Simplified Assay for Gentamicin in the Presence of Other Antibiotics

DAVID V. ALCID AND STEPHEN J. SELIGMAN

Department of Medicine, State University of New York, Downstate Medical Center, Brooklyn, New York 11203

Received for publication 26 February 1973

Gentamicin concentration was estimated by using a clinical isolate of a multiple antibiotic-resistant strain of *Staphylococcus epidermidis* in an agar well diffusion assay. The presence of clinically attainable concentrations of cephalothin, ampicillin, carbenicillin, clindamycin, or chloramphenicol did not affect the assay.

Gentamicin is an aminoglycoside antibiotic effective especially in the treatment of gram-negative infections. For many bacteria, the therapeutic range is between 4 and 8 $\mu\text{g/ml}$. Sustained levels above 12 $\mu\text{g/ml}$, however, are potentially toxic. Variability in dose response curve from patient to patient occurs. Riff and Jackson concluded that, by using dosage schedules based on weight, 10% of patients with normal renal function would receive potentially toxic doses and that 30% would have inadequate levels (6). Blood levels are influenced by hematocrit (6) and injection site (10). In the presence of renal impairment, gentamicin levels are increased. Alterations of dosage schedules based either on creatinine (2) or creatinine clearance (3) have been proposed; however, gentamicin levels in the presence of renal insufficiency are even more variable than in patients with normal renal function. Consequently, maintenance of gentamicin levels within the therapeutic range, but with avoidance of toxicity, is difficult if creatinine or creatinine clearance is relied upon. A simple and reliable way of regulating gentamicin dosage is by following serum concentrations. Several assays are available; however, the use of enzymes (8), radioactive substrates (9), or radioactive antibiotics (5) makes these assays complicated for routine clinical application. In the current method, the use of a multiple antibiotic-resistant organism results in a simplified assay which is accurate even in the presence of other antibiotics.

MATERIALS AND METHODS

Assay organism. The assay organism was a clinical isolate, *Staphylococcus epidermidis* GA, resistant to ampicillin, carbenicillin, cephalothin, chloram-

phenicol, tetracycline, kanamycin, streptomycin, colistimethate, sulfisoxazole, lincomycin, and clindamycin and sensitive to gentamicin and vancomycin as determined by the agar diffusion test of Bauer et al. (1). The sensitivity of the assay organism was checked with each passage of the stock culture. The stock culture was maintained by streaking an isolated colony onto agar at 6-week intervals.

The organism has been deposited with the American Type Culture Collection (ATCC 27626).

Preparation of seeded agar. A 0.5-ml amount of an overnight growth of *S. epidermidis* GA was mixed with 18 ml of liquid Trypticase soy agar (BBL) previously cooled to 52 C. The mixture was quickly poured into a 150-mm plastic petri dish, allowed to solidify, and cooled at 4 C. Wells were made with suction by using a thin-walled glass tubing with an outer-wall diameter of 2.5 mm. Well spacing was facilitated by using a grid system under the plates. Twenty-eight wells were made per plate. Seeded agar was stored at 4 C in closed plastic bags.

Preparation of standards. Standard gentamicin solution was made by mixing gentamicin powder of known potency with 0.1 M phosphate buffer, pH 7.4. Working standards were made by diluting the stock solution in pooled human serum to final concentrations of 1.0, 8.0, and 24.0 $\mu\text{g/ml}$. Wells were filled by using nonheparinized capillary tubes (75 $\times$ 1.4 to 1.6 mm in diameter). Each assay was done in triplicate. A well required 4 μl of assay solution. Overfilled and underfilled wells were marked and were not read. Plates were incubated at 37 C. Zone diameters were measured after 5 h and overnight incubation by using a Fisher-Lilly zone size reader which permitted readings to the nearest 0.1 mm. Results were plotted on semilogarithmic paper.

Collecting sera from pediatric patients. Heel blood was collected in a nonheparinized capillary tube (75 $\times$ 1.4 to 1.6 mm in diameter), allowed to clot, and then was centrifuged (Adams autocrit). The capillary tube was broken at the junction of red cells and serum. The serum-containing portion of the capillary tube was used to fill wells in the seeded agar.

RESULTS

When zone size on a linear scale against gentamicin concentration on a semilogarithmic scale was plotted, the dose response curve was linear for concentrations as low as 1.0 $\mu\text{g/ml}$ to concentrations as high as 24 $\mu\text{g/ml}$ (Fig. 1). In a typical experiment, a change of 1.8 mm in zone size indicated a twofold change in gentamicin concentration. With 18 ml of agar and a 150-mm petri dish, the resulting agar thickness was 1.5 mm. Increasing agar thickness to 3 mm resulted both in smaller zone sizes and two zones of inhibition; consequently, the thinner agar was preferable. Zone diameters, after 24 h of incubation, were larger with seeded agar stored for 6 weeks than with freshly prepared agar (Fig. 2). The resulting increase in sensitivity of the assay was offset by the lack of distinct zones at 5 h, so that rapid readings were not possible with 6-week-old agar. Freshly prepared agar and 4-week-old agar gave similar results both at 5 and 24 h.

Although bacteria are more susceptible to gentamicin at pH 7.8 than at 7.4 and although, in general, aminoglycoside antibiotics are more active at the more alkaline pH (7), zone sizes were larger with standards at pH 7.4 than at pH 7.8. Standards were kept at 4 C for at least 4 months without loss of potency. The zone diameters with standards diluted in buffer were identical to those of standards diluted in serum; however, the color and increased viscosity of serum made filling of wells easier. Sera from normal subjects, from patients with blood urea nitrogen as high as 150 mg%, acute hemolytic

FIG. 1. Zone sizes after 5 and 24 h of incubation with varying concentrations of gentamicin. Seeded agar was freshly prepared.

FIG. 2. Zone sizes after 24 h of incubation with seeded agar stored at 4 C for varying lengths of time.

anemia, or diabetic ketoacidosis did not show inhibitory activity against the test organism. Heparinized capillary tubes decreased zone size, an observation consistent with the findings of Kunin that heparin interfered with the diffusion of aminoglycoside antibiotics (4). The assay was tested with different antibiotics in combination with gentamicin. Zone size resulting from 8 μg of gentamicin per ml was not altered by 150 μg of cephalothin, ampicillin, carbenicillin, and clindamycin per ml or by 30 μg of chloramphenicol per ml (Table 1). The addition of a known amount of gentamicin to sera obtained from patients receiving gentamicin showed that the resulting concentration did not differ significantly from that expected, i.e., the sum of the added gentamicin plus the pre-existing gentamicin concentration (Table 2).

DISCUSSION

A simplified bioassay for gentamicin has been described. By using a multiple antibiotic-resistant strain of *S. epidermidis*, gentamicin concentrations were estimated in the presence of other antibiotics without the use of enzymes, radioactive material, or elaborate equipment and techniques. The assay is so simple that relatively untrained personnel have been able to perform it. Results can be obtained after 5 h of incubation. Seeded agar plates can be stored for 6 weeks, and antibiotic standards can be stored for at least 4 months; accordingly, both can be made in advance and stored at 4 C until needed. Thus, the assay can be performed at night, with results available by morning. The assay is especially useful as a guide to therapy of seriously ill patients with renal insufficiency. Because small amounts of sera are necessary, multiple determinations can be done easily in infants.

TABLE 1. Zone sizes with 8 μ g of gentamicin/ml with and without ampicillin, cephalothin, carbenicillin, clindamycin, or chloramphenicol

Additional antibiotic	μ g/ml	Zone size (mm)
None		13.2
Ampicillin	150	13.0
Cephalothin	150	13.0
Carbenicillin	150	13.0
Clindamycin	150	13.4
Chloramphenicol	30	13.0

TABLE 2. Gentamicin assay of gentamicin-containing sera to which known amounts of gentamicin were added

Pre-existing gentamicin activity (μ g/ml)	Concentration of gentamicin enrichment	Observed gentamicin concentration	Expected gentamicin concentration	Difference ^a
0.0	4.0	3.8	4.0	-0.2
0.0	4.0	3.6	4.0	-0.4
0.0	4.0	4.4	4.0	+0.4
1.1	4.0	4.6	5.1	-0.5
1.2	4.0	5.2	5.2	0.0
1.2	4.0	4.6	5.2	-0.6
1.6	2.4	3.6	4.0	-0.4
1.8	4.0	5.0	5.8	-0.8
2.0	4.0	6.2	6.0	+0.2
2.4	2.4	4.8	4.8	0.0
2.4	3.5	5.2	5.9	-0.7
2.4	4.0	6.8	6.4	+0.4
2.5	4.0	6.8	6.5	+0.3
3.0	4.0	6.3	7.0	-0.7
3.8	4.0	8.0	7.8	+0.2
4.2	4.0	7.4	8.2	-0.8
4.6	4.0	8.0	8.6	-0.6
4.8	3.5	7.0	8.3	-1.3
5.6	4.0	10.0	9.6	+0.4

^a Derived by the two-tailed, matched student's *t* test; *P* = 0.10.

The technique of using multiple antibiotic-resistant strains should be applicable to other antibiotics. Clinically significant concentrations of tobramycin or vancomycin did not give sufficiently large zone sizes with *S. epidermidis* GA; however, another *S. epidermidis* strain was found which was suitable for tobramycin.

ACKNOWLEDGMENTS

We thank Wen-huey Su for statistical assistance. Gentamicin powder was a gift from the Schering Corp., Bloomfield, N.J.

LITERATURE CITED

1. Bauer, A. W., W. M. Kirby, J. C. Sherris, and M. Turk. 1966. Antibiotic susceptibility by a standardized single disc method. *Amer. J. Clin. Pathol.* 45:493-496.
2. Chan, R. A., E. J. Benner, and P. D. Hoeprich. 1972. Gentamicin therapy in renal failure; a nomogram for dosage. *Ann. Intern. Med.* 76:773-778.
3. Gingell, J. C., and P. M. Waterworth. 1968. Dose of gentamicin in patients with normal renal function and renal impairment. *Brit. Med. J.* 2:19.
4. Kunin, C. M. 1970. Binding of antibiotics to tissue homogenates. *J. Infect. Dis.* 121:55-64.
5. Lewis, J. E., J. C. Nelson, and H. A. Elder. 1972. Radioimmunoassay of an antibiotic, gentamicin. *Nature N. Biol.* 239:214-216.
6. Riff, L. J., and G. G. Jackson. 1971. Pharmacology of gentamicin in man. *J. Infect. Dis.* 124:S98-105.
7. Rubenis, M., V. M. Kozij, and G. G. Jackson. 1964. Laboratory studies on gentamicin. *Antimicrob. Ag. Chemother.* 1963, p. 153.
8. Sabath, L. D., J. I. Casey, P. A. Rich, and M. Finland. 1971. Rapid microassay for circulating nephrotoxic antibiotics. *Antimicrob. Ag. Chemother.* 1970, p. 83.
9. Smith, D. A., B. Van Otto, and A. L. Smith. 1972. Rapid chemical assay for gentamicin. *New Engl. J. Med.* 286:583-596.
10. Winters, R. E., K. D. Litwack, and W. L. Hewitt. 1971. Relation between dose and level of gentamicin in blood. *J. Infect. Dis.* 124:S90-95.