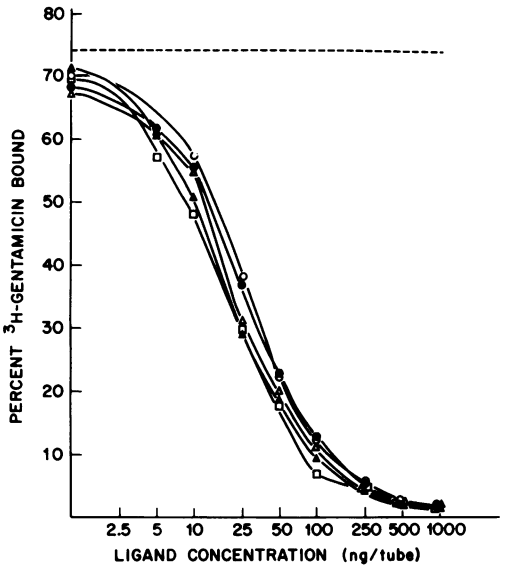

FIG. 1. *Specificity of radioimmunoassay for gentamicin. Similar standard curves were obtained with gentamicin complex (●), gentamicin C1 (Δ), gentamicin C1a (□), gentamicin C2 (×), and sisomicin (○). The dashed line represents data obtained with tobramycin and demonstrates that tobramycin at concentrations up to 2,000 ng/ml does not cross-react with gentamicin.*

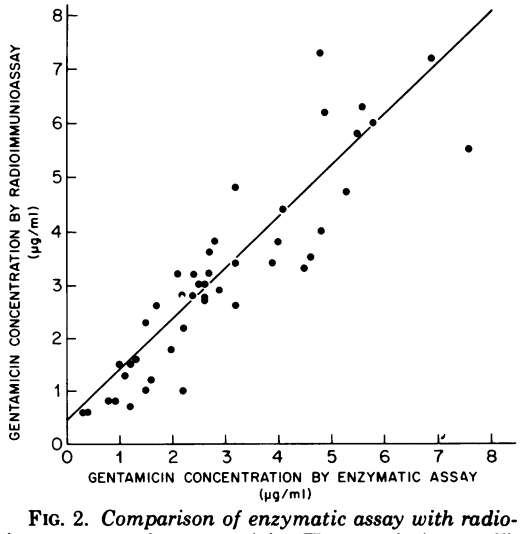

FIG. 2. *Comparison of enzymatic assay with radioimmunoassay for gentamicin. The correlation coefficient is 0.90. The linear regression line determined by the method of least squares is $y = 0.99x + 0.35$.*

The data presented in Fig. 2 demonstrate that the enzymatic and radioimmunoassays for gentamicin described here provide consistent values for gentamicin concentrations in serum specimens. Published studies have also demonstrated good correlations between radioimmunoassays and microbiological assays (7, 9) and between enzymatic and microbiological assays (12) for gentamicin. Thus, microbiological, enzymatic, and radioimmunoassays for gentamicin all give satisfactory results in research laboratories. For routine determinations of gentamicin in clinical laboratories, considerations of speed, cost, specificity, sensitivity, and reproducibility will determine the method(s) of choice. The high specificity and the rapidity of the enzymatic assays and radioimmunoassays for gentamicin are attractive features. We prefer enzymatic assays rather than radioimmunoassays for aminoglycosides because the reagents are easier to prepare, the experimental protocols are somewhat simpler, and in our experience the results are less affected by minor variations in the experimental procedures.

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