

Effect of Time and Concentration Upon Interaction Between Gentamicin, Tobramycin, Netilmicin, or Amikacin and Carbenicillin or Ticarcillin

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An aminoglycoside antibiotic and carbenicillin or ticarcillin are widely used in the treatment of patients with gram-negative bacillus infections. This study evaluated the effect of time upon in vitro interaction between mixtures of four aminoglycosides at two concentrations with carbenicillin or ticarcillin at four concentrations. By linear regression analysis, the inactivation of each aminoglycoside was shown to be directly proportional to the concentration of carbenicillin ($P < 0.001$). Inactivation was significantly ($P < 0.01$) greater for gentamicin and tobramycin than for amikacin or netilmicin at all carbenicillin concentrations. At carbenicillin concentrations of 300 and 600 $\mu\text{g}/\text{ml}$, significantly ($P < 0.005$) less inactivation of amikacin occurred when compared to netilmicin. Ticarcillin produced a significant ($P < 0.025$) inactivation of gentamicin and tobramycin, with inactivation being directly proportional to ticarcillin concentration. No inactivation of amikacin or netilmicin activity occurred unless the ticarcillin concentration was 600 $\mu\text{g}/\text{ml}$. No significant change in aminoglycoside activity occurred when stored with ticarcillin or carbenicillin at concentrations ranging from 100 to 600 $\mu\text{g}/\text{ml}$ at -70°C for 56 days. When an aminoglycoside and carbenicillin or ticarcillin are indicated in patients with renal failure, this study supports the use of ticarcillin with either amikacin or netilmicin.

Aminoglycoside antibiotics in combination with carbenicillin or ticarcillin frequently are used for treatment of patients with infections due to gram-negative bacilli (3, 8, 12; J. L. Hoecker, L. K. Pickering, D. Groschel, S. Kohl, and J. van Eys, *Cancer*, in press). The combination of carbenicillin or ticarcillin with an aminoglycoside provides a broad spectrum of activity against gram-negative bacilli and has been shown to be synergistic against *Pseudomonas aeruginosa* and various *Enterobacteriaceae* (1, 2, 6, 11, 13, 17). Conversely, inactivation of aminoglycoside antibiotics has been shown to be caused by either ticarcillin or carbenicillin (7, 10, 15, 16, 18, 21). McLaughlin and Reeves (16) demonstrated in vitro inactivation of gentamicin by carbenicillin and suggested that some antagonistic effect might occur in vivo by a combination that had been regarded as synergistic. Riff and Jackson (21) studied the half-life of gentamicin in four patients receiving hemodialysis and found that when carbenicillin and gentamicin were administered concomitantly in a dosage ratio of 80:1, the half-life of gentamicin was markedly reduced. Davies and associates (7) evaluated eight patients with renal failure and

found that a 25 to 74% reduction in the half-life of gentamicin resulted from the concomitant administration of therapeutic doses of carbenicillin and ticarcillin. Holt et al. (10) showed that carbenicillin and ticarcillin inactivated gentamicin, tobramycin, sisomicin, and amikacin at a concentration of 50:1 and that this inactivation was least in pooled human sera and greatest in phosphate buffer at pH 7.4. This study was conducted to determine the effect of time and concentration upon the inactivation of four aminoglycoside antibiotics when exposed to various concentrations of ticarcillin or carbenicillin.

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MATERIALS AND METHODS

Amikacin base (902 $\mu\text{g}/\text{mg}$; Bristol Laboratories, Syracuse, N.Y.), gentamicin sulfate (586 $\mu\text{g}/\text{mg}$), and netilmicin sulfate (583 $\mu\text{g}/\text{mg}$; Schering Corporation, Bloomfield, N.J.), tobramycin sulfate (968 $\mu\text{g}/\text{mg}$; Eli Lilly & Co., Indianapolis, Ind.), disodium ticarcillin (870 $\mu\text{g}/\text{mg}$; Beecham Laboratories, Bristol, Tenn.), and disodium carbenicillin (Roerig, New York, N.Y.)

were dissolved in pooled human sera obtained from three donors to achieve final concentrations of 5 and 10 $\mu\text{g/ml}$ for gentamicin, netilmicin, and tobramycin, and 10 and 20 $\mu\text{g/ml}$ for amikacin. At the same time, these concentrations of each aminoglycoside antibiotic were mixed separately with 100, 200, 300, and 600 $\mu\text{g/ml}$ concentrations of ticarcillin or carbenicillin in pooled human sera. All samples were adjusted to a pH of 7.4.

Immediately after mixing, a portion of each specimen was assayed for specific aminoglycoside activity. Each mixture then was divided and subjected to the following: (i) incubation at 37°C, with further portions being assayed for aminoglycoside activity at 1 and 3 days; (ii) storage at -70°C, with portions being thawed and assayed for residual aminoglycoside activity at 1, 3, 7, and 56 days. Results of residual aminoglycoside activity for each sample are expressed as percent activity when compared to activity at zero time. All samples were assayed in duplicate.

Concentrations of gentamicin and tobramycin were determined by radioenzymatic assay, using an adenyltransferase enzyme (4, 22). Netilmicin and amikacin were determined by radioenzymatic assay, using an acetyltransferase enzyme (5). Each assay has a standard curve linear from 1 to 100 $\mu\text{g/ml}$ and a within- and between-assay precision of 6%. Samples containing tobramycin and netilmicin also were assayed for activity by using microbiological and radioimmune assays as previously reported by us (4, 5). *Bacillus globogii* and *Klebsiella pneumoniae* were used as the test organisms for tobramycin and netilmicin, respectively. Results were compared and correlated with the radioenzymatic assay results.

Statistical analysis was performed by using a paired *t* test within and between groups and linear regression analysis to determine the effect of carbenicillin concentrations (Fig. 1). Values expressed represent mean ± 1 standard error of the mean of three to four separate experiments.

RESULTS

Incubation at 37°C. The percent activity of the various aminoglycoside antibiotics after incubation with carbenicillin at 37°C at concentrations ranging from 100 to 600 $\mu\text{g/ml}$ for periods of 1 to 3 days are shown in Table 1. The activity of gentamicin, tobramycin, netilmicin, and amikacin showed no significant decrease with time when carbenicillin was not present. When either 5 or 10 μg of gentamicin per ml was incubated with 100 μg of carbenicillin per ml, a significant inactivation of gentamicin occurred after 24 h ($P < 0.02$) and 72 h ($P < 0.005$). As the concentration of carbenicillin increased, the inactivation of gentamicin became more significant, with the greatest inactivation occurring at carbenicillin concentrations of 600 $\mu\text{g/ml}$. Tobramycin and carbenicillin interaction results were similar to those of gentamicin and carbenicillin, with tobramycin inactivation being directly proportional to carbenicillin concentration ($P <$

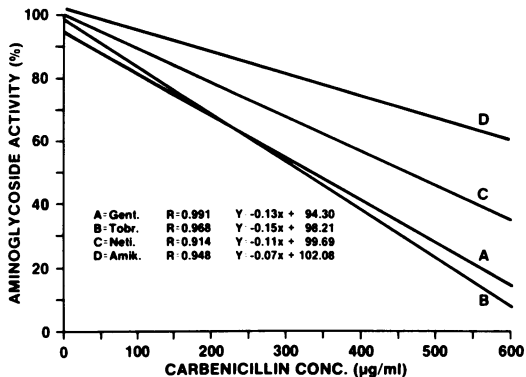


FIG. 1. Linear regression analysis of gentamicin (Gent.), tobramycin (Tobr.), netilmicin (Neti.), and amikacin (Amik.) activity after exposure to various concentrations of carbenicillin at 37°C for 72 h ($n = 16$ for each aminoglycoside).

0.001). A similar inactivation of tobramycin occurred when 5 and 10 μg of tobramycin per ml were incubated with 100 μg of carbenicillin per ml (1:20 and 1:10, respectively) or when 5 and 10 μg of tobramycin per ml were exposed to 300 μg of carbenicillin per ml (1:60 and 1:30, respectively). No change in netilmicin activity occurred when exposed to 100 μg of carbenicillin per ml; however, as the concentration of carbenicillin increased, inactivation occurred, most noticeably after 72 h of incubation. This inactivation was significantly ($P < 0.01$) less than seen in gentamicin or tobramycin at all carbenicillin concentrations studied. A similar degree of inactivation occurred when 5 and 10 μg of netilmicin per ml were incubated with 300 μg of carbenicillin per ml (1:60 and 1:30, respectively). Decreases in amikacin activity were significantly ($P < 0.001$) less at all times and concentrations tested than changes that occurred in gentamicin and tobramycin activity. At clinically relevant concentrations, amikacin inactivation was not different than netilmicin inactivation at carbenicillin concentrations of 100 and 200 $\mu\text{g/ml}$; however, at carbenicillin concentrations of 300 and 600 $\mu\text{g/ml}$, significantly ($P < 0.005$) less inactivation of amikacin occurred when compared to netilmicin.

Figure 1 depicts linear regression analysis of gentamicin, tobramycin, netilmicin, and amikacin activity when exposed to various concentrations of carbenicillin at 37°C for 72 h. There was a direct relationship between the concentration of carbenicillin and the inactivation of each aminoglycoside. The higher the concentration of carbenicillin in the reaction mixture, the greater the inactivation of gentamicin, tobramycin, netilmicin, and amikacin ($P < 0.001$).

TABLE 1. Aminoglycoside-carbenicillin interaction after a 24- and 72-h mixture at 37°C

Aminoglycoside	Concn ($\mu\text{g/ml}$)	Activity at the following carbenicillin concn ($\mu\text{g/ml}$): ^a												
		24 h						72 h						
		0	100	200	300	600	0	100	200	300	600			
Gentamicin	5	100 ± 1.0	91 ± 1.2	80 ± 2.1	67 ± 1.8	41 ± 1.9	97 ± 2.1	81 ± 2.0	68 ± 1.9	49 ± 1.7	17 ± 4.3			
	10	99 ± 1.0	88 ± 1.1	ND	64 ± 2.1	ND	98 ± 2.1	80 ± 1.4	ND	46 ± 1.9	ND			
Tobramycin	5	99 ± 1.1	97 ± 2.1	84 ± 1.7	63 ± 1.9	33 ± 3.0	99 ± 2.1	88 ± 2.0	72 ± 2.2	39 ± 2.3	13 ± 4.2			
	10	98 ± 1.0	93 ± 2.3	ND	68 ± 2.4	ND	98 ± 2.2	88 ± 2.3	ND	49 ± 1.9	ND			
Netilmicin	5	100 ± 1.2	98 ± 1.3	93 ± 1.9	78 ± 2.4	74 ± 2.7	97 ± 1.9	95 ± 2.0	88 ± 3.3	52 ± 2.1	39 ± 3.1			
	10	99 ± 1.0	98 ± 1.1	ND	80 ± 2.1	ND	98 ± 2.1	96 ± 1.9	ND	68 ± 2.2	ND			
Amikacin	10	100 ± 1.3	99 ± 1.2	96 ± 1.8	89 ± 2.2	81 ± 3.0	100 ± 1.1	96 ± 1.7	93 ± 1.2	88 ± 2.6	60 ± 3.9			
	20	98 ± 1.0	97 ± 1.3	ND	90 ± 1.9	ND	99 ± 2.1	97 ± 1.3	ND	89 ± 2.1	ND			

^a Values represent percent aminoglycoside activity, expressed as mean ± standard error of the mean. ND, Not done

Table 2 shows the percent activity of gentamicin, tobramycin, netilmicin, and amikacin when incubated with ticarcillin at concentrations of 100 to 600 $\mu\text{g/ml}$ for 72 h at 37°C. No significant change in aminoglycoside activity occurred when ticarcillin was not present. Ticarcillin concentrations of 200, 300, and 600 $\mu\text{g/ml}$ produced significant ($P < 0.025$) inactivation of gentamicin and tobramycin, with inactivation being directly proportional to the ticarcillin concentration. The activity of netilmicin (5 and 10 $\mu\text{g/ml}$) and amikacin (10 and 20 $\mu\text{g/ml}$) was constant at ticarcillin concentrations of 100, 200, and 300 $\mu\text{g/ml}$. At a ticarcillin concentration of 600 $\mu\text{g/ml}$, netilmicin and amikacin maintained $83 \pm 2.4\%$ and $90 \pm 2.6\%$ activity, respectively. These activities were significantly ($P < 0.001$) greater than remaining activity of gentamicin and tobramycin at the same concentration.

Table 3 shows percent aminoglycoside activity after mixing with 300 or 600 μg of carbenicillin or ticarcillin per ml and storage at -70°C for 8 weeks. Amikacin and netilmicin underwent no significant change when stored with either carbenicillin or ticarcillin at either concentration. Gentamicin and tobramycin underwent a slight decrease in activity when stored with carbenicillin or ticarcillin at 300 or 600 $\mu\text{g/ml}$ concentrations; however, these changes were not significant. No changes were noted when carbenicillin or ticarcillin concentrations of 200 μg or less per ml were used.

Comparison of the tobramycin radioenzymatic assay with the microbiological assay and the radioimmune assay gave correlation coefficients of 0.97 and 0.95, respectively ($n = 24$). Comparison of the netilmicin radioenzymatic assay with the microbiological assay and the radioimmune assay yielded correlation coefficients of 0.82 and 0.90, respectively ($n = 22$).

DISCUSSION

Patients with proven or suspected infections due to *Pseudomonas aeruginosa* often receive carbenicillin or ticarcillin in addition to an aminoglycoside antibiotic (3, 8, 12; Hoecker et al., in press). The combination of one of these penicillin derivatives with an aminoglycoside has been shown to be synergistic against *P. aeruginosa*. Conversely, inactivation of aminoglycosides such as gentamicin occurs in the presence of carbenicillin (7, 16).

In patients with normal renal function, studies have shown no apparent inactivation of gentamicin by carbenicillin (9, 21, 23), provided the antibiotics were administered separately and not mixed in the same bottle (7, 15, 18). In most patients, these drugs are excreted at a rate faster

TABLE 2. Aminoglycoside-ticarcillin interaction after a 72-h mixture at 37°C

Aminoglycoside	Concn ($\mu\text{g/ml}$)	Activity at the following ticarcillin concn ($\mu\text{g/ml}$): ^a				
		0	100	200	300	600
Gentamicin	5	100 \pm 1.0	100 \pm 0.5	90 \pm 2.9	80 \pm 2.4	30 \pm 2.1
	10	98 \pm 2.1	95 \pm 1.5	ND	81 \pm 3.6	ND
Tobramycin	5	98 \pm 2.1	89 \pm 3.0	85 \pm 1.8	74 \pm 3.1	25 \pm 4.0
	10	100 \pm 1.1	89 \pm 1.5	ND	76 \pm 2.3	ND
Netilmicin	5	100 \pm 1.6	100 \pm 0.5	96 \pm 1.9	94 \pm 1.0	83 \pm 2.4
	10	99 \pm 1.7	97 \pm 1.5	ND	96 \pm 1.9	ND
Amikacin	10	100 \pm 2.1	98 \pm 1.3	98 \pm 2.0	97 \pm 1.1	90 \pm 2.6
	20	99 \pm 1.6	100 \pm 2.0	ND	98 \pm 2.0	ND

^a Values represent percent aminoglycoside activity expressed as mean \pm standard error of the mean. ND, Not done.

TABLE 3. Aminoglycoside activity after mixture with carbenicillin or ticarcillin at -70°C for 8 weeks

Aminoglycoside	Activity with: ^a				
	Carbenicillin ($\mu\text{g/ml}$)			Ticarcillin ($\mu\text{g/ml}$)	
	0	300	600	300	600
Amikacin	98 \pm 2.8	96 \pm 2.4	96 \pm 1.4	100 \pm 1.2	100 \pm 0.8
Netilmicin	98 \pm 1.9	100 \pm 2.1	100 \pm 0.8	100 \pm 2.1	100 \pm 1.8
Gentamicin	97 \pm 1.6	92 \pm 2.7	90 \pm 2.7	90 \pm 2.3	89 \pm 3.4
Tobramycin	98 \pm 1.4	94 \pm 3.1	89 \pm 2.8	86 \pm 2.9	87 \pm 3.1

^a Values represent percent aminoglycoside activity expressed as mean \pm standard error of the mean.

than the interaction between them. Presumably, the effect of the aminoglycoside on bacteria takes place before any significant loss of activity due to inactivation. However, in patients with impaired renal function, an accumulation of these antibiotics will occur if the dosage schedule is not modified. Appropriate use of aminoglycoside antibiotics in patients with renal failure is often achieved by determining concentrations in serum; however, carbenicillin and ticarcillin concentrations in these patients are rarely monitored, since the therapeutic-to-toxic ratio is wide. If the dosage schedule of ticarcillin or carbenicillin is not modified (19) in patients with renal failure, serum concentrations could be well above the therapeutic range for extended periods of time. This could result in a significant inactivation of the concurrently administered aminoglycoside antibiotic. McLaughlin and Reeves (16) observed gentamicin inactivation *in vivo* in a patient who received a daily 20-g dose of carbenicillin by continuous infusion over 24 h, which provided a carbenicillin concentration of 1,000 $\mu\text{g/ml}$ in blood.

Inactivation of each of the aminoglycoside antibiotics in our study was shown to occur by using three different assay procedures. Comparison of the results of these assays showed a significant correlation. One of the methods was a microbiological assay indicating that any in-

activation products which were formed were not microbiologically active; however, the potential renal and ototoxicity of these products is unknown. Further work needs to be undertaken to define these products and to determine whether they are capable of producing side effects.

The finding of inactivation of these aminoglycosides in combination with carbenicillin and ticarcillin at 37°C indicates that these specimens should be centrifuged and frozen immediately to ensure accurate determination when performed. If the sample to be tested does not contain a penicillin derivative in addition to the aminoglycoside, then transfer or storage of the sterile sample at room temperature will not cause or result in any decrease in aminoglycoside activity (20). However, if a penicillic derivative such as carbenicillin or ticarcillin is present, then inactivation may occur (4), and the specimen should be frozen for transport. Some antibiotics such as chloramphenicol undergo no change in activity when incubated and stored at room temperature with numerous other antibiotics, including penicillin derivatives (20a).

The results of our *in vitro* studies show that ticarcillin caused significantly less inactivation of all aminoglycosides, particularly amikacin and netilmicin, than did carbenicillin. When an aminoglycoside and either carbenicillin or ticarcillin are indicated in a patient with renal failure,

either amikacin or netilmicin and ticarcillin should be used.

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