Animal Model Distinguishing In Vitro from In Vivo Carbenicillin-Aminoglycoside Interactions

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Aminoglycosides and carbenicillin are frequently co-administered to patients with serious gram-negative infections. Aminoglycosides are inactivated by carbenicillin in vitro, and a loss of antibacterial activity of both antibiotics results. Although these interactions are presumed to occur in vivo, previous studies have not used assay methodology that can distinguish inactivation occurring prior to and during microbiological assay from inactivation in vivo. To address this problem, we gave seven bilaterally nephrectomized mongrel dogs doses designed to achieve simultaneous therapeutic serum concentrations of aminoglycosides and carbenicillin. Serum samples were tested by radioimmunoassay on three occasions: immediately, to determine in vivo interactions, and at 24 h and 1 week to assess the time course of in vitro inactivation. In comparison with immediate radioimmunoassay, gentamicin and tobramycin concentrations decreased by 39 and 53%, respectively, when assayed at 24 h ($P < 0.05$) and by 75 and 82% when assayed at 7 days ($P < 0.001$). In contrast, amikacin concentrations were reduced by only 9 and 30% at 24 h and 7 days. Tobramycin concentrations were also determined by immediate microbiological assay and were found to be similar to those in samples stored for 24 h before radioimmunoassay. Immediate radioimmunoassay demonstrated that carbenicillin reduced in vivo serum half-lives of gentamicin and tobramycin by 40% ($P < 0.05$). The half-life of amikacin in vivo was not significantly altered. In the presence of carbenicillin, amikacin was the most stable aminoglycoside both in vivo and in vitro, and it is the aminoglycoside of choice in patients with renal failure who require this combination.

Concurrent carbenicillin and aminoglycoside therapy is frequently used in the treatment of gram-negative infections. This combination is synergistic in vitro against *Pseudomonas* (1, 6). In 1971, McLaughlin and Reeves (7) demonstrated that gentamicin can be inactivated by carbenicillin in vitro. Subsequent studies have shown that both carbenicillin and ticarcillin can inactivate aminoglycosides in vitro, with the rate of inactivation influenced by temperature, time, concentration, and the medium employed (5, 10, 11, 13). This interaction causes inactivation of both antibiotics and loss of their antibacterial effect (10).

Ervin and colleagues (3) reported that, after carbenicillin administration, gentamicin half-life decreased from a control mean of 46 h to 22 h in patients with end-stage renal disease. Davies and associates (2) studied patients with renal failure and found that the half-life of gentamicin decreased from a mean value of 54 h to 21 h after carbenicillin or ticarcillin therapy. However, neither of these studies used sampling and assay techniques which reliably differentiate inactivation occurring prior to assay from actual in vivo inactivation. If the reaction occurs only

in vitro, then dosage increases based on in vitro effects pose danger to patients.

Because serum slows the rate of in vitro inactivation (11), it was even possible that no actual in vivo inactivation occurred. This study, then, was performed to develop analytical and sampling methods which would distinguish in vitro from in vivo interactions and to determine the comparative in vivo effects of carbenicillin on gentamicin, tobramycin, and amikacin disposition during renal failure in an animal model.

(This paper was presented at the 11th International Congress of Chemotherapy and 19th Interscience Conference on Antimicrobial Agents and Chemotherapy, 1-5 October 1979, Boston, Mass.)

MATERIALS AND METHODS

Animals. Seven mongrel dogs, weighing 22.3 ± 2.1 kg, were bilaterally nephrectomized and divided into three groups. The first group (dogs 1, 2, and 3) received an intravenous bolus dose (7.5 mg/kg) and continuous infusion (4.5 mg/kg per h) of carbenicillin, via a volumetric infusion pump. After 2 h, they were given an intravenous bolus composed of gentamicin (2.0 mg/kg), tobramycin (2.0 mg/kg), and amikacin (6.0 mg/kg).

Animals in the second group (dogs 4, 5, and 6) were each given an intravenous bolus of tobramycin (2.0 mg/kg) followed after 10 h by a bolus (7.5 mg/kg) and infusion (4.5 mg/kg per h) of carbenicillin. The last animal (dog 7) received only the intravenous mixture of gentamicin (2 mg/kg), tobramycin (2 mg/kg), and amikacin (6 mg/kg). All of these doses were chosen to achieve simultaneous therapeutic serum concentrations as follows: gentamicin, 10 µg/ml; tobramycin, 10 µg/ml; amikacin, 30 µg/ml; and carbenicillin, 200 to 300 µg/ml.

Blood sampling. Venous blood was drawn from a cannula in the right femoral vein at intervals of 15 min for the first 2 h after administration of either carbenicillin or aminoglycoside and then at intervals of 2 to 4 h for the next 24 to 48 h.

At each blood collection time, one-third of the sample (3 to 4 ml) was collected into a Vacutainer containing penicillinase (5,000 U/ml of sample), one-third was collected into a test tube containing cellulose phosphate powder (100 mg/ml of sample), and a third portion was collected and refrigerated. These procedures permitted the determination of aminoglycoside concentrations, carbenicillin concentrations, and the effect of storage, respectively.

Assays. Cellulose phosphate powder was added to samples to selectively bind aminoglycosides (12). The procedure consisted of adding cellulose phosphate powder (ICN Biochemicals, Cleveland, Ohio), 100 mg/ml, to blood. The mixture was blended on a Vortex mixer and centrifuged at $2,000 \times g$ for 5 min. Carbenicillin concentrations in the supernatant were measured (as total antibiotic activity) by a modified agar well diffusion technique with the use of *Bacillus subtilis* spores. This microbiological procedure requires that assay plates be incubated for 12 h. Tobramycin activity in dogs 4, 5, and 6 was assayed by use of *B. subtilis* with penicillinase incorporated into the media (14). Bioassay standard concentrations were 0.5, 1.0, 2.0, 4.0, and 8.0 µg/ml, with a coefficient of variation of 12% at 0.5 µg/ml and 8% at 8.0 µg/ml. With some samples, a high-pressure liquid chromatographic (HPLC) procedure was also used (4). A radioimmunoassay (RIA, Monitor Sciences, Newport Beach, Calif.) was used to determine aminoglycoside concentrations in all samples.

Assay specificity. The possibility that inactivated tobramycin could give a partial response by RIA or HPLC was assessed by spiking 50 ml of blank dog serum with tobramycin to a final concentration of 10 µg/ml. The sample was divided, and 500 µg of carbenicillin per ml was added to one part. Both tobramycin and tobramycin-plus-carbenicillin samples were placed in a water bath at 37°C. Immediately after carbenicillin was added and again at 12 h, tobramycin concentrations were determined in triplicate by RIA, HPLC, and the *B. subtilis* bioassay.

Effects of penicillinase on carbenicillin inactivation. An in vitro study was performed to assess the role of penicillinase lysis of carbenicillin in tobramycin inactivation. The experiment was performed twice, in dog serum and in whole blood. A 200-ml tube of serum or blood was divided into four portions, and antibiotics were added as follows: tube 1, tobramycin (10 µg/ml) and penicillinase (500,000 U/ml); tube 2, the same as tube 1 plus carbenicillin (500 µg/ml); tube 3, tobramycin and carbenicillin but no penicillinase; and tube

4, tobramycin (10 µg/ml) alone. All tubes were incubated at 37°C, and the contents were assayed for both tobramycin and carbenicillin at 0, 2, 4, 6, 8, 12, and 24 h. Tobramycin levels were measured by immediate RIA; carbenicillin levels, were measured by bioassay after inactivation of aminoglycoside with cellulose phosphate.

Effects of sample storage. Samples from dogs 1, 2, and 3 were assayed for aminoglycoside and carbenicillin immediately (10 min), after 24 h of refrigeration, and again at 7 days. Samples from dogs 4, 5, 6, and 7 were assayed immediately.

Analysis. Computer data fitting (8) and two-compartment linear modeling were used to describe the observed biexponential decline in aminoglycoside concentrations and to determine aminoglycoside serum half-lives. Statistical analysis was performed by use of the unpaired Student *t* test, with *P* < 0.05 considered significant.

RESULTS

The ability of cellulose phosphate to selectively bind and remove aminoglycosides was tested by adding therapeutic concentrations of gentamicin (10 µg/ml), tobramycin (10 µg/ml), and amikacin (30 µg/ml) to dog serum samples containing or not containing carbenicillin (500 µg/ml). Aminoglycoside and carbenicillin activity were determined by bioassay. Carbenicillin was not affected by cellulose powder, since zone sizes were not significantly different between samples containing only carbenicillin (13.8 ± 0.8 mm) and samples containing carbenicillin and aminoglycosides plus cellulose powder (13.8 ± 0.6 mm). No activity was noted in samples containing only aminoglycosides after treatment with cellulose powder.

Specificity of RIA and HPLC tobramycin assays. Tobramycin concentrations in the control sample free from carbenicillin were 9.89 ± 0.13 µg/ml determined by RIA, 9.93 ± 0.09 µg/ml by HPLC, and 9.84 ± 0.90 µg/ml by bioassay.

Immediately after carbenicillin addition, concentrations assayed were 9.94 ± 0.18 µg/ml by RIA, 9.96 ± 0.10 µg/ml by HPLC, and 6.63 ± 0.56 µg/ml by bioassay. These bioassay results were available after 12 h of incubation at 37°C.

Tobramycin concentrations determined after 12 h of incubation with carbenicillin were 6.63 ± 0.16 µg/ml by RIA, 6.17 ± 0.30 µg/ml by HPLC, and 4.29 ± 0.54 µg/ml by bioassay. Since concentrations measured by RIA and HPLC at time zero closely approximated the known initial concentration, and because RIA and HPLC results determined after 12 h were similar to bioassay results read at 12 h (but prepared at time zero), it appears that there was significant loss during in vitro incubation, and that RIA, HPLC, and bioassay all measured only active tobramycin.

Penicillinase effects. As shown in Fig. 1, tobramycin was quite stable for 24 h under the

FIG. 1. Tobramycin concentrations determined by RIA in blood and serum samples containing tobramycin alone in the presence (●) or absence (□) of penicillinase; in serum samples containing tobramycin (10 μ g/ml), carbenicillin (500 μ g/ml), and penicillinase (500,000 U/ml) (○); and in blood samples containing the same (■).

incubation conditions and when exposed to serum or blood containing 500,000 U of penicillinase per ml. In all cases, when carbenicillin was added to this mixture of tobramycin and penicillinase, carbenicillin concentrations were undetectable. However, in spite of the absence of biologically active carbenicillin, the rate of tobramycin degradation increased. Of interest, this inactivation reaction was more pronounced in blood than in serum. Figure 1 also shows the very rapid destruction of tobramycin at 37°C without carbenicillin destruction by penicillinase. From this experiment it was concluded that the destruction of carbenicillin dramatically slows the inactivation but does not completely stop it.

Effects of sample collection and storage. The effects of carbenicillin on serum concentrations of gentamicin (Fig. 2), tobramycin (Fig. 3), and amikacin (Fig. 4) were determined by assaying aminoglycoside concentrations 10 min after collection, after 24 h of refrigeration, and again after 7 days of refrigeration. When compared with the 10-min RIA results, gentamicin concentrations were reduced 39% and tobramycin concentrations were reduced 53% after 24 h of refrigeration ($P < 0.05$) and by 75 and 82% when assayed by RIA after 7 days ($P < 0.001$).

The rate of *in vitro* inactivation of gentamicin and tobramycin was nearly constant over the clinical concentration range, because there was no significant difference in the slope of the 10-min, 24-h, or 7-day curves, and the standard

deviation of percent decrease in aminoglycoside concentrations at each sample time was small.

In contrast to the marked inactivation of gentamicin and tobramycin, amikacin concentrations were reduced only 9% from the 10-min to the 24-h assay, and only a 30% decrease in amikacin activity occurred when samples were assayed again after 7 days of refrigeration ($0.1 > P > 0.05$).

Inactivation during microbiological assay incubation. Significant loss of tobramycin activity also occurred during the 12-h incubation of our microbiological assay, despite penicillinase treatment. Figure 5 shows the mean serum concentrations of tobramycin before and after carbenicillin administration. The solid line represents the mean immediate RIA concentrations, and the dashed line is the mean immediate microbiological assay concentration. In samples taken prior to carbenicillin administration, tobramycin concentrations determined by RIA and microbiological assay were nearly identical, with correlation coefficients above 0.95 in each of the three dogs. After carbenicillin administration, tobramycin concentrations determined by immediate RIA and HPLC were nearly identical ($r = 0.95$) and were significantly higher than microbiological assay results. Tobramycin concentrations determined by bioassay consistently

FIG. 2. Mean ($\pm$ standard deviation) serum concentrations of gentamicin in dogs 1, 2 and 3, determined by immediate RIA (●) and after refrigeration for 24 h (○) and 7 days (■). Mean carbenicillin concentrations are also shown. The serum half-life of gentamicin averaged 18.2 h in the three anephric dogs, a decrease from the control value of 30.4 h.

FIG. 3. Mean ($\pm$ standard deviation) serum concentrations of tobramycin in dogs 1, 2 and 3, determined by immediate RIA ($\bullet$) and after refrigeration for 24 h ($\circ$) and 7 days ($\blacksquare$). Mean carbenicillin concentrations are also shown. The serum half-life of tobramycin averaged 17.5 h in the three anephric dogs, a decrease from the control value of 29.2 h.

FIG. 4. Mean ($\pm$ standard deviation) serum concentrations of amikacin in dogs 1, 2, and 3, determined by immediate RIA ($\bullet$) and after refrigeration for 24 h ($\circ$) and 7 days ($\blacksquare$). Mean carbenicillin concentrations are also shown. The serum half-life of amikacin averaged 28.6 h in the three anephric dogs, a decrease from the control value of 33.8 h.

FIG. 5. Mean ($\pm$ standard deviation) serum concentrations of tobramycin in dogs 4, 5, and 6 determined by both microbiological assay and immediate RIA prior to ($\bullet$) carbenicillin. By immediate RIA after carbenicillin ($\circ$), and by immediate microbiological assay after carbenicillin ($\circ$). The data show both the *in vitro* (immediate RIA versus microbiological assay) and *in vivo* (serum half-life of tobramycin before and after carbenicillin) effects of carbenicillin.

averaged 65% of those determined by immediate RIA or HPLC.

In vivo inactivation of gentamicin, tobramycin, and amikacin. The three aminoglycosides were administered as a simultaneous mixture to dogs 1, 2, 3, and 7 so that their comparative *in vivo* disposition could be assessed under identical physiological conditions.

Table 1 compares the effect of carbenicillin on the *in vivo* serum half-lives of gentamicin, tobramycin, and amikacin. In the control animal, the *in vivo* serum half-lives of these three aminoglycosides were 30.4, 29.2, and 33.8 h, respectively. However, in the presence of carbenicillin, the mean *in vivo* serum half-lives of gentamicin and tobramycin were reduced to 18.2 and 17.5 h ($P < 0.05$), as compared with the controls. In contrast to the *in vivo* inactivation of gentamicin and tobramycin, there was no significant difference in half-life between the amikacin control (33.8 h) and animals treated with amikacin and carbenicillin (28.6 h).

TABLE 1. *In vivo* aminoglycoside half-life values in dogs^a

Amino-glycoside	Half-life (h)		Decrease on carbenicillin administration (%)
	Control (dog 7)	During carbenicillin (dogs 1, 2, 3)	
Gentamicin	30.4	18.2 ± 2.6	40.1
Tobramycin	29.2	17.5 ± 2.3 ^b	39.3
Amikacin	33.8	28.6 ± 2.6	15.4

^a All concentrations used to calculate the values shown were determined by immediate RIA, as inactivation in vitro renders microbiological assay values inaccurate.

^b Statistical significance: $P < 0.05$, control versus during carbenicillin.

The *in vivo* effects of carbenicillin on the half-life of tobramycin alone were also assessed in dogs 4, 5, and 6. The mean half-life was significantly reduced from 27.8 ± 1.2 h before to 16.9 ± 1.8 h after carbenicillin administration ($P < 0.01$). This reduction was nearly identical to that observed when dogs 1, 2, and 3 were compared with the control.

DISCUSSION

Carbenicillin and an aminoglycoside are frequently given to patients with suspected or proven gram-negative infections. Although this combination has been shown to be synergistic against *P. aeruginosa*, *in vitro* inactivation of aminoglycosides has been reported. This interaction does not contradict the reports of synergism, because in most patients with normal renal function, the antibacterial effects of aminoglycosides and carbenicillin are thought to occur prior to any significant loss of activity. However, Murillo and associates have recently observed ticarcillin-associated reductions in gentamicin concentrations in granulocytopenic patients with normal serum creatinines (9). In contrast to the few reports of the interaction in patients with normal renal function, there are numerous reports of interactions in patients with renal failure. These are presumably facilitated by high concentrations of these antimicrobial agents, in contact for extended periods of time (10). However, none of these studies used specific sampling and assay methodologies which would reliably differentiate *in vitro* from *in vivo* interactions.

In this study, we distinguished *in vitro* from *in vivo* inactivation of aminoglycosides by carbenicillin, as is best illustrated in Fig. 5. The *in vitro* inactivation was shown by comparison of aminoglycoside concentrations quantitated by immediate RIA with those determined by either

immediate microbiological assay or delayed RIA.

As shown by immediate RIA, true *in vivo* interaction was observed and quantitated in both groups of animals. In the first group, gentamicin and tobramycin half-lives were reduced by approximately 40% as compared with the control. *In vivo* tobramycin serum half-life was similarly reduced after carbenicillin administration in the second group. However, these reductions were less than those reported by Riff (11) or Davies (2), who employed microbiological assays exclusively.

Since most previous studies of this interaction have used the bioassay, inactivation reported as *in vivo* may have been an *in vitro* problem or a combination of *in vitro* and *in vivo* inactivation. We have demonstrated that the RIA and HPLC procedures measure biologically active tobramycin, as does the microbiological procedure if a correction is made for the inactivation that occurs over the time required for bioassay incubation. However, we point out that calculation of a correction factor for bioassay is not possible, since the rate of reaction is also dependent on the carbenicillin concentration in the blood sample, and time between sampling and application to bioassay plates is usually not known. We have also shown that *in vitro* destruction of carbenicillin dramatically slows the rate of the inactivation, but we were somewhat surprised that it did not completely stop it. This conclusion was also apparent from our *in vivo* dog experiments. It was of great interest that whole blood apparently protects the carbenicillin in some fashion, as the reaction proceeds even more quickly in this medium. Potential explanations for this accelerated effect include intracellular carbenicillin shielded from lysis by the penicillinase, tobramycin sequestration inside or binding to erythrocytes, resulting in loss of activity, or possibly some component of erythrocytes acting as a catalyst for this reaction.

Amikacin was the most stable aminoglycoside in the presence of carbenicillin, both *in vitro* and *in vivo*. Concentrations were reduced by only 9 and 30% *in vitro* when assayed after 24 h and 7 days of refrigeration, over the carbenicillin/amikacin concentration range of 5:1 to 25:1. These findings are in agreement with *in vitro* data previously reported (5, 10), which showed amikacin to be the most stable. Also, because amikacin concentrations determined by delayed RIA and microbiological assay correlated well with immediate RIA results ($r = 0.98$), the bioassay is useful here, and the need for rapid assay is less acute than with tobramycin or gentamicin.

In contrast to gentamicin and tobramycin, *in vivo* amikacin half-life in the carbenicillin-

treated animals was not significantly different from the control. Although several investigators have demonstrated that amikacin is relatively resistant to inactivation by carbenicillin *in vitro*, this study is the first to present *in vivo* data showing that amikacin half-life in renal failure is not significantly reduced in the presence of therapeutic carbenicillin concentrations. When patients with renal failure require both an aminoglycoside and carbenicillin, and the infecting bacteria are sensitive to all three aminoglycosides, amikacin is the aminoglycoside of choice.

The significance of these findings for patients with normal renal function deserves further investigation, and such studies must employ immediate RIA to differentiate *in vitro* from *in vivo* inactivation.

Finally, the therapeutic significance of our results is that, if tobramycin or gentamicin dosage increases are based on serum concentrations determined by microbiological assay, inactivation due to incubation will yield a falsely low value, which could mislead and result in an increased dosage. Actual *in vivo* levels may rise into the toxic range as a result. The significance of these findings for patients with normal renal function deserves further investigation, and such studies must employ immediate RIA to differentiate *in vitro* from *in vivo* inactivation.

ACKNOWLEDGMENTS

This investigation was supported in part by Public Health Service grant 20852 from the National Institute of General Medical Sciences.

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