Stability of Gentamicin, Tobramycin, and Amikacin in Combination with Four β-Lactam Antibiotics

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The stability of the aminoglycosides gentamicin, tobramycin, and amikacin stored in combination with carbenicillin, piperacillin, cefotaxime, and moxalactam was evaluated at four temperatures (25, 4, −8, and −70°C) over a 3-week period. Amikacin was the most stable of the aminoglycosides and demonstrated no loss of activity when stored with either carbenicillin or piperacillin. Gentamicin and tobramycin were inactivated by carbenicillin and piperacillin at 25 and 4°C, with aminoglycoside activity declining substantially after 8 to 48 h of storage; virtually no loss of gentamicin or tobramycin activity occurred with storage at −8 or −70°C. Cefotaxime and moxalactam produced no degradation of any of the three aminoglycosides.

Coadministration of a penicillin or cephalosporin antibiotic with an aminoglycosidic aminocyclitol is often used in the treatment of serious gram-negative rod infections, particularly those caused by Pseudomonas aeruginosa. These antibiotic combinations have been shown to be synergistic in vitro, in experimental infections in animals, and in clinical studies in humans (4, 8, 9, 17). However, in vitro studies have demonstrated that at high concentrations penicillins such as carbenicillin and ticarcillin can inactivate aminoglycosides (2, 5, 10, 11, 13). Evidence suggests that the inactivation mechanism involves the nucleophilic opening of the penicillin beta-lactam ring, which then combines with an amino group of the aminoglycoside, resulting in the formation of a biologically inactive amide (3, 16). The rate of in vitro inactivation is dependent upon temperature, time, the composition of the medium, and the concentration of the beta-lactam (11, 13, 14, 16). Aminoglycoside inactivation also can occur in vivo when excretion of both drugs is delayed, as in patients with renal insufficiency (5, 6, 14).

In light of the increasing use of the newer beta-lactam antibiotics and the clinical importance of accurately monitoring aminoglycoside levels in sera, we examined the stability of three aminoglycosides (gentamicin, tobramycin, and amikacin) stored alone and in combination with each of four beta-lactam antibiotics (carbenicillin, piperacillin, cefotaxime, and moxalactam) at four laboratory temperatures (25, 4, −8, and −70°C) over a 3-week period to assess the effects of time and temperature on the measurement of aminoglycoside concentrations in sera.

(Materials and Methods)

Stock solutions in pooled human sera of gentamicin sulfate (Schering Corp., Bloomfield, N.J.), tobramycin sulfate (Eli Lilly & Co., Indianapolis, Ind.), and amikacin sulfate (Bristol Laboratories, Syracuse, N.Y.) were reconstituted from clinical laboratory standards. For each aminoglycoside, portions of the stock solution were then mixed with each of the following beta-lactams: carbenicillin disodium (J. B. Roerig, New York, N.Y.), piperacillin sodium (Lederle Laboratories, Pearl River, N.Y.), cefotaxime sodium (Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J.), and moxalactam disodium (Eli Lilly & Co.). The resulting drug concentrations were chosen to fall within a clinically achievable range, with gentamicin and tobramycin at 5 μg/ml, amikacin at 20 μg/ml, carbenicillin and piperacillin at 200 μg/ml, and cefotaxime and moxalactam at 100 μg/ml.

Immediately after mixing, a portion of each test solution, containing an aminoglycoside either alone or in combination with a beta-lactam, was removed and assayed for aminoglycoside activity (0 h). The remainder of each solution was divided into samples and stored in glass vials at the following temperatures: 25°C, room temperature; 4°C, refrigerator temperature; −8°C, a standard household freezer; and −70°C, an ultracold freezer (Revco Inc., West Columbia, S.C.). Samples were assayed for residual aminoglycoside activity after 8 h, 48 h, 1 week, and 3 weeks of storage. All measurements were performed in duplicate.

Concentrations of gentamicin, tobramycin, and amikacin were determined by a homogeneous enzyme immunoassay technique in which EMIT-AMD kits (Syva Co., Palo Alto, Calif.) were used according to
the instructions of the manufacturer. EMIT procedures have been shown to be accurate and specific, and they correlate well with results of radioimmunoassay and microbiological assays (7, 12). Optimal sensitivity of the EMIT assay is between 2 and 10 μg/ml for gentamicin and tobramycin and between 5 and 50 μg/ml for amikacin. After measurement of aminoglycoside concentrations, the percentage of residual activity (measured value/intended value × 100) was determined for each aminoglycoside alone and in combination with each beta-lactam.

RESULTS

Immediately after mixing the samples (0 h), 100% activity was demonstrated for each aminoglycoside alone and in combination with each beta-lactam. For all three of the aminoglycosides stored alone, virtually no loss of aminoglycoside activity occurred (>92% residual activity) at any temperature over the 3-week period of study.

Effect of beta-lactam antibiotics. No inactivation of amikacin occurred during storage in combination with any of the beta-lactam antibiotics at any temperature over the 3-week period. However, substantial inactivation of gentamicin and tobramycin occurred in the presence of carbenicillin or piperacillin during storage at 25 and 4°C. Figure 1 shows the percentages of residual activity for all aminoglycosides alone and in combination with beta-lactam antibiotics stored at 4°C. The combination of gentamicin and carbenicillin demonstrated 72% residual aminoglycoside activity after 3 weeks of storage, whereas the combination of tobramycin and carbenicillin declined to 64% residual aminoglycoside activity over this same period.

In comparison with carbenicillin, piperacillin caused less inactivation of gentamicin, with 92% activity remaining at the end of the sample period, but produced a 40% loss of tobramycin activity after 3 weeks of storage at 4°C. Similarly, at room temperature, carbenicillin resulted in more profound inactivation of gentamicin and tobramycin than did piperacillin. In fact, with only one exception, less inactivation of gentamicin and tobramycin occurred in the presence of piperacillin than carbenicillin at every temperature studied over the 3-week period.

Unlike carbenicillin and piperacillin, cefotaxime and moxalactam resulted in virtually no inactivation of the three aminoglycosides studied. At each temperature, residual aminoglycoside activity ranged between 92 and 100% over the 3-week period for each aminoglycoside in combination with either cefotaxime or moxalactam.

Effect of temperature. Figure 2 shows the effect of temperature on the interaction between tobramycin and carbenicillin, the combination which showed the greatest loss of aminoglycoside activity during the test period. The most notable decline in tobramycin activity occurred at room temperature, with 64% residual activity after 48 h of storage and 56% residual activity after 1 week of storage. At refrigerator temperature, 84% aminoglycoside activity remained after 48 h, 68% activity remained after 1 week, and 64% activity remained after 3 weeks of storage. Minimal loss of tobramycin activity occurred (88% residual activity) after 3 weeks of storage at −8°C. More notably, 100% tobramycin activity remained throughout the sample period for the tobramycin-plus-carbenicillin combination at −70°C.

Similar time- and temperature-dependent trends were observed for the combinations of tobramycin or gentamicin plus piperacillin and gentamicin plus carbenicillin. Significant losses of aminoglycoside activity were detected at 25 and 4°C, particularly after more than 48 h of storage. No loss of aminoglycoside activity was obtained with any of the combinations at −70°C.

Relative stability of the aminoglycosides. Amikacin was the most stable of the aminoglycosides studied. No inactivation occurred when amikacin was stored in combination with each of the four beta-lactams (Fig. 1) at all temperatures studied. Gentamicin and tobramycin were inactivated only in combination with carbenicillin or
piperacillin at 25 and 4°C, and in virtually all instances, tobramycin demonstrated a greater loss of activity than did gentamicin.

**DISCUSSION**

The in vitro inactivation of an aminoglycoside by a penicillin or cephalosporin is of concern when processing blood samples for aminoglycoside determinations in laboratories. Carbenicillin has been shown by many investigators to cause inactivation of gentamicin in vitro (5, 10, 11, 13, 16). In our study, both carbenicillin and piperacillin inactivated gentamicin and tobramycin in sera at room temperature (25°C) and at refrigerator temperature (4°C). The loss of aminoglycoside activity was greater with carbenicillin than with piperacillin. The instability of both gentamicin and tobramycin in combination with carbenicillin or piperacillin was temperature dependent. The loss of aminoglycoside activity was more prominent at room temperature than at refrigerator temperature, with only minor degradation occurring at -8°C. Samples frozen at -70°C were stable for all aminoglycosides studied. These findings are in agreement with those of Pickering and Gearhart (13), who detected no significant inactivation of gentamicin, tobramycin, amikacin, or netilmicin when stored in combination with 200 µg of carbenicillin or ticarcillin per ml at -70°C for up to 8 weeks.

Cefotaxime and moxalactam caused no significant inactivation of any of the aminoglycosides studied over a 3-week period at any temperature. Similarly, Teil and co-workers (15) found no substantial differences in either gentamicin or cefamandole concentrations when these drugs were stored together in sera at 24, 6, and -17°C over 24 h, and Noone and Pattison (11) detected no inactivation of gentamicin when stored in sera at 37°C with either cephaloridine or cephalothin at a concentration of 200 µg/ml.

Of the three aminoglycosides studied, the greatest stability was demonstrated by amikacin, with 100% amikacin activity detected for every combination at each temperature over a 3-week period. Similarily, Pickering and Gearhart (13) found that inactivation of gentamicin and tobramycin was significantly greater than that of amikacin in combination with various concen-

**FIG. 2.** Effect of temperature on residual tobramycin activity when stored in sera in combination with carbenicillin.
trations of carbenicillin at 37°C for 72 h. Likewise, Adam and Haneder (1) noted that piperacillin had the greatest effect on the activity of tobramycin, followed by gentamicin, with amikacin being inactivated least after storage in phosphate buffer at 37°C.

In summary, in laboratories performing determinations of aminoglycoside concentrations in sera, serum samples containing a semisynthetic penicillin derivative should be assayed for gentamicin or tobramycin activity as soon as possible after collection. If a delay of more than 8 h is anticipated, the serum samples should be frozen, preferably at −70°C or, if not possible, at −8°C. Failure to freeze serum samples may result in falsely low gentamicin or tobramycin levels due to in vitro inactivation by the semisynthetic penicillin. On the contrary, cefotaxime and moxalactam do not cause significant inactivation of aminoglycosides in vitro, and time and temperature factors are not critical for accurate amikacin determinations.

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LITERATURE CITED