^{125}I-RADIOIMMUNOASSAY OF AMIKACIN AND COMPARISON WITH A MICROBIOASSAY

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A radioimmunoassay (RIA) has been developed using ^{125}I-amikacin. Amikacin was iodinated by a modified BOLTON and HUNTER method. Dextran-charcoal was used to separate bound from free drug. The standard curve was linear on a logit-log plot in the range of 0.5 ng to 4 ng amikacin per tube. There was no cross-reactivity of amikacin antisera to the aminoglycosides gentamicin, tobramycin, netilmicin, and sisomicin but a 70% cross-reaction was observed with kanamycin, the compound from which amikacin is synthetically derived. Correlation of the RIA with a microbioassay for the determination of serum amikacin levels in 18 patient samples was excellent (r=0.94). This new RIA technique is more sensitive, rapid, versatile, and less costly than the RIA using ^{3}H-amikacin, and is far more sensitive and faster than microbioassay.

Amikacin is a semi-synthetic aminoglycoside derived from the acylation of the one amino (1-NH_2) group of the 2-deoxystreptamine portion of kanamycin A.[1] Amikacin is active against most clinically significant Gram-negative bacilli[2] and as other aminoglycosides, is potentially ototoxic and nephrotoxic[3,4]. Serial monitoring of serum levels may be useful in averting toxicity and selecting adequate therapeutic doses[3]. Aminoglycosides can be measured in serum by microbioassay, radioenzymatic assay, and radioimmunoassay (RIA) but RIA has been shown to be the most rapid, sensitive and more importantly, the most specific method for the measurement of serum aminoglycosides[5,6]. Our present purpose is to report an RIA of amikacin using ^{125}I-labelled amikacin which is more rapid, sensitive, versatile and potentially less costly than the RIA originally described using ^{3}H-amikacin[7] and to compare this RIA with a standard microbiological assay for amikacin.

Materials and Methods

Conjugate Preparation and Immunization

Amikacin (provided by Mr. EDWARD YEVAK, Bristol Laboratories, Syracuse, New York) was conjugated to porcine thyroglobulin by the carbodiimide reaction[8,9] as described previously for gentamicin[6] and amikacin[7] using dialysis for purification. New Zealand white rabbits were immunized with 100 μg of the conjugate per animal by the method of VAITIKAITUS, *et al.*[10] After one month the rabbits were boosted intramuscularly into all four flanks with 100 μg conjugate in complete FREUND's adjuvant per animal. Antiserum was obtained 10 days after the booster injections and stored at $-20°C$. Antisera was appropriately diluted to obtain an initial 50% maximum binding (Bo) of ^{125}I-amikacin in the assay mixture.

Iodinated Amikacin

Amikacin was commercially iodinated by Diagnostic Product Corporation, Los Angeles, California, using a modified BOLTON and HUNTER[11] method and had an approximate specific activity of 200 Ci/mmole.

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Radioimmunoassay

The assay was performed in 12×75 mm polystyrene tubes. Standards of 0, 5, 10, 20, and 40 μg amikacin per ml were prepared in normal human serum. All standards and serum samples were diluted 1/2,000 in 0.05 M phosphate, 0.15 M NaCl pH 7.4 (PBS) for the assay. The following was added to the tubes for the reaction: 200 μl of the diluted standards or samples, 100 μl of ^{125}I-amikacin containing approximately 35,000 cpm and 100 μl of appropriately diluted rabbit antisera. The tubes were gently shaken and incubated at 37°C for 15 minutes. For separation of bound from free amikacin, 1 ml of ice-cold dextran charcoal suspension was added (1% Norit A charcoal, 0.1% dextran, M. W. 500,000 and 0.1% gelatin in PBS). The tubes were vortexed and centrifuged immediately at 4°C and 3,000×g for 10 minutes. The supernatant containing bound amikacin was decanted into scintillation vials containing 10 ml of scintillation fluid and counted in a liquid scintillation spectrophotometer[6]. Efficiency of counting was approximately 98%. Alternatively, the supernatant could be decanted into appropriate tubes for counting in a gamma counter. The standard curve was constructed using logit-log graph paper (Team Inc., Tamworth, New Hampshire) by correlating the logit of the net cpm of each standard (B) divided by the net cpm of the maximum binding (Bo) of ^{125}I-amikacin in the absence of standard amikacin with the logarithmic concentration of each standard. Values for unknown serum samples were interpolated from the standard curve.

The reliability of the RIA in the measurement of serum amikacin was determined by comparison with a microbioassay using *Klebsiella pneumoniae* as the test organism[12]. Eighteen patient sera were selected covering a range of 5 μg~40 μg of amikacin per ml of serum. The sera were assayed in duplicate on two different days and the correlation coefficient (r value) determined between the methods by linear regression analysis.

Results

Standard Curve

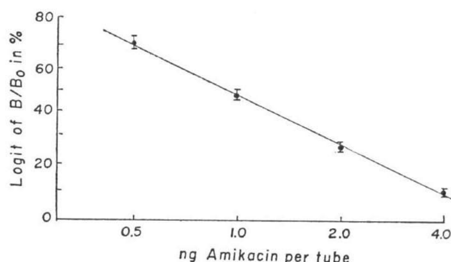
The mean standard curve±SEM of four different standard curves of the RIA of amikacin in which the logit of B/Bo in percentage of relative bound ^{125}I-amikacin was plotted versus the logarithmic amount of amikacin in nanograms per assay tube (Fig. 1). The sensitivity of the assay can be adjusted by variations in the amount of ^{125}I-labelled amikacin and antisera but for the assay presented the lowest amount detectable is 200 pg/tube.

Precision and Reproducibility

To assess precision, six replicate determinations were run on three samples representative of low, middle, and high concentrations of serum amikacin. The representative samples with mean concentration in μg/ml±SD with coefficient of variation (CV) were as follows: low 5.25±0.34, CV 6.5%; middle 16.32±0.85, CV 5.2%, and high 32.45±1.41, CV 4.4%. To determine day-to-day variation, *i.e.*, reproducibility, the same three representative samples were assayed in duplicate on four different assay days. The mean concentration in μg/ml±SD with the CV was as follows: low 5.65±0.74, CV 13.1%, middle 15.48±1.13, CV 7.3%: and high 32.75±1.26, CV 3.9%.

Fig. 1. Mean standard curve of the RIA of amikacin correlating the logarithmic amount of standard amikacin with the logit of B/Bo in relative percent binding.

Bars indicate±SEM of four standard curves.



Specificity

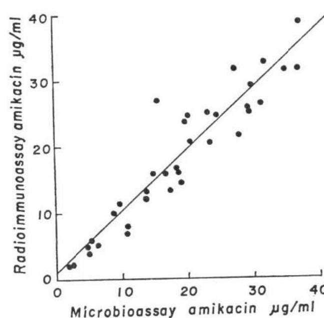
There was no cross-reactivity or interfer-

ence with the following antibiotics at a concentration at least 50 times greater than would be expected therapeutically: sisomicin, gentamicin, tobramycin, netilmicin, cephalothin, carbenicillin, oxacillin, cefazolin, and chloramphenicol. There was, however, a 70% cross-reaction with kanamycin.

Comparison

Comparison of the RIA and microbioassay as determined by linear regression analysis is shown in Fig. 2. The correlation coefficient (r value) was 0.94 and was significant at P< 0.00005. The line of regression had a slope of 0.91 and a y intercept of 0.93. Consequently, there was no significant difference in the measurement of amikacin between these two methods.

Fig. 2. Comparison between the RIA and microbioassay in the measurement of serum amikacin as analyzed by linear regression.
r=0.94
slope=0.91
y intercept=0.93



Discussion

RIA of amikacin has been developed in response to the need to measure amikacin rapidly and accurately as a guide to proper therapy.[3,4] It should be noted that the advantage of the RIA for gentamicin[6,13,14,15] and amikacin[7] is not the superior sensitivity of this method, which is the major justification for using RIA to measure agents such as digoxin and morphine but is related primarily to the rapidity, accuracy and high degree of specificity of RIA as compared to the slower and less specific microbioassay or radioenzymatic assays.[5,6] The RIA reported here is more rapid, versatile, and less costly than that described when using ^{3}H-amikacin.[7] Based on the time to determine a standard curve, excluding computation time, the RIA using an iodinated antigen can be performed in about 80 minutes less than the RIA using ^{3}H-amikacin. The reasons for this are a shorter incubation and equilibration time, elimination of the cumbersome additional step of solubilizing the antibody-bound amikacin, and marked reduction in counting time. Further, since ^{125}I in contrast to ^{3}H can be counted efficiently in either a liquid scintillation spectrophotometer or gamma counter, the method is more versatile because one has more flexibility in choosing available laboratory counting equipment. The RIA using ^{125}I-amikacin is potentially less costly because expensive vials, scintillation fluids and protein solubilizers are unnecessary. Additionally, less amikacin antisera is required due to the higher specific activity of ^{125}I-amikacin. Even though the assay can detect as little as 200 pg this sensitivity offers no real clinical advantage over the microbioassay or radioenzymatic assays in terms of the volume of serum necessary to measure serum amikacin but may have utility as a research tool.

The real advantage of the RIA other than rapidity is its high degree of specificity which permits measurement of amikacin in the presence of other antimicrobials. This specificity is particularly important since RIA, unlike microbioassay or radioenzymatic assay, is not affected by concomitant antimicrobials, with the exception of kanamycin whose simultaneous use would be unlikely. Technical personnel working with clinical specimens may not have accurate information about all the antibacterial agents a patient is receiving. Consequently when using a bioassay one may obtain erroneously high levels due to the presence of drugs such as clindamycin, chloramphenicol, or co-trimoxazole that cannot be readily inactivated. Further, it has been reported that chloramphenicol can interfere with the acetylating radioenzymatic assay of amikacin.[5]

The extensive cross-reaction of kanamycin is not surprising since amikacin is a semi-synthetic derivative of kanamycin.[1] Due to this cross-reactivity an RIA could easily be adapted to measure kanamycin using this amikacin antisera. An analogous situation was recently reported in the RIA of sisomicin using gentamicin antisera.[16]

This assay is precise and reproducible with quite low coefficients of variation at the three major portions of the standard curve. The high degree of correlation with the microbiological assay ($r=0.94$) and the additional data presented indicates that this RIA is a reliable, useful, rapid and specific method for the determination of serum amikacin and should be considered by those laboratories with adequate patient volume and suitable facilities for using such a method.

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