BIOLOGICAL ASPECTS OF THE INTERACTION BETWEEN GENTAMICIN AND CARBENICILLIN

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(Received for publication November 22, 1971)

Carbenicillin is capable of inactivating gentamicin *in vitro*. This effect is time, temperature and medium dependent. *In vitro* antibacterial tests demonstrate greater than additive activity in some instances and inactivation in others, particularly after prolonged incubation. Inactivation was not observed *in vivo* in mouse protection tests. Additive or more than additive combined activity in mouse protection tests occurred only infrequently. Intravenous administration of carbenicillin had no effect on gentamicin serum levels (given i.m.) in dogs although it did result in reduced recovery of gentamicin in the urine. This may have occurred after the urine had left the bladder. It is important that serum samples containing both gentamicin and carbenicillin be assayed shortly after drawing since inactivation can occur in these samples which then might be falsely interpreted as *in vivo* inactivation.

A number of studies have suggested that gentamicin and carbenicillin *in vitro* may act synergistically. Clinical studies have also appeared reporting the combined use of the two agents particularly in cases of *Pseudomonas* infections.

An article by MCLAUGHLIN and REEVES[1] and comments[2~5] and reply[6] following it demonstrate that combining carbenicillin with gentamicin, at least in some *in vitro* situations, results in inactivation of gentamicin the rate of which is time, temperature and media depedent.

This report describes our observations related to biological aspects of the interaction of gentamicin and carbenicillin. Physico-chemical studies of this interaction suggest that the reaction involves nucleophilic opening of the β-lactam ring of the penicillin by an amino group of the gentamicin with formation of a biologically inactive amide. Similar inactivation occurs with other aminoglycosides and with other penicillins. Details of these chemical studies will be published elsewhere[7].

Materials and Methods

Gentamicin was used in the form of the sulfate; however all data are expressed in terms of the base. The carbenicillin used was obtained commercially (Roerig lot 09332, and Beecham lot A 15060 G). Antibiotic assays for gentamicin were performed according to the procedures of ODEN *et al*[8]. When gentamicin and carbenicillin were present together, penicillinase treatment preceded gentamicin assays. Assays using a carbenicillin-resistant strain of *Klebsiella pneumoniae* confirmed that penicillinase treatment was not releasing gentamicin from an inactive complex. Carbenicillin was assayed according to methods described for penicillin[9] using *Staphylococcus aureus* ATCC 6538 P. The combi-

nation of low pH and sample dilution in this assay assured that the usual levels of gentamicin did not interfere.

In vitro sensitivity and joint activity tests were done in MUELLER-HINTON broth (B.B.L.) using a volume of 3 ml and 0.05 ml of a 1 : 1,000 dilution of an overnight broth culture as an inoculum (10^4~10^5 organisms). Incubation was at 37°C and end points were determined visually after 24 and 48 hours. The calculation of fractional MIC's to determine joint action is described below. In all animal tests, both gentamicin and carbenicillin were given as solutions in sterile distilled water. Mouse protection tests were performed as described previously[10,11] using 20 g male CF-1 mice in groups of 7~10 each. Mice were treated subcutaneously 1 hour after intraperitoneal injection of organisms. Separate injection sites were used for the two antibiotics. Survivors were determined 48 hours after infection and PD_{50} values were calculated by probit procedures.

For absorption tests in dogs, beagle type mongrels of both sexes weighing approximately 10 kg each were utilized. Gentamicin was administered intramuscularly while carbenicillin was given intravenously as a bolus.

Results and Discussion

In Vitro Stability Studies

Solutions of gentamicin, carbenicillin and penicillin G alone and in combination were prepared in distilled water, in pH 7.0 phosphate buffer and in pH 7.0 phosphate-citrate buffer. These were incubated at 20°C, 35°C and 56°C for 24, 48 and 72 hours. Initial samples and aliquots at each time period were assayed for gentamicin after penicillinase treatment to destroy penicillin G and carbenicillin activity. The results of this study are shown in Table 1. Gentamicin alone was stable in each of the buffers and in distilled water at all temperatures for all time periods. Carbenicillin inactivated

Table 1. Effect of temperature on activities of solutions of gentamicin and carbenicillin or gentamicin and penicillin in various diluents

Antibiotic(s)	Incubation time (hrs)	Gentamicin potency* (mcg/ml)								
		Distilled water			Phosphate buffer pH 7.0			Phosphate-citrate buffer pH 7.0		
		20°C	35°C	56°C	20°C	35°C	56°C	20°C	35°C	56°C
Gentamicin, 5 mcg/ml (control)	0	4.6			4.8			4.1		
	24	4.1	4.3	4.4	4.2	4.3	4.4	4.1	4.2	3.9
	48	4.8	4.2	4.2	4.2	4.0	4.3	4.3	4.4	4.2
	72	4.7	4.6	4.9	4.1	4.2	3.9	4.1	4.0	4.2
Gentamicin, 5 mcg/ml+ carbenicillin, 100 mcg/ml	0	4.3			4.6			3.8		
	24	2.2	1.2	0	4.1	1.8	0.6	3.9	3.6	2.8
	48	1.6	0.4	0	3.6	2.1	0	3.8	3.4	2.9
	72	1.4	0	0	3.4	2.0	0	3.7	3.4	2.7
Gentamicin, 5 mcg/ml+ penicillin G, 100 mcg/ml	0	4.4			4.3			3.8		
	24	4.4	3.2	2.4	3.7	3.9	0.8	4.3	4.1	3.4
	48	4.2	3.3	2.4	4.6	3.2	0	3.9	3.6	3.3
	72	4.5	2.9	1.9	4.4	2.9	0	4.0	3.8	3.3
Carbenicillin, 100 mcg/ml (penicillinase control)	0	0			0			0		
	24	0	0	0	0	0	0	0	0	0
	48	0	0	0	0	0	0	0	0	0
	72	0	0	0	0	0	0	0	0	0
Penicillin G, 100 mcg/ml (penicillinase control)	0	0			0			0		
	24	0	0	0	0	0	0	0	0	0
	48	0	0	0	0	0	0	0	0	0
	72	0	0	0	0	0	0	0	0	0

* In all assays, carbenicillin or penicillin G activity destroyed by treatment with penicillinase prior to assay.

gentamicin to a varying degree in each system. The inactivation was greater at higher temperatures and increased with increased incubation time. Inactivation was less in phosphate buffer and phosphate-citrate buffer than in distilled water. Penicillin G also inactivated gentamicin although to a lesser degree than observed for carbenicillin in this system. The ability of phosphate-citrate buffer to decrease the inactivation rate of gentamicin by both carbenicillin and penicillin G was confirmed in additional studies (Table 2).

Table 2. Stability of carbenicillin and penicillin G in presence of gentamicin at 37°C in McIlvaines pH 7 phosphate-citrate buffer

Antibiotic	Quantity per ml	Assay*			
		0 hour	24 hours	48 hours	72 hours
Gentamicin	5 mcg	3.6	3.4	3.3	3.3
Carbenicillin	200 mcg	178	153	111	95
Penicillin G	300 units	354	252	182	145
Gentamicin	5 mcg	3.2	2.7	2.7	2.7
+Carbenicillin	+200 mcg	177	148	109	89
Gentamicin	5 mcg	3.4	3.1	3.0	2.8
+Penicillin G	+300 units	260	267	177	146

* mcg or units per ml

The suggestion that the inactivation was to some extent media dependent, was examined in serum and in Mueller-Hinton broth. In this test, early inactivation such as might be anticipated *in vivo* was of interest. As shown in Table 3, the degree of inactivation of gentamicin by carbenicillin was dependent on the relative concentration of each component and was reduced in serum and Mueller-Hinton broth as compared with distilled water.

Table 3. Inactivation of gentamicin in serum and in Mueller-Hinton broth*

Media	Gentamicin mcg/ml	Carbenicillin mcg/ml	Percent activity remaining after 4 hours
Serum	2	—	100
	2	5	90
	2	10	66
	2	20	87
	2	40	80
	2	80	87
Serum	5	—	100
	5	5	63
	5	10	49
	5	20	51
	5	40	34
	5	80	36
Serum	10	—	100
	10	5	90
	10	10	90
	10	20	81
	10	40	61
	10	80	68
Mueller-Hinton broth	5	—	100
	5	5	96
	5	10	78
	5	20	89
	5	40	74
	5	80	74

* Incubation at 37°C.

In Vitro Antibacterial Tests

Since many investigators have reported *in vitro* synergism between gentamicin and carbenicillin, this was also examined. A checkerboard design was used with eight levels of gentamicin and four levels of carbenicillin as well as gentamicin and carbenicillin alone resulting in a 9×5 block design. Of 98 clinical isolates of *Pseudomonas aeruginosa*, 30 were found with carbenicillin MIC values of 50 mcg/ml or less. These included gentamicin-sensitive and gentamicin-resistant organisms. For each of these strains, the MIC of each antibiotic alone as well as the lowest sum of the fractional MIC's was determined. The latter was calculated as follows:

If in combination, the strain was inhibited by $^1/_2$ the MIC of each antibiotic necessary to inhibit alone, a value of 1.0 ($^1/_2$+$^1/_2$) was assigned. A value of 0.5 would suggest that the organism was inhibited by the antibiotics together at levels $^1/_4$ the

MIC of each antibiotic alone. A value of 1.0 thus represents additive activity, a value greater than 1.0 represents less than additive activity and a value of 0.5 or less represents greater than additive activity.

Results of tests with 30 *Pseudomonas* are shown in Table 4. Of the 30 *Pseudomonas* strains, 14 were gentamicin-resistant and 16 were sensitive. Greater than additive activity was seen with 11 of the 30 strains, primarily amongst the gentamicin-resistant strains.

A peculiar response was observed in 29 of the 98 *Pseudomonas* strains but not in any of 33 *Escherichia coli* strains. This is illustrated in Table 5. The lower line of this table shows the effect of gentamicin alone against this strain which is considered resistant to gentamicin. After 24 hours incubation the organism was inhibited by 6.25 mcg/ml while after 48 hours it was inhibited by 25 mcg/ml. However, in all tubes containing carbenicillin, the 48-hour MIC's were higher than 75 mcg/ml. This is thought to represent inactivation of gentamicin by carbenicillin which apparently occurred after the 24-hour reading.

Table 4. *In vitro* activity of gentamicin and carbenicillin alone and together.
9×5 Checkerboard design in MUELLER-HINTON broth

Strains of *Pseudomonas aeruginosa*	MIC alone		Lowest sum of fractional MIC* together
	Gentamicin	Carbenicillin	
206	50.0	50.0	0.5
10010	3.12	25.0	>1.0
10006	6.25	50.0	0.5
9954	6.25	50.0	0.625
9928	6.25	25.0	>1.0
10502	6.25	25.0	0.75
10330	6.25	50.0	0.50
10268	6.25	50.0	0.75
10178	6.25	25.0	>1.0
10144	6.25	25.0	>1.0
10101-2	6.25	25.0	>1.0
10126	6.25	50.0	1.0
10009	6.25	50.0	0.5
10075	3.12	50.0	>1.0
85	3.12	50.0	0.625
99	50.0	50.0	0.625
130	50.0	50.0	0.5
9978	1.6	50.0	1.0
12	3.12	50.0	1.0
208	25.0	50.0	0.5
327	25.0	50.0	0.5
338	12.5	50.0	0.625
205	50.0	50.0	0.24
348	25.0	50.0	0.5
C-18	50.0	50.0	0.5
30	25.0	50.0	0.75
75	25.0	50.0	0.75
79	50.0	50.0	1.0
137	50.0	50.0	0.5
9978	25.0	50.0	1.0

* 1.0-together the antibiotics inhibit at $^1/_2$ the MIC value of each alone, additive activity; >1.0-less than additive activity; ≤ 0.5-synergism.

In Vivo Studies in Mice

In vivo mouse protection tests were conducted using infections with 15 different *Pseudomonas* strains representing gentamicin-resistant strains from several sources. A

Table 5. Activity of gentamicin and carbenicillin against *Pseudomonas aeruginosa* strain 10074 in MUELLER-HINTON broth (Growth after 24 and 48 hours)

Carbenicillin mcg/ml	Gentamicin mcg/ml																	
	75		50		25		12.5		6.25		3.12		1.6		0.8		0	
	24	48	24	48	24	48	24	48	24	48	24	48	24	48	24	48	24	48
50	−	*+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	+	+
25	−	+	−	+	−	+	−	+	−	+	−	+	−	+	+	+	+	+
12.5	−	+	−	+	−	+	−	+	−	+	−	+	+	+	+	+	+	+
6.25	−	+	−	+	−	+	−	+	−	+	−	+	+	+	+	+	+	+
0	−	−	−	−	−	−	−	+	−	+	+	+	+	+	+	+	+	+

+ =growth. − =no growth.

checkerboard design was set up using 5 levels of gentamicin and 6 levels of carbenicillin. Groups of 7 mice each were treated subcutaneously in separate sites with each drug 1 hour after i.p. infection. Survivors were determined 48 hours after infection and PD_{50} values were calculated by probit procedures. Thirteen of the 15 strains failed to show any evidence of antagonism, additive activity or more than additive activity. Two strains showed evidence of combined activity and are detailed in Table 6. With both of these strains 500 mg/kg of carbenicillin alone failed to protect any mice against death. These strains were also resistant to 50 mcg/ml of carbenicillin *in vitro*. However, gentamicin PD_{50} values decreased with both strains as carbenicillin levels were increased. With one of two strains the decrease was marginal (strain 836) while with the other (strain 37) it was more dramatic. These were the only two instances of enhanced combined activity *in vivo* in the 15 strains studied here and some 30 strains (sensitive and resistant) studied earlier using other techniques. No evidence for antagonism has been observed in this type of mouse protection test.

Table 6. *In vivo* activity of gentamicin and carbenicillin against two gentamicin-resistant *Pseudomonas* strains.

Each drug given separately, s.c., 1 hour after i.p. infection

	Carbenicillin level mg/kg	Gentamicin PD_{50} (mg/kg) (95 % C.L.)
Strain 37*	0	40 (28~59)
	15.6	49 (34~79)
	31.0	35 (21~79)
	62.0	38 (14~82)
	125.0	14 (7~25)
	250.0	14 (9~21)
	500.0	8 (1~19)
Strain 836*	0	32 (24~44)
	15.6	23 (13~43)
	31.0	30 (20~46)
	62.0	18 (10~34)
	125.0	17 (11~24)
	250.0	6 (1~13)
	500.0	14 (8~24)

* No mice infected with either strain were protected by 500 mg/kg of carbenicillin alone.

Absorption Tests in Dogs

A final study investigated serum and urine levels in dogs dosed with gentamicin i.m. (100 mg=10 mg/kg) and carbenicillin i.v. (500 or 2,000 mg=50 or 200 mg/kg). Assays of both antibiotics were done and the results are shown in Table 7. Gentamicin at 10 mg/kg i.m. produced average peak serum levels of 15.5 mcg/ml 1 hour after dosing. These declined to approximately 1.0 mcg/ml 6 hours after dosing. An average of 60% of the dose was excreted in the urine the first 24 hours after dosing with an additional 3 % excreted in the 25~48-hour period. Carbenicillin i.v. at a dose of 50 mg/kg produced peak levels of 34.5 mcg/ml which declined to 1.1 mcg/ml 6 hours after dosing. Approximately 40 % of the carbenicillin dose was recovered in the urine.

Carbenicillin (50 mg/kg) given i.v. 1 hour before, at the same time, or 1 hour after the 10 mg/kg gentamicin dose had no effect on gentamicin serum levels. Carbenicillin i.v. at 200 mg/kg given at the same time as 10 mg/kg of gentamicin likewise had no effect on gentamicin serum levels. Urine recovery of gentamicin however was significantly lower in all dogs treated with carbenicillin. This was most noticeable with the high carbenicillin level and when carbenicillin was given before the gentamicin. It is not known if this represents inactivation in the bladder or in the collection bottle prior to being collected for assay.

Serum samples from dogs showed a decrease in gentamicin levels when held at room temperature for more than a day prior to assay. It is important therefore that

Table 7. Serum and urine levels of gentamicin and carbenicillin in dogs

Treatment	Dog No.	Assay for*	Serum levels (mcg/ml) after dosing (hours)						Urine levels (mg excreted)	
			0	1	2	4	6	24	1~24 hours	25~48 hours
Gentamicin 100 mg i.m.	1	G	0	18.0	15.0	2.8	0.7	0	46.5	1.8
	2	G	0	15.0	11.5	4.1	0.9	0	64.5	2.2
	3	G	0	16.0	10.6	4.1	1.3	0	77.5	2.0
	4	G	0	14.5	13.2	2.4	1.3	0	68.2	1.7
	290	G	0	21.2	11.0	3.7	0.9	0	69.9	2.8
	J 3	G	0	16.0	13.0	2.0	0.6	0	52.5	2.4
	J 17	G	0	19.0	15.0	3.1	0.7	0	54.0	4.1
	K86	G	0	14.5	12.0	2.5	1.0	0	59.4	2.5
	J 17	G	0	9.3	9.3	2.7	1.2	0	49.8	5.4
	K86	G	0	12.0	10.5	3.1	0.7	0	56.0	2.1
	Average	G	0	15.5	12.1	3.0	0.9	0	59.9	2.7
Carbenicillin 500 mg i.v.	90	C	0	34.5	17.2	2.4	1.1	0	202.0	0.2
Carbenicillin 500 mg i.v.	290									
Gentamicin 100 mg i.m. 1 hour later		G	0	17.0	10.1	4.7	1.1	0	4.3	1.3
		C	0	28.0	15.0	2.2	1.2	0	0.8	0.3
	90	G	0	18.5	12.0	3.2	0.7	0	5.4	0.6
		C	0	26.0	10.5	1.6	0.9	0	3.9	0.3
	Average	G	0	17.2	11.0	3.9	0.9	0	4.8	1.0
		C	0	27.0	12.7	1.9	1.0	0	2.4	0.3
Gentamicin 100 mg i.m.	1	G	0	14.0	14.0	2.2	0.6	0	16.1	0.9
Carbenicillin 500 mg i.v. at same time		C	0	32.0	9.8	2.1	0.9	0	118.0	0.3
	2	G	0	11.5	6.5	2.4	0.4	0	24.4	0.7
		C	0	32.0	13.0	2.7	0.9	0	16.7	0.2
	Average	G	0	12.7	10.2	2.3	0.5	0	20.2	0.8
		C	0	32.0	11.4	2.4	0.9	0	67.4	0.2
Gentamicin 100 mg i.m.	3	G	0	15.0	9.0	1.9	0.4	0	24.2	1.3
Carbenicillin 500 mg i.v. 1 hour later		C	0	—	26.0	3.1	0.9	0	17.2	0.4
	4	G	0	13.0	7.5	2.3	0.4	0	20.9	1.6
		C	0	—	31.0	2.9	1.0	0	56.0	0.2
	Average	G	0	14.0	8.3	2.1	0.4	0	22.6	1.4
		C	0	—	26.5	3.0	0.9	0	36.6	0.3
Gentamicin 100 mg i.m.	63	G	0	14.0	6.5	1.8	0.4	0	3.3	1.6
Carbenicillin 2,000 mg i.v. at same time		C	0	100.0	60.0	8.5	5.7	0	1326.0	14.3
	25	G	0	12.3	7.8	1.4	0.2	0	3.7	0.8
		C	0	204.0	42.0	9.2	2.7	0	74.9	20.8
	Average	G	0	12.1	7.1	1.6	0.3	0	3.5	1.2
		C	0	152.0	51.0	8.8	4.2	0	700.0	17.0

G=gentamicin. C=carbenicillin.

assays be done shortly after drawing the sample and that serum samples be frozen until assayed.

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