ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 1977, p. 126-131
Vol. 11, No. 1

Printed in U.S.A.

In Vitro Activity of Netilmicin, Gentamicin, and Amikacin

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Received for publication 11 August 1976

The in vitro activity of netilmicin (Sch 20569), a new semisynthetic derivative of gentamicin, was compared with that of gentamicin and amikacin. One hundred and ninety-two clinical isolates of *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were tested using both agar and broth dilution techniques. Netilmicin was comparable to gentamicin, with the following exceptions: (i) for *Serratia marcescens* and *P. aeruginosa*, gentamicin was more active than netilmicin; (ii) all strains of *Escherichia coli*, *Klebsiella*, *Enterobacter*, *Proteus mirabilis*, and *Citrobacter freundii*, which were resistant to gentamicin, were susceptible to netilmicin; (iii) some strains of *S. marcescens*, indole-positive *Proteus*, and *Providencia*, which were resistant to gentamicin, were susceptible to netilmicin. Netilmicin was more active than amikacin for all *Enterobacteriaceae* and *S. aureus* and equal to amikacin in activity against gentamicin-susceptible strains of *P. aeruginosa*. All strains of *P. aeruginosa*, resistant to gentamicin, were also resistant to netilmicin but were susceptible to amikacin. Minimal inhibitory concentrations (MICs) obtained with broth and agar showed no significant differences except for *P. mirabilis*, where broth MICs were twofold greater than agar MICs, and for *P. aeruginosa*, where agar MICs were twofold higher than broth MICs. The minimal bactericidal concentration (MBC) was either identical to or within one twofold dilution of the MIC for the strains tested. A 100-fold increase in inoculum size produced less increase in MIC and MBC with netilmicin than with gentamicin or amikacin.

Although gentamicin has proven to be an effective antibiotic for treatment of serious infections due to gram-negative bacteria, several problems have developed which limit its usefulness. First, administration of the maximum nontoxic doses of gentamicin often results in serum concentrations that are just above the minimal inhibitory concentrations (MICs) for many pathogens. Second, the ratio between therapeutic and toxic levels of gentamicin is narrow and the level obtained after a given dose is not always predictable, thus necessitating frequent monitoring of blood levels. Third, nosocomial infections are being caused with increasing frequency by organisms resistant to gentamicin. Newer antibiotics are being sought and tested in an effort to overcome these problems. One such agent is netilmicin (formerly Sch 20569), a semisynthetic aminoglycoside which is a derivative of sisomycin (4). This report presents the results of in vitro susceptibility testing of netilmicin, amikacin, and gentamicin for 192 clinical isolates. The MICs as determined in broth and agar were compared, the effect of increased inoculum size on MIC and minimal bactericidal concentration (MBC) was determined, and the bactericidal activity of the three antibiotics was measured.

MATERIALS AND METHODS

Antimicrobial agents. Netilmicin and gentamicin were supplied by the Schering Corp., and amikacin was supplied by Bristol Laboratories. The 1,000-μg/ml standards, prepared in 0.1 M phosphate buffer (pH 8.0), were stored at −70°C. Subsequent dilutions were made in 0.1 M phosphate buffer (pH 8.0).

Bacterial isolates. One hundred and eighty-two strains were obtained from isolates from clinical material submitted to the microbiology laboratory at Montefiore Hospital, Pittsburgh, Pa. Additional organisms resistant to gentamicin were obtained from specimens submitted to the microbiology laboratories at Mercy Hospital, Pittsburgh, Pa. (four *Pseudomonas aeruginosa*), Presbyterian University Hospital, Pittsburgh, Pa. (one indole-positive *Proteus*), and the Veteran's Administration Hospital, Pittsburgh, Pa. (two *Escherichia coli*, one *Klebsiella*, one *Enterobacter aerogenes*, and one *Providencia*).

A total of 192 bacterial strains were tested, including: 31 *E. coli*, 28 *Klebsiella*, 22 *Proteus mirabilis*, 16 indole-positive *Proteus*, 48 *P. aeruginosa*, 23 *Staphylococcus aureus*, 11 *Serratia marcescens*, and 13 miscellaneous (7 *Providencia*, 1 *E. aerogenes*, and

5 *Citrobacter freundii*). For analysis, the strains were divided into two groups: 133 that were susceptible to gentamicin (MIC ≤8.0 μg/ml) and 59 (34 *Enterobacteriaceae* and 25 *P. aeruginosa*) that were resistant to gentamicin (MIC >8.0 μg/ml). The 34 *Enterobacteriaceae* that were resistant to gentamicin included: 5 *E. coli*, 3 *Klebsiella*, 1 *E. aerogenes*, 3 *S. marcescens*, 8 indole-positive *Proteus*, 7 *Providencia*, 2 *P. mirabilis*, and 5 *C. freundii*.

Susceptibility testing. MICs for netilmicin, gentamicin, and amikacin were determined by both the agar and broth dilution methods.

(i) Agar dilution method. Square petri dishes were each filled with 20 ml of Mueller-Hinton agar containing twofold dilutions of antibiotic ranging from 0.12 to 256 μg/ml. The bacterial inoculum was prepared by growing individual strains overnight in 1 ml of Mueller-Hinton broth (MHB) and then diluting 1:100 in MHB just prior to inoculation onto drug-containing plates. A Steers replicating device was used which delivered 36 inocula, each containing approximately 0.002 ml, onto the surface of the agar plates. The plates were then incubated at 37°C for 18 h. The MIC was defined as that concentration of antibiotic at which fewer than five colonies grew at the site of inoculation.

(ii) Broth dilution methods. Disposable plastic microtiter U plates with 96 wells were used. Antibiotics were diluted in MHB, and 50 μl was placed in each well. A culture, grown overnight in MHB, was diluted 1:10,000, and 50 μl was then added to each well containing antibiotic. The trays were incubated at 37°C for 18 h. The MIC was defined as that concentration at which there was no visible growth. After determining the MIC, 0.01 ml was removed from each well containing no visible growth, plated on antibiotic-free media, and incubated for 18 h. The MBC was defined as the concentration of antibiotic at which 99.9% of the organisms were killed.

Bactericidal activity. One strain of *Klebsiella* and one strain of *P. aeruginosa* were each grown overnight in MHB. Each suspension was then diluted 1:100 in MHB and added to an equal volume of antibiotic to make a final concentration that was eight times the MIC for that organism. The mixtures were incubated at 37°C, and the number of colony-forming units (CFU) was determined by pour-plate dilutions just after mixing and 3, 6, and 24 h later.

RESULTS

Susceptibility of gentamicin-susceptible organisms to netilmicin and amikacin. The activity of netilmicin, gentamicin, and amikacin was compared for 133 clinical isolates that were susceptible to gentamicin (MIC ≤8 μg/ml). Results of MICs as measured in broth and agar were generally comparable except as noted later. Unless specified otherwise, all results were obtained with the broth dilution method.

Netilmicin showed a wide spectrum of activity against the gram-negative organisms and *S. aureus* (Fig. 1). At a concentration of 1 μg/ml, netilmicin inhibited 100% of *E. coli*, *K. pneumoniae*, and *S. aureus*, 75% of indole-positive *Proteus* and *S. marcescens*, 58% of *P. aeruginosa*, and 30% of *P. mirabilis*. At a concentration of 2 μg/ml, 85% or more of strains of *P. mirabilis*, indole-positive *Proteus*, *S. marcescens*, and *P. aeruginosa* were inhibited. At a concentration of 4 μg/ml, 100% of gentamicin-susceptible strains tested were inhibited by netilmicin except for two isolates of *P. aeruginosa*, which had MICs of 8 and 64 μg/ml; the MICs to gentamicin for these isolates were 2 and 8 μg/ml, respectively.

In general, netilmicin was comparable to gentamicin but more active than amikacin for *Enterobacteriaceae* and *S. aureus* (Table 1; Fig. 2). With *S. marcescens*, however, gentamicin was more active than both netilmicin and amikacin. At 1 μg/ml, 100% of *Serratia* strains were inhibited by gentamicin, with a geometric mean MIC of 0.46 μg/ml. At this same concentration, only 75% of *Serratia* strains were inhibited by netilmicin (geometric mean MIC, 1.09 μg/ml) and 63% by amikacin (geometric mean MIC, 1.68 μg/ml).

Netilmicin was found to be comparable to amikacin but less active than gentamicin against *P. aeruginosa*. At 1 μg/ml, 87% of *Pseudomonas* strains were inhibited by gentamicin (geometric mean MIC, 0.59 μg/ml) compared to 66% by amikacin (geometric mean MIC, 1.22 μg/ml) and 58% by netilmicin (geometric mean MIC, 1.41 μg/ml).

Susceptibility of gentamicin-resistant organisms to netilmicin and amikacin. The activity of netilmicin was compared with that of amikacin for 34 *Enterobacteriaceae* and 25 *P. aeruginosa* that were resistant to gentamicin

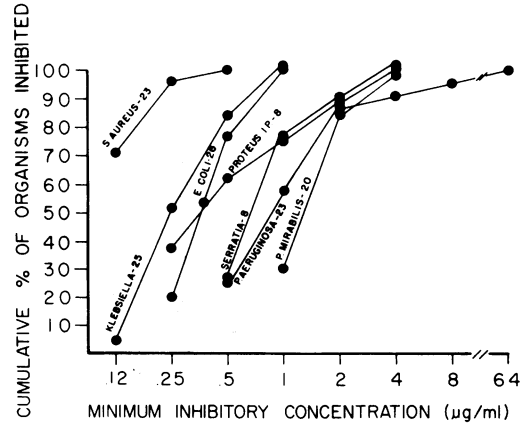

FIG. 1. *Susceptibility of gentamicin-susceptible isolates to netilmicin. The number of strains tested is indicated.*

TABLE 1.
Comparison of in vitro activity in broth and agar for netilmicin, gentamicin, and amikacin against gentamicin-susceptible isolates

Isolates (no.)	Geometric mean MIC (range)					
	Netilmicin		Gentamicin		Amikacin	
	Broth	Agar	Broth	Agar	Broth	Agar
E. coli (26)	0.51 (0.25–1)	0.51 (0.25–1)	0.57 (0.25–1)	0.50 (0.25–2)	2.20 (1–4)	1.90 (1–8)
K. pneumoniae (25)	0.38 (0.12–1)	0.40 (0.25–1)	0.57 (0.125–8)	0.45 (0.125–8)	1.50 (1–4)	1.28 (0.5–4)
P. mirabilis (20)	1.80 (1–4)	0.81 (0.5–2)	1.27 (0.5–4)	0.66 (0.25–1)	4 (2–8)	2.30 (1–4)
Indole-positive *Proteus* (8)	0.65 (0.25–4)	0.56 (0.12–4)	0.71 (0.125–4)	0.60 (0.25–4)	1.19 (0.25–4)	1.19 (0.25–4)
S. marcescens (8)	1.09 (0.5–4)	1.30 (0.5–4)	0.46 (0.25–1)	0.50 (0.25–1)	1.68 (1–4)	1.83 (1–4)
P. aeruginosa (23)	1.41 (0.5–64)	2.87 (0.5–64)	0.59 (0.125–8)	1.20 (0.5–8)	1.22 (0.5–4)	1.73 (1–4)
S. aureus (23)	0.16 (0.125–0.5)	0.18 (0.125–0.5)	0.15 (0.125–0.25)	0.15 (0.125–0.5)	0.46 (0.25–2)	0.75 (0.125–2)

(MIC >8 μg/ml) (Table 2). At 8 μg/ml, amikacin inhibited 32 (94%) of 34 *Enterobacteriaceae* isolates, compared to 24 (71%) for netilmicin. Five isolates of indole-positive *Proteus* were resistant to netilmicin but susceptible to amikacin, with MICs ranging from 1 to 4 μg/ml. Four isolates of *Providencia* were resistant to netilmicin with MICs ≥64 μg/ml; the MICs to amikacin ranged from 2 to 16 μg/ml. One isolate of *S. marcescens* was resistant to netilmicin, with an MIC of 16 μg/ml; the MIC to amikacin was 4 μg/ml.

All 25 isolates of *P. aeruginosa* were resistant to netilmicin and had comparable MICs to gentamicin. Amikacin inhibited 24 (96%) of 25 isolates at 4 μg/ml and inhibited all isolates at 8 μg/ml.

Comparison of MICs in agar and broth. Comparison of the MICs obtained with MHB and agar showed no significant differences for *Enterobacteriaceae* except for *P. mirabilis* strains, where broth MICs were usually twofold greater than agar MICs (Table 1). The opposite effect was seen for *P. aeruginosa* in that the agar MIC generally averaged twofold greater. Amikacin showed less discrepancy between agar and broth MICs for *Pseudomonas* than did netilmicin or gentamicin. For *S. aureus*, broth and agar MICs were comparable with netilmicin and gentamicin; however, for amikacin, MICs measured by the broth dilution technique were slightly lower than when measured in agar.

Comparison of MBC to MIC. The MBC was determined by the broth dilution technique for eight *Enterobacteriaceae* and three *P. aeruginosa* strains. Inocula of 10^5 and 10^7 CFU/ml were tested. The MBC was identical to or within one tube difference of the MIC for the three aminoglycosides regardless of inoculum size; however, for one strain of *E. coli*, the MBC to amikacin was fourfold greater than the MIC at an inoculum size of 10^7 CFU/ml.

Effect of increasing the inoculum size on MIC and MBC. Twenty-four strains (5 *E. coli*, 5 *Klebsiella*, 5 *P. mirabilis*, 5 *P. aeruginosa*, 2 *S. marcescens*, 2 indole-positive *Proteus*) were tested to determine the effect on the MIC (measured by broth dilution) of increasing the inoculum size 100-fold, i.e., from 10^5 to 10^7 CFU/ml (Table 3). A fourfold increase was seen in four strains with netilmicin, 11 strains with gentamicin, and 14 strains with amikacin.

Eleven strains (2 *E. coli*, 3 *Klebsiella*, 2 *S. marcescens*, 4 *P. aeruginosa*) were tested to determine the effect on the MBC of increasing the inoculum size from 10^5 to 10^7 CFU/ml. A fourfold increase was seen in two strains with netilmicin, five strains with gentamicin, and seven strains with amikacin.

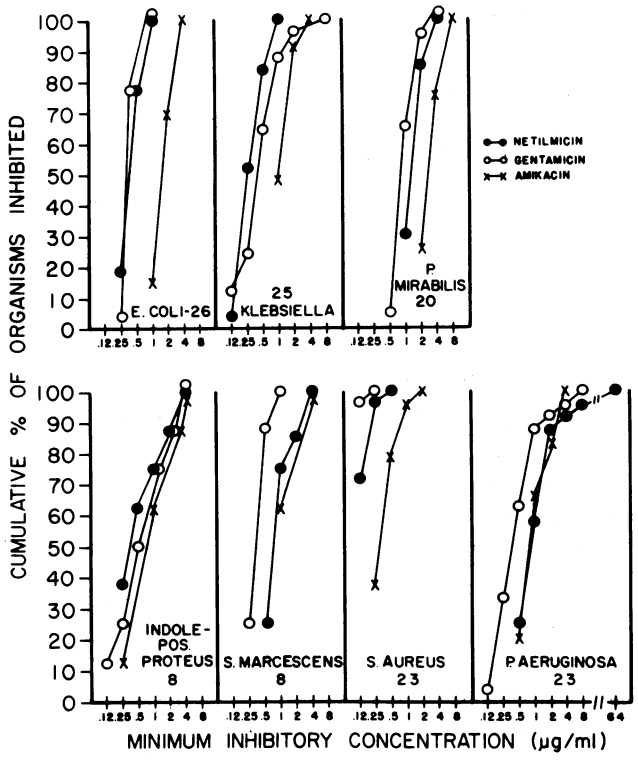

FIG. 2. *Comparison of activity of netilmicin with gentamicin and amikacin against gentamicin-susceptible isolates. The number of strains tested is indicated.*

TABLE 2. *Comparison of MICs to netilmicin and amikacin for organisms resistant to gentamicin*

Isolates (no.)	Antibiotic[a]	No. of strains with MIC (μg/ml) equal to:									
		0.12	0.25	0.5	1	2	4	8	16	32	64[b]
E. coli (5)	N			2	2		1				
	A					2	3				
Klebsiella (3)	N						2	1			
	A					1		2			
E. aerogenes (1)	N			1							
	A					1					
S. marcescens (3)	N					1		1	1		
	A					1	1	1			
C. freundii (5)	N			1	1	3					
	A			2	2		1				
P. mirabilis (2)	N						1	1			
	A					1	1				
Indole-positive *Proteus* (8)	N						1	2	2		3
	A				3	3	2				
Providencia (7)	N							3			4
	A	1				3		1	2		
P. aeruginosa (25)	N									5	20
	A			4	3	7	10	1			

[a] N, Netilmicin; A, amikacin.
[b] MIC is 64 μg/ml or greater.

TABLE 3. *Effect of a 100-fold increase in inoculum size on the MIC for netilmicin, gentamicin, and amikacin*

Isolates (no.)	No. of isolates with fourfold or greater increase in MIC		
	Netilmicin	Gentamicin	Amikacin
E. coli (5)	1	2	2
Klebsiella (5)	0	1	2
P. mirabilis (5)	0	3	4
Indole-positive *Proteus* (2)	1	2	2
S. marcescens (2)	2	2	2
P. aeruginosa (5)	0	1	2

Results of in vitro killing studies. Bactericidal activity, as a function of time, was similar for the three antibiotics. Rapid killing of *K. pneumoniae* occurred (Fig. 3); there was a 4-log reduction in organisms at 3 h, and no colonies were detected at 6 and 24 h. Slower killing was seen with *P. aeruginosa*; there was a 4-log reduction at 6 h, and no colonies were detected at 24 h (Fig. 3).

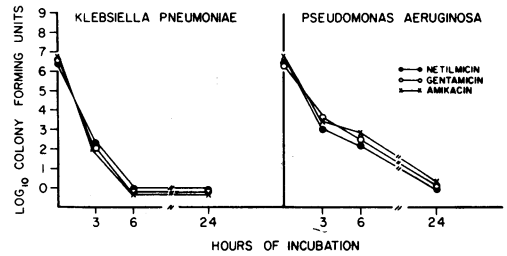

FIG. 3. *Comparison of bactericidal activity as a function of time for netilmicin, gentamicin, and amikacin against one strain each of Klebsiella and P. aeruginosa.*

DISCUSSION

In this study, the activity of netilmicin, gentamicin, and amikacin was measured against 192 clinical isolates. Our data support and extend the observations of Rahal et al. (4), Kabins et al. (1), and Watanakunakorn (6), who previously reported on the in vitro activity of netilmicin. Netilmicin was comparable to gentamicin in in vitro activity, with the following exceptions: (i) for *S. marcescens*, gentamicin was more active than netilmicin; (ii) for strains of *P. aeruginosa*, which were susceptible to gentamicin, gentamicin was more active than netilmicin; (iii) all strains of *E. coli*, *Klebsiella*, *Enterobacter*, *P. mirabilis*, and *C. freundii* that were resistant to gentamicin were susceptible to netilmicin; (iv) some strains of *Serratia*, indole-positive *Proteus*, and *Providencia*, which were resistant to gentamicin, were susceptible to netilmicin. Netilmicin was more active than amikacin for all *Enterobacteriaceae* and *S. aureus*. For strains of *P. aeruginosa* susceptible to gentamicin, netilmicin and amikacin were equal in activity. All strains of *P. aeruginosa* resistant to gentamicin that were tested in this study were resistant to netilmicin but susceptible to amikacin.

Our studies with netilmicin and *Pseudomonas* show the MICs to be higher when measured by agar dilution than by broth dilution techniques. This discrepancy has previously been reported with aminoglycosides (1, 3, 7) and is presumably due to the different concentrations of calcium and magnesium in broth and agar (3).

Several studies have shown the effect of increasing inoculum size on the MICs for amikacin and gentamicin (2, 3, 8). Our study suggests that there was less of an effect on the MICs and MBCs (as measured in broth) for netilmicin than for gentamicin or amikacin when the inoculum was increased 100-fold.

This in vitro study shows that netilmicin has a wide spectrum of activity against clinical pathogens. The frequency with which resistance to different aminoglycosides is reported will vary depending on which inactivating enzymes are present in the bacterial population being investigated (1). Among the isolates we studied, resistance to netilmicin was uncommon with *E. coli*, *Klebsiella*, *Enterobacter*, *Serratia*, *P. mirabilis*, and *C. freundii* but frequent with *P. aeruginosa*, *Providencia*, and indole-positive *Proteus*. Initial studies in animals indicated that the ototoxic and nephrotoxic potential of netilmicin is considerably less than that of gentamicin (5). Because of its broad range of in vitro activity and the possibility of decreased toxicity in clinical use, netilmicin may have advantages over present aminoglycosides and warrants further evaluation.

ACKNOWLEDGMENTS

This study was supported by a grant from the Schering Corp.

We wish to thank Yvonne Korica for identification of the clinical isolates tested, Arnold Brown and Spyros Kominos for providing several clinical isolates, JoAnne Reitz for preparing the manuscript, and Frederick Ruben for critically reviewing the manuscript.

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