Comparison of Radioimmunoassay and Enzyme Immunoassay Methods in Determining Gentamicin Pharmacokinetic Parameters and Dosages

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Radioimmunoassay and enzyme immunoassay methods for analysis of serum gentamicin levels have been shown to be comparable. The purpose of this study was to determine if serum concentration-time data from the same patient assayed by radioimmunoassay and enzyme immunoassay would provide the same estimates for half-life, elimination rate constant, distribution volume, drug clearance, and gentamicin dose. A total of 103 pre- and postinfusion serum samples were obtained from 32 patients. The samples were divided and assayed by radioimmunoassay and enzyme immunoassay. Serum concentration-time data were fitted to a one-compartment model, and kinetic calculations were performed using the method of Sawchuk et al. (Clin. Pharmacol. Ther. 21:362-369, 1977). While good correlation was established between the two assay methods, significant (P < 0.05) mean differences were seen in distribution volume (25%), gentamicin clearance (15%), and half-life (11%), using the quantitative data from both methods. Because of differences noted in these pharmacokinetic parameters, significant differences were also noted in dosage calculations. We conclude that there are differences in the pharmacokinetic parameters obtained using results from the radioimmunoassay and enzyme immunoassay. These differences also translate into significant differences between dosage recommendations when individualization of the gentamicin regimen is attempted.

Gentamicin is a commonly used antibiotic for the treatment of serious gram-negative bacillary infections. Many authors have suggested improved clinical outcomes when particular serum concentration ranges of gentamicin are reached and maintained (1, 4, 5, 8). Serum concentration monitoring has also been suggested to minimize the potential of nephrotoxicity and ototoxicity (2, 3). Zaske et al. have identified substantial interpatient variation among 1,640 gentamicin patients and have suggested individualizing gentamicin therapy based upon each patient's pharmacokinetic parameters (12).

Two assay methods widely used for the quantitation of serum gentamicin concentration are the radioimmunoassay (RIA) and enzyme immunoassay (EMIT). These methods of gentamicin serum analysis have been shown to be comparable in precision, accuracy, and sensitivity (6, 9–11). The purpose of this study was to determine if gentamicin serum concentration-time data obtained from the same patient assayed by RIA and EMIT would provide the same estimate for gentamicin half-life (T1/2), elimination rate constant (Kd), distribution volume (Vd), total body clearance (TBC), and dosage calculation.

MATERIALS AND METHODS

A total of 32 patients (27 females and 5 males) receiving intravenous gentamicin were studied. Patients ranged in age from 15 to 90 years and were receiving gentamicin for the treatment of pelvic inflammatory disease, pneumonia, bacteremia, pyelonephritis, and cholecystitis. All patients received other antibiotics (clindamycin, penicillin G, ampicillin, or cefazolin) in addition to gentamicin. Of the 32 patients, 30 had a normal serum creatinine value (≤1.4 mg/dl), and 2 had abnormal values (5.1 and 1.5 mg/dl). Patients received 1.5 mg of gentamicin per kg infused intravenously over a 1-h period. Serum samples for measuring gentamicin concentration were obtained before the gentamicin infusion and at 0.25, 1.0, and 3 h postinfusion. An extended sampling interval was utilized in patients with abnormal renal function. Exact infusion and sampling times were recorded and used in the data analysis. A total of 103 serum samples were obtained from the 32 patients. Each sample was separated by centrifugation and divided into two specimens. The first half of the sample was assayed immediately by RIA, and the other half of the specimen was frozen at
FIG. 1. Comparison of EMIT with RIA for gentamicin over three concentration ranges (<2, ≥2 and ≤5, and >5 μg/ml). Orthogonal regression values are found in Table 1.

-70°C until EMIT was performed in accordance with the manufacturer’s specifications.

Assays. RIA of the gentamicin serum concentrations was performed using a commercially available assay (American Diagnostics Corp., Newport Beach, Calif.). Each determination was made on a Packard Prias model auto-gamma counter, run in duplicate, and reported as the mean. A standard curve was constructed using six calibrators of 0.5, 1.0, 2.0, 4.0, 8.0, and 16.0 μg/ml. Three controls (2.5, 5, and 10 μg/ml) were utilized as part of the daily assay procedure. The daily coefficient of variation ranged from 8 to 9% for the three control values.

The second portion of the original specimen was also measured using an EMIT (Syva Corp., Palo Alto, Calif.). Gentamicin serum level determinations were performed on a Gilford Statarm-3 spectrophotometer, run in duplicate, and reported as the mean. Gentamicin concentrations of 1.0, 2.0, 4.0, 8.0, and 16.0 μg/ml were used to construct the standard curve. One control value of 6 μg/ml was utilized for which the coefficient of variation between injections was 2.3%. Care was taken to assure that calibrators, controls, and other reagents used in both assay techniques were freshly prepared and were used well in advance of the product’s expiration date.

Pharmacokinetic calculations. Each patient’s postinfusion serum concentration-time data were fitted using linear regression analysis to a standard one-compartment pharmacokinetic model (7). These data provided an estimate of the gentamicin $K_d$, $T_{1/2}$, $V_d$, and TBC for each patient. Calculations for dose and dosage interval were performed for each patient by the method of Sawchuk et al. (7). The percent difference in pharmacokinetic parameters ($K_d$, $T_{1/2}$, $V_d$, and TBC) derived from RIA and EMIT data was calculated as the RIA value minus the EMIT value divided by the RIA value, with this quantity multiplied by 100.

Data analysis. The 103 gentamicin serum samples were stratified for descriptive analytical purposes into three groups (<2, ≥2 and ≤5, and >5 μg/ml). Separate analyses were performed on the 37 samples that were <2 μg/ml, the 45 serum samples that were ≥2 and ≤5 μg/ml, and the 21 serum samples that were >5 μg/ml. Each serum sample measured by RIA and EMIT was considered an independent observation. The grouping of the serum level data was determined using the RIA value for each serum level pair. The correlation between gentamicin concentrations as determined by EMIT and RIA was determined using Pearson’s product moment correlation coefficient. The relationship between serum concentrations determined by EMIT and RIA was described by orthogonal regression analysis.

Differences between gentamicin concentrations measured by RIA and EMIT (RIA minus EMIT results) were calculated for each of these 103 serum samples. These calculated differences for the three concentration ranges were compared with zero difference using a Student’s $t$ test for single samples. To determine if individual serum concentration-time data assayed by RIA and then by EMIT would provide the same estimates for the various pharmacokinetic parameters, estimates for $T_{1/2}$, $K_d$, $V_d$, TBC, and dosage were calculated and compared using a Student’s $t$ test for paired data. A 5% level of significance was used to define statistical differences between pharmacokinetic parameters derived from RIA and EMIT data.

RESULTS

A comparison of gentamicin concentration as determined by the two assay techniques is presented in Fig. 1. Orthogonal regression analysis for the three groups of stratified data is presented in Table 1. The greatest variation in slope,
TABLE 1. Orthogonal regression analysis of stratified gentamicin concentration data

<table>
<thead>
<tr>
<th>Group n</th>
<th>Concentration range (µg/ml)</th>
<th>Orthogonal regression</th>
<th>Line equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>I 37</td>
<td>&lt;2</td>
<td>0.94</td>
<td>y = 0.86x - 0.19</td>
</tr>
<tr>
<td>II 45</td>
<td>≥2 and ≤5</td>
<td>0.88</td>
<td>y = 0.77x + 0.30</td>
</tr>
<tr>
<td>III 21</td>
<td>&gt;5</td>
<td>0.94</td>
<td>y = 0.98x - 0.06</td>
</tr>
</tbody>
</table>

intercept, and correlation for the three groups of data occurred in group II (Fig. 1 and Table 1).

Gentamicin serum concentrations were consistently higher with RIA when compared with EMIT. Absolute differences between gentamicin serum concentrations (RIA minus EMIT concentrations) were significantly greater (P < 0.005) than zero for all three serum concentration groups (Fig. 2). The tendency towards a positive difference increased with the gentamicin concentration and was skewed such that in group III, zero was not included in the distribution of the differences of the serum concentration-time data.

Pharmacokinetic parameters estimated for each of the 32 patients using RIA serum concentration data and EMIT data are presented in Table 2. Despite the fact that analyses by these two quantitative techniques were made on the same patient sera, significant differences (P < 0.05) were seen in Kd, Vd, and TBC (Table 2). Gentamicin concentration data as measured by EMIT gave higher mean values for Vd, T1/2, and TBC. The mean percent differences for the respective pharmacokinetic parameters using these data for the 32 patients were 25, 11, and 15% higher as compared with RIA data determinations. RIA data, however, gave on the average 6% higher values for Kd in these patients as compared with EMIT determinations.

Dosage calculations producing a desired gentamicin peak concentration of 6 µg/ml were significantly different (P < 0.001) for the 32 patients when RIA and EMIT concentration data were used. The mean gentamicin dose was 85 ± 5 mg for the RIA data and 101 ± 6 mg for the EMIT data.

DISCUSSION

RIA and EMIT methods for quantitative analysis of serum gentamicin levels have been shown to be comparable techniques in precision, accuracy, and sensitivity (6, 9-11). Traditionally, assay comparability is determined by regressing the newer assay technology upon a more established assay method. Establishing a linear correlation of one, a linear regression slope of one, and a y intercept of zero are evidence of assay duplicability. To our knowledge, there has been no attempt to duplicate estimates of pharmacokinetic parameters when making assay comparisons.

Stratifying these data into three ranges of serum concentrations results in a discrepancy between the two assay methods in groups II and III. Instead of the expected normal distribution about zero difference, there is a marked proclivity towards a positive difference in assay results. In group III, zero is not included in the distribution of the data (Fig. 2). Statistically, these differences were greater than zero, indicating the constantly higher serum concentration estimates made by RIA as compared with EMIT.

Pharmacokinetic parameters determined for individual patients using the same patient sera and analyzing the specimens quantitatively by two techniques resulted in significantly different mean estimates of Vd, Kd, and TBC. Gentamicin dosage calculations using pharmacokinetic pa-
rameters obtained from the two assay techniques were also significantly different (Table 2).

\[ V_d = K_d (1 - e^{-K_d t}) / \int K_d \ \text{CP}_{	ext{max}} - \text{CP}_{	ext{min}}(e^{-K_d t}) \]  

where \( V_d \) is the infusion rate (milligrams per hour), \( t' \) is the length of infusion (hours), \( K_d \) is the elimination rate constant (hours\(^{-1}\)), \( \text{CP}_{	ext{max}} \) is the peak serum concentration (micrograms per milliliter), and \( \text{CP}_{	ext{min}} \) is the trough serum concentration (micrograms per milliliter). \( K_d \) derived for each patient is the result of linear regression analysis of the three postinfusion (0.25, 1.0, and 3.0 postinfusion) gentamicin levels. With RIA providing higher estimates of gentamicin concentration than EMIT in groups II and III, the result of linear regression analysis through the three postinfusion gentamicin concentration values will provide different estimates for \( K_d \). Also, because RIA will produce higher estimates of the peak serum concentration, the difference in the denominator \( \int \text{CP}_{	ext{max}} - \text{CP}_{	ext{min}}(e^{-K_d t}) \) will be larger resulting in a smaller \( V_d \) with the RIA technique. Clearance is also affected by volume, as the calculation for this parameter is the product of \( K_d \) and \( V_d \).

Intrinsic differences between the two assay techniques might explain the variation observed in the concentration reported for the same patient sera. Each of these assay methods employs an antigen-antibody reaction. Differences in the affinity of the antigen and antibody utilized between these two methods might be responsible for part of the differences observed in serum concentration. Although essentially the same number and concentrations of gentamicin calibrators are used to construct the standard curve, different mathematical models are utilized to determine the exact nature of the curve. Thus, variation may be expected in the reported gentamicin concentration for the same specimen of serum when different mathematical methods are used.

From our study, it would appear that methods for comparing assay techniques for clinical use should involve a detailed comparative analysis of pharmacokinetic parameters and doses subsequently derived. Laboratories changing assay techniques for gentamicin might expect to see differences in pharmacokinetic parameters. These differences will affect dosages recommended for patients receiving gentamicin and may result in subtherapeutic or potentially toxic serum concentrations.

**ACKNOWLEDGMENT**

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**LITERATURE CITED**


**TABLE 2.** Pharmacokinetic parameters determined from EMIT or RIA serum concentration—time data

<table>
<thead>
<tr>
<th>Assay</th>
<th>Pharmacokinetic parameter value determined*</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIA</td>
<td>( V_d ) (liters)</td>
</tr>
<tr>
<td>11.5 ± 3.6</td>
<td>0.44 ± 0.19</td>
</tr>
<tr>
<td>14.1 ± 4.2</td>
<td>0.41 ± 0.17</td>
</tr>
</tbody>
</table>

* The values represent the mean ± the standard deviation. The \( P \) values for each of the parameters from left to right in the table are 0.001, 0.009, 0.171, 0.001, and 0.001.