125I Radioimmunoassay of Netilmicin

PAUL STEVENS,* LOWELL S. YOUNG, AND WILLIAM L. HEWITT

Department of Medicine, Division of Infectious Diseases, University of California School of Medicine, Los Angeles, California 90024

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We report a radioimmunoassay for the new semisynthetic aminoglycoside netilmicin with a sensitivity of 480 pg per tube and a correlation coefficient of 0.94 between this radioimmunoassay and a microbioassay for measurement of netilmicin in serum.

Netilmicin is a new semisynthetic aminoglycoside produced by ethylation of the one amino group of the deoxystreptamine moiety of sisomicin. Like gentamicin, netilmicin is active against most clinically significant gram-negative bacilli (5). Although preliminary data suggest that netilmicin is less nephrotoxic in rats (7) than is gentamicin, it has the potential for ototoxicity. Methods to determine serum or body fluid levels of netilmicin could aid in management of human therapy by helping to avert untoward reactions, select adequate dosage regimens, and provide useful pharmacokinetic data.

Netilmicin (provided by Schering Corporation) was conjugated to porcine thyroglobulin by the carbodiimide reaction (4, 6), and New Zealand white rabbits were immunized with this conjugate as described previously (9, 10). Netilmicin antiserum was obtained 7 to 10 days after the booster injections and stored at −20°C.

Netilmicin was commercially iodinated by Diagnostic Products Corp., Los Angeles, Calif., using a modified Bolton and Hunter method. This procedure is similar to that recently described for the iodination of gentamicin (2). The netilmicin had an initial approximate specific activity of 200 to 250 Ci/mmol.

The radioimmunoassay (RIA) procedure used was similar to the RIA of amikacin (10) using 125I-labeled antigen. For the assay, the reaction mixture consisted of the following: 200 μl of the diluted standard or samples, 100 μl of 125I-labeled netilmicin, which contained anywhere from 30,000 to 60,000 cpm, and 100 μl of appropriately diluted rabbit antiserum that would bind approximately 40 to 50% of 125I-labeled netilmicin in the absence of unlabeled netilmicin. The tubes were incubated at 22°C for 30 min. To separate bound from free netilmicin, dextran-charcoal was used as described previously (10). The standard curve was constructed using logit-log graph paper (10). Values for unknown serum samples were interpolated from the standard curve. The RIA for netilmicin was compared with an agar diffusion microbioassay using Klebsiella pneumoniae as the seed organism (11). Twenty-five patient sera that covered a range from 1 to 10 μg of netilmicin per ml were selected. The sera were assayed simultaneously by both methods in duplicate on two different days, and the correlation coefficient (r value) was determined between the methods by least squares linear regression.

A typical standard curve of the RIA of netilmicin in which the logit of B/B₀ in percentage of relative bound 125I-labeled netilmicin was plotted versus the logarithmic amount of netilmicin in nanograms per assay tube as shown in Fig. 1. The sensitivity of the assay was determined to be 480 pg per tube. This was based on a theoretical mean value which was two standard deviations (SD) below the mean of the maximum binding tube (B₀).

To assess precision, five replicate determinations were run on three samples that represented the low, middle, and high concentrations of clinically expected serum netilmicin levels. The samples with mean concentrations in micrograms per milliliters ± the SD with the coefficient of variation (CV) were as follows: low, 1.02 ± 0.04, CV 3.9%; middle, 4.42 ± 0.18, CV 4.1%; and high, 7.78 ± 0.25, CV 3.2%. Reproducibility of the assay was determined by assaying the same three representative samples in duplicate on five different assay days. The mean concentration in micrograms per milliliter ± SD with the CV was as follows: low, 1.2 ± 0.13, CV 13.3%; middle, 4.66 ± 0.42, CV 9.1%; and high, 8.48 ± 0.86, CV 10.2%.

There was no cross-reaction of the netilmicin antiserum with the following antimicrobial agents at serum levels at least 20 times greater than would be expected therapeutically: tobramycin, amikacin, cephalothin, chloramphenicol, cefazolin, carbenicillin, or oxacillin. There
was, however, a 3% cross-reaction with gentamicin and sisomicin.

The comparison of the RIA with the microbiobioassay, as determined by linear regression analysis, is depicted in Fig. 2. The correlation coefficient was 0.94 and was significant at $P < 0.00005$. The line of regression had the equation of $y = 0.94x + 0.96$. Statistically, therefore, there was no significant difference in the measurement of netilmicin between these two methods.

RIA is a rapid, sensitive and, more importantly, the most specific method for the determination of aminoglycosides in serum. The desirability of having more rapid and specific assay methods for aminoglycosides is obvious and has been discussed in previous investigations (8, 9). Clinically, the major advantage to this assay other than rapidity is its specificity which obviates the need for laboratory personnel to obtain information regarding other antimicrobial agents that the patient may be receiving.

Unlike the extensive cross-reaction (70%) between kanamycin and antisera produced to the semisynthetic derivative of kanamycin, amikacin (10), we find little cross-reactivity (3%) between netilmicin antisera and its parent compound sisomicin. The possible reasons for this are numerous but most likely relate to the groups or portions of the aminoglycoside molecule that are immunodeterminant after the aminoglycoside molecule has been conjugated to porcine thyroglobulin.

Recent work has shown the usefulness of cross-reacting gentamicin antisera for dual use in an RIA for sisomicin (3). This novel use of cross-reacting antisera was primarily due to the reported 65% cross-reaction of gentamicin antisera with sisomicin. Analogous multiple use of netilmicin antisera for RIA of other aminoglycosides, while theoretically possible, is neither practical nor economical since the cross-reactivity of netilmicin antisera with gentamicin and sisomicin is only 3% and would require large amounts of antisera to obtain any type of useful standard curve.

The RIA reported here is precise and reproducible and well within limits of acceptable coefficients of variation for clinical laboratories. It correlates well with the microbiobioassay, and the high degree of sensitivity of this method will permit quantification of concentrations not possible with microbiological and radioenzymatic techniques.

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LITERATURE CITED


