1-N HAPA GENTAMICIN B, A NEW AMINOGLYCOSIDE ACTIVE AGAINST GENTAMICIN RESISTANT ISOLATES—ACTIVITY COMPARED TO OTHER AMINOGLYCOSIDES

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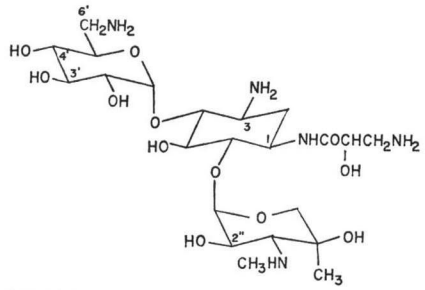
1-N HAPA gentamicin B is a new aminoglycoside active against most Enterobacteriaceae, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Among 504 clinical isolates at a concentration of 12.5 μg/ml all *Staph. aureus, Escherichia coli, Klebsiella, Enterobacter, Proteus rettgeri, Providencia* and 78% of *Pseudomonas*, 86% of *Proteus morganii* were inhibited. Like other aminoglycosides, the activity was greatest at an alkaline pH and reduced by high cations concentrations. 1-N HAPA gentamicin B was equal in activity to amikacin against both gentamicin-sensitive and resistant isolates. It inhibited bacteria containing many of the aminoglycoside inactivating enzymes. When combined with carbenicillin it inhibited in a synergistic manner many Gram-negative bacteria, particularly *Pseudomonas* and *Serratia*.

The resistance of Gram-negative bacteria to many of the currently available antibiotics has continued to increase[5,8,10]. Gentamicin resistance of both the members of the Enterobacteriaceae and of *Pseudomonas aeruginosa* is significant in some hospital centers. Tobramycin inhibits some *Pseudomonas* resistant to gentamicin, but it is inactivated by most of the enzymes in the Enterobacteriaceae which inactivate gentamicin. Sisomicin also has not extended the antibacterial spectrum of gentamicin[6]. Amikacin has proved to be resistant to many of the aminoglycoside inactivating enzymes[7], but resistant isolates have appeared[5,8]. Furthermore, concern over the nephrotoxicity and ototoxicity of agents in this class[1,9] makes it important to evaluate new agents which might possess increased antibacterial activity while having lower toxicity.

Fig. 1. Structure of 1-N HAPA gentamicin B.



1-N-[S-3-Amino-2-hydroxypropionyl]-gentamicin B

The purpose of this study was to evaluate the *in vitro* activity of 1-N HAPA gentamicin B (Fig. 1) and to compare its activity with the activity of amikacin, gentamicin, netilmicin and tobramycin against recent clinical isolates as well as against bacteria with known aminoglycoside inactivating enzymes.

Materials and Methods

Antibiotics

1-N HAPA gentamicin B, gentamicin complex, netilmicin and sisomicin were gifts from Schering

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Corporation. Tobramycin was given by Eli Lilly and Company, and amikacin by Bristol Laboratories. Carbenicillin was obtained from Beecham Laboratories. Fresh dilutions of all antibiotics were prepared daily in sterile medium or distilled water. Bacterial isolates were obtained from the Diagnostic Laboratory of the Columbia Presbyterian Medical Center, N.Y.C. The isolates were from blood, sputum and urine specimens and represented individual strains insofar as could be determined by general antibiograms and epidemiologic survey.

Susceptibility Tests

The antimicrobial activity was measured primarily by the agar dilution method. Serial two-fold dilutions of antibiotic in MUELLER-HINTON agar (BBL) were used. An inoculum of 10^5 colony forming units (CFU) was applied with a replicating device. Standard strains were included in every run. The minimum inhibitory concentration (MIC) was the lowest concentration of antibiotic on which there was not visible growth or less than five colonies. Medium, inoculum effects and bactericidal levels were obtained by use of broth dilution. The standard inoculum size was 10^5 CFU. The MIC level was the lowest concentration that inhibited development of visible turbidity. The minimum bactericidal concentration (MBC) was determined by plating 0.01 ml from clear tubes to agar. The MBC was the concentration at which less than five colonies grew.

Synergy Studies

Synergy studies were performed with the antibiotics combined in equal concentrations or at ratios similar to those that would be obtained after intravenous infusion. Synergy was defined as a four-fold reduction in MIC of both agents. Partial synergy is defined as a four-fold reduction in MIC of one agent and a two-fold or no reduction in MIC of the other agent. The details of the procedure are published[8].

Results

The *in vitro* activity of 1-N HAPA gentamicin B against 504 Gram-positive and Gram-negative organisms is summarized in Table 1. 1-N HAPA gentamicin B inhibited only 29% of *Staph. aureus* at a concentration of 1.6 μg/ml, but at levels of 12.5 μg/ml which could easily be achieved in man (personal communication, Schering Corp.) it inhibited 100% of the *Staph. aureus* which included methicillin and kanamycin resistant isolates. The activity of the compound against both enterococci, *Streptococcus faecalis* and β-hemolytic streptococci, *Strep. pyogenes* and *Strep. aglactiae* was poor, with only 12% of β-hemolytic streptococci and 32% of enterococci inhibited by 25 μg/ml. The compound had excellent activity against most hospital pathogenic members of the Enterobacteriaceae inhibiting 97% of *E. coli*, 97% of *Klebsiella pneumoniae*, 97% of *Enterobacter* and 88% of *Citrobacter* at a level of 1.6 μg/ml. It is interesting that the activity of the compound against *Proteus mirabilis* was much less, although 91% were inhibited at 12.5 μg/ml. Indeed, 1-N HAPA gentamicin B was more active against indole-positive *Proteus* and *Providencia* inhibiting 100% of *Providencia stuartii* and *Proteus vulgaris* and 93% of *P. rettgeri* and 88% of *P. morganii* at 6.3 μg/ml. The activity of the compound against *Pseudomonas* was only fair with only 20% inhibited at 1.6 μg/ml and 78% at 12.5 μg/ml. The activity against *Acinetobacter*, which is becoming more important as a respiratory pathogen, was similar to the activity against the Enterobacteriaceae with 88% inhibited by 3.1 μg/ml. Similar to all aminoglycosides, 1-N HAPA gentamicin B had no activity against *Bacteroides*.

The effect of the growth medium used to determine the MIC and MBC is given in Table 2 for selected representative strains. The lowest MIC and MBC values were obtained in nutrient broth which has the lowest concentration of both monovalent and divalent cations. MUELLER-HINTON broth yielded values which were intermediate between those found in nutrient broth and those found in brain heart infusion broth. *Proteus mirabilis, Serratia marcescens* and some *Pseudomonas aeruginosa*

Table 1. Activity of 1-N HAPA gentamicin B against Gram-negative and Gram-positive microorganisms

Organism	No. of isolates	Susceptible isolates (%) Minimum inhibitory concentration (μg/ml)									
		0.1	0.2	0.4	0.8	1.6	3.1	6.3	12.5	25	≥50
Staphylococcus aureus	30				6	29	88	97	100		
Streptococcus faecalis	32					3		9	15	32	100
β-Hemolytic streptococci	32						3	6		12	100
Escherichia coli	34		3	32	82	97		100			
Shigella	23				4	20	78	96	100		
Klebsiella	32			75	94	97	100				
Enterobacter	37		3	8	89	97			100		
Serratia	37				3	22	38	59	97		100
Salmonella	22			3	96	100					
Citrobacter	24	4	33	67	88		96				100
Proteus mirabilis	31				3	12	35	48	81	100	
Proteus rettgeri	15				47	67	73	93	100		
Proteus vulgaris	6				33	50	67	100			
Proteus morganii	26				15	62	85	88	96	100	
Providencia	29			3	21	83	93	100			
Pseudomonas aeruginosa	46				5	20	39	61	78	85	100
Acinetobacter	16				13	69	88				100
Bacteroides fragilis	32										100

Table 2. Effect of growth medium on the activity of 1-N HAPA gentamicin B

Organism	MIC (MBC) μg/ml		
	Nutrient broth	MUELLER-HINTON broth	Brain heart infusion
Escherichia coli 3939	0.8 (1.6)	6.3 (12.5)	6.3 (25)
Klebsiella 3929	0.8 (0.8)	0.8 (1.6)	1.6 (3.1)
Proteus rettgeri 3919	3.1 (3.1)	6.3 (6.3)	12.5 (25)
Proteus mirabilis 3378	0.4 (1.6)	12.5 (25)	≥50 (≥50)
Serratia marcescens 3915	3.1 (≥50)	6.3 (≥50)	25 (≥50)
Pseudomonas aeruginosa 3696	0.8 (3.1)	3.1 (3.1)	≥50 (≥50)
Pseudomonas aeruginosa 3948	1.6 (6.3)	0.8 (6.3)	1.6 (6.3)
Pseudomonas aeruginosa 3950	3.1 (12.5)	1.6 (3.1)	3.1 (≥50)

Table 3. Effect of pH upon the activity of 1-N HAPA gentamicin B

Organism	MIC (MBC) μg/ml		
	pH 6	pH 7	pH 8
Proteus mirabilis 3378	25 (≥50)	12.5 (25)	12.5 (12.5)
Serratia marcescens 3915	12.5 (≥50)	6.3 (≥50)	3.1 (≥50)
Klebsiella pneumoniae 3929	3.1 (3.1)	0.8 (1.6)	0.4 (1.6)
Escherichia coli 3939	12.5 (25)	6.3 (12.5)	1.6 (1.6)
Pseudomonas aeruginosa 3902	1.6 (3.1)	0.8 (1.6)	0.4 (0.8)

MUELLER-HINTON broth was used with the pH adjusted to the value shown.

Table 4. Comparative activity of 1-N HAPA gentamicin B with known aminoglycoside antibiotics

Organism	Antibiotic	Cumulative percent inhibited Minimum inhibitory concentration (μg/ml)									
		0.1	0.2	0.4	0.8	1.6	3.1	6.3	12.5	25	≥50
Staph. aureus (34)	1-N HAPA gentamicin B				6	29	88	97	100		
	Gentamicin			50	88	97					100
	Tobramycin				26	91	94				100
	Amikacin					18	88	100			
	Netilmicin			74	91	97	100				
Enterococci (32)	1-N HAPA gentamicin B							3	9	34	100
	Gentamicin					6	15	34	85	100	
	Tobramycin					13	17	37	77	97	100
	Amikacin					3	6	12		38	100
	Netilmicin				3	12	22	76	100		
E. coli (34)	1-N HAPA gentamicin B		3	32	82	97		100			
	Gentamicin			12	74	76		79	82	88	100
	Amikacin			6	56	90	100				
	Netilmicin		3	24	79	90	91	94	97	100	
Klebsiella pneumoniae (32)	1-N HAPA gentamicin B			75	94	97	100				
	Gentamicin			63	75	81				84	100
	Amikacin			6	88	87	100				
	Netilmicin			63	94	97					100
Proteus rettgeri (15)	1-N HAPA gentamicin B				47	67	73	93	100		
	Gentamicin			7	33		40	67	77	93	100
	Amikacin				67		77	93	100		
	Netilmicin			27	33		40	53	80	100	
Proteus morganii (26)	1-N HAPA gentamicin B				15	62	85	88	96	100	
	Gentamicin			19	50	81	88	96			100
	Amikacin				27	58	77	88	100		
	Netilmicin			11	50	69	85	92	96	100	
Providencia (29)	1-N HAPA gentamicin B			3	21	83	93	100			
	Gentamicin						21	66	90	96	100
	Amikacin				38	79	96	100			
	Netilmicin					10	14	45	90	96	100
Proteus mirabilis (31)	1-N HAPA gentamicin B				3	12	35	48	81	100	
	Gentamicin			3	22	90	97	100			
	Amikacin				6	22	58	84	90	100	100
	Netilmicin			3	29	45	87	100			100

(to be continued)

Table 4. (Continued)

Organism	Antibiotic	Cumulative percent inhibited Minimum inhibitory concentration (μg/ml)									
		0.1	0.2	0.4	0.8	1.6	3.1	6.3	12.5	25	≥50
Enterobacter (37)	1-N HAPA gentamicin B		3	8	89	97					
	Gentamicin		5	32	86	95				97	100
	Amikacin				24	86	100				
	Netilmicin			27	95	97				100	
Serratia marcescens (37)	1-N HAPA gentamicin B				3	22	38	59	97		100
	Gentamicin				19	22	57			70	100
	Amikacin					8	22	38	49	95	100
	Netilmicin					14			19	32	100
Citrobacter (24)	1-N HAPA gentamicin B	4	33	67	88		96				100
	Gentamicin			58	88	96					100
	Amikacin		4	12	67	92		96			100
	Netilmicin	4	21	79	88	96					100
Acinetobacter (16)	1-N HAPA gentamicin B				13	69	88				100
	Gentamicin			6	44	63	75	81		88	100
	Amikacin				31	69	81	88		94	100
	Netilmicin			6	19	50	56	75	88		100
Pseudomonas (39)	1-N HAPA gentamicin B				5	20	39	61	78	85	100
	Gentamicin		2		5	44	67	74	79	89	100
	Amikacin				2	36	64	74	79	89	100
	Netilmicin						59	71	79	87	100
	Tobramycin		2	10	72	87			94	100	

showed markedly different results for the MIC depending upon the medium. In all of the media the MBC value usually was only two-fold greater than the MIC value, with the exception of *Serratia* in which there was consistently a marked discrepancy of MIC and MBC values. With *Pseudomonas* assay in medium of higher calcium and magnesium content yielded large differences between MIC and MBC values.

Similar to other aminoglycosides, this compound is most active at an alkaline pH (Table 3). The difference in MIC and MBC values from pH 8 to pH 6 ranged from two-fold to sixteen-fold. The pH effect was noted for *E. coli* as well as for *Pseudomonas* and *Serratia*.

The comparative *in vitro* activity of 1-N HAPA gentamicin B and other aminoglycosides is given in Table 4. Against *Staph. aureus* the activity of 1-N HAPA gentamicin B was similar to amikacin, with both compounds four-fold less active than gentamicin or tobramycin. Against enterococci netilmicin was the most active compound, followed by gentamicin and tobramycin, and again amikacin and 1-N HAPA gentamicin B were four-fold less active than the other compounds. 1-N HAPA gentamicin B was as active as gentamicin against gentamicin-sensitive *E. coli* isolates, two-fold more active than amikacin, and equal in activity to amikacin and netilmicin against the gentamicin-resistant isolates. 1-N HAPA gentamicin B was equal in activity to netilmicin and amikacin against *Klebsiella pneumoniae*. All of the compounds had similar activity against *Enterobacter* and *Citrobacter* although 1-N HAPA gentamicin B was two-fold more active than amikacin. The *Serratia marcescens* isolates were collected not only from our own institution but from several other institutions in the City and many were highly gentamicin-resistant. 1-N HAPA gentamicin B was the most active compound. The activity of the compounds against *Acinetobacter* was similar with netilmicin the least active agent. Tobramycin was the most active aminoglycoside tested against the *Pseudomonas aeruginosa* isolates. Amikacin and this compound had similar activity against *Pseudomonas* with both eight-fold less active than tobramycin. 1-N HAPA gentamicin B and amikacin had almost identical *in vitro* activity against the indole-positive *Proteus* and *Providencia* with both appreciably more active than gentamicin or netilmicin.

Direct comparison of the activity of 1-N HAPA gentamicin B, amikacin and netilmicin against gentamicin resistant clinical isolates is given in Table 5. Overall 1-N HAPA gentamicin B and amikacin differed only two-fold in either direction against most of the isolates listed.

Both compounds were active at a concentration which could be achieved in man. Netilmicin was not as active as the other two agents against the indole-positive *Proteus, Providencia* and *Serratia*.

Fig. 2 is a plot comparison of 1-N HAPA gentamicin B versus amikacin and versus netilmicin tested against 43 non-*Pseudomonas* clinical isolates of ten species of bacteria which were gentamicin resistant. Such a plot shows that 1-N HAPA gentamicin B is considerably more active than netilmicin against gentamicin-resistant bacteria. It is also clear that 1-N HAPA gentamicin B is more potent than amikacin since half the isolates had lower MIC values for 1-N HAPA gentamicin B, and only 23% had lower amikacin MIC values.

The comparative MIC and MBC values of all of the aminoglycosides against isolates with known inactivating enzymes as well as a *Serratia* isolate that has a defect in transport of aminoglycosides (FU and NEU in preparation) is given in Table 6. 1-N HAPA gentamicin B was active against *Klebsiella* and *E. coli* which contain adenylating enzyme ANT (2″) and are resistant to tobramycin, gentamicin and kanamycin. It was also active against *Klebsiella* and *Serratia* which contained

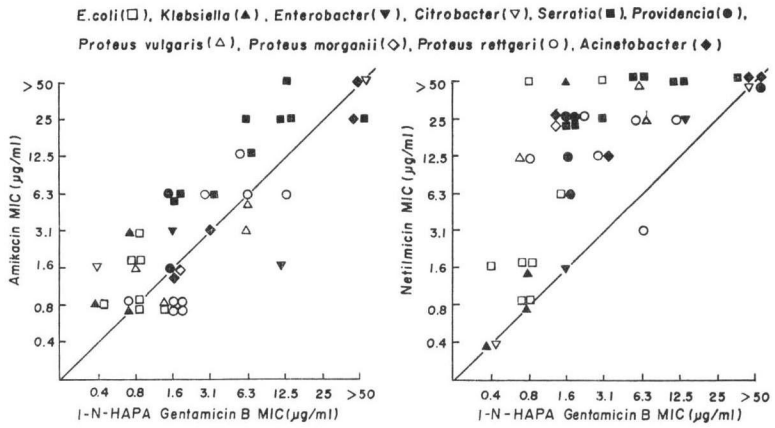
Table 5. Activity of 1-N HAPA gentamicin B against gentamicin resistant organisms

Organism	MIC (μg/ml)			
	Gentamicin	Amikacin	Netilmicin	1-N HAPA gentamicin B
Staphylococcus aureus	≥50	6.3	3.1	12.5
Escherichia coli	≥50	0.8	6.3	1.6
Escherichia coli	≥50	1.6	0.8	0.8
Enterobacter	25	3.1	1.6	1.6
Citrobacter	≥50	1.6	0.4	0.4
Klebsiella	≥50	3.1	1.6	0.8
Proteus morganii	25	1.6	25	1.6
Proteus vulgaris	≥50	3.1	≥50	6.3
Proteus rettgeri	≥50	6.3	25	12.5
Providencia	≥50	1.6	≥50	1.6
Acinetobacter	≥50	25	≥50	≥50
Serratia	25	6.3	25	1.6
Pseudomonas aeruginosa	≥50	1.6	1.6	1.6
Pseudomonas aeruginosa	25	25	25	25

Table 6. Activity of 1-N HAPA gentamicin B against organisms with known aminoglycoside modifying enzymes

Organism	Enzyme	MIC (MBC) μg/ml						
		1-N HAPA gentamicin B	Gentamicin	Sisomicin	Amikacin	Tobramycin	Netilmicin	Kanamycin
Klebsiella	ANT (2″)	0.8 (3.1)	12.5 (12.5)	6.3 (6.3)	0.8 (1.6)	25 (25)	0.4 (0.8)	>100 (>100)
Escherichia coli	ANT (2″)	0.2 (3.1)	12.5 (12.5)	6.3 (25)	0.4 (0.8)	25 (100)	0.4 (0.4)	50 (100)
Klebsiella	APH (3′)-II	0.8 (6.3)	0.4 (3.1)	0.4 (0.4)	3.1 (6.3)	0.8 (0.8)	0.4 (0.4)	>100 (>100)
Serratia	APH (3′)-II	0.8 (6.3)	0.4 (0.8)	0.2 (0.2)	0.8 (0.8)	3.1 (6.3)	0.8 (0.8)	>100 (>100)
Escherichia coli	APH (3′)-I	3.1 (12.5)	25 (25)	12.5 (25)	6.3 (12.5)	>100 (>100)	1.6 (1.6)	>100 (>100)
Providencia	AAC (2′)	25 (≥50)	100 (100)	50 (100)	12.5 (25)	50 (100)	100 (100)	—
Escherichia coli	AAC (6′)	25 (25)	1.6 (3.1)	—	50 (100)	100 (100)	100 (100)	>100 (>100)
Pseudomonas aeruginosa	AAC(3)-II	3.1 (6.3)	>100 (>100)	>100 (>100)	0.8 (3.1)	50 (100)	>100 (>100)	>100 (>100)
Serratia	None	12.5 (25)	>100 (>100)	>100 (>100)	>100 (>100)	>100 (>100)	>100 (>100)	>100 (>100)

Fig. 2. Comparative activity of 1-N HAPA gentamicin B with amikacin and netilmicin against gentamicin resistant (MIC $\geq$12.5 μg/ml) non-*Pseudomonas* clinical isolates.



phosphorylating enzyme APH(3′)-II which are resistant to kanamycin. It was active against *Pseudomonas aeruginosa* which contained acetylating enzyme AAC(3)-II but not against bacteria containing AAC(2′) and AAC(6′). Interestingly, 1-N HAPA gentamicin B had the lowest MIC against the strain which was resistant because of a permeability barrier.

1-N HAPA gentamicin B was combined with carbenicillin and evaluated against 201 of the clinical isolates (Table 7). Complete synergy was seen against 11% of the isolates with partial synergy against 29% of the isolates. The greatest degree of synergy was found against *Pseudomonas* and *Serratia*. Indeed, some form of synergy was found against 69% of *Serratia*, 35% of *Pseudomonas* and 56% of the *Acinetobacter* tested.

Table 7. Synergy of 1-N HAPA gentamicin B when combined with carbenicillin

	No. tested	Synergy %	Partial synergy %
Escherichia coli	34	0	26
Klebsiella pneumoniae	31	0	16
Enterobacter	39	20	26
Serratia	32	16	53
Citrobacter	4	25	0
Providencia	15	7	7
Acinetobacter	16	6	50
Pseudomonas	30	23	12
Total	201	11	29

Discussion

1-N HAPA gentamicin B has been shown to have excellent *in vitro* activity against the members of the Enterobacteriaceae. Similar to other aminoglycosides, its activity was affected by the cation content of the test medium[4]. It was equal in activity to amikacin against both gentamicin sensitive and most gentamicin resistant isolates, and was more active against many *E. coli*. This compound was similar in activity to amikacin against the highly resistant indole-positve *Proteus* and *Providencia* and it was more active against *Serratia*, although it should be noted that there are large differences between the MIC and MBC values with *Serratia* which we are investigating.

It is intriguing that 1-N HAPA gentamicin B has activity against some of our isolates which are resistant because of a permeability barrier. If animal toxicologic studies show that this compound has low nephrotoxicity and ototoxicity, its excellent *in vitro* activity would indicate that it should undergo further clinical evaluation in man.

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