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Clinical and Bacteriological Evaluation of Netilmicin in Gram-Negative Infections

J. KLASTERSKY,* F. MEUNIER-CARPENTIER, L. COPPENS-KAHAN, D. DANEAU, AND J. M. PREVOST

Service de Médecine et Laboratoire d'Investigation Clinique H. J. Tagnon (Section des Maladies Infectieuses), Institut Jules Bordet, Centre des Tumeurs de l'Université Libre de Bruxelles, Brussels, Belgium

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A preliminary study was conducted with netilmicin, a new aminoglycoside antibiotic. Its effectiveness was evaluated in vitro against 546 strains of *Enterobacteriaceae* and *Pseudomonas aeruginosa* isolated from clinical material. Its effectiveness against gentamicin-susceptible strains was roughly similar to that of gentamicin and other newer aminoglycoside antibiotics. Cross-resistance to netilmicin was found in 9 of 44 gentamicin-resistant strains. A favorable clinical response was observed in 70% of the patients treated with a dose of netilmicin ranging from 4.5 to 7.5 mg/kg. However, the appearance of granular casts was seen in 7 of 10 patients receiving the higher dosage of netilmicin (7.5 mg/kg) and a rise in blood urea nitrogen or serum creatinine levels was observed in 5 of these patients.

Netilmicin is a new semisynthetic aminoglycoside antibiotic that is active in vitro against a wide variety of gram-negative bacteria. This new compound is derived from sisomicin by ethylation of the 1-*N* position of the deoxystreptamine ring.

For most clinically significant *Enterobacteriaceae* and *Pseudomonas aeruginosa*, the activity of netilmicin in vitro was comparable or superior to that of gentamicin, tobramycin, and amikacin with respect to potency by weight and achievable blood levels. Against gentamicin-resistant strains, the activity of netilmicin usually paralleled that of amikacin (3). These in vitro studies were repeated here using the strains isolated from clinical material in our hospital, with special attention to gentamicin-resistant strains. No reports of clinical experience with netilmicin have yet been published; that is why the present investigation was undertaken as an increasing-dose trial.

MATERIALS AND METHODS

Bacteriological studies. All the aminoglycosides (gentamicin, sisomicin, tobramycin, netilmiçin, amikacin) were supplied as sterile powders by the respective manufacturers. Dilutions were prepared in Trypticase soy broth (Difco). The microorganisms examined comprised 163 strains of *Klebsiella*, 58 strains of *Proteus mirabilis*, 21 strains of *Enterobacter*, 178 strains of *Escherichia coli*, and 94 strains of *P. aeruginosa*. All these gram-negative rods were isolated from patients who were hospitalized at the Institut Bordet, which is the cancer hospital of Brussels University. The microorganisms were isolated from sputum, urine, wounds, and blood cultures. They were identified by the usual methods. The minimum inhibitory concentrations (MICs) were determined for each aminoglycoside by the inocula replicating method of Steers et al. (15) on a Mueller-Hinton medium (pH 7.4), with an inoculum containing approximately 10^4 microorganisms per ml. The reading was made after 18 h of incubation at 37°C.

In addition, the cross-resistance between gentamicin and the other aminoglycosides studied was investigated in the microorganisms resistant to gentamicin. All microorganisms that were resistant to one or more of the antibiotics sisomicin, tobramycin, netilmicin, and amikacin were also resistant to gentamicin. Resistance to gentamicin, tobramycin, sisomicin, and netilmicin was defined by an MIC of ≥ 6 μg/ml. Resistance to amikacin was defined by an MIC of ≥ 12 μg/ml. Similar techniques were used to determine the MIC of the bacteria isolated from the patients who had been included in the clinical study.

Serum was obtained from most patients 1 and 6 h after the administration of netilmicin on various days after the onset of the treatment. These sera were tested for bacteriostatic and bactericidal activity against the organism responsible for the infection in each case: a suspension of these microorganisms (approximately 10^5 per ml) in Trypticase soy broth was added to serial twofold dilutions of the sera. The highest dilution that failed to show evidence of macroscopic growth was considered to represent the bacteriostatic end point. Clear tubes were plated on blood agar plates and incubated for 18 h at 37°C to determine the bactericidal activity.

Assays for the concentration of netilmicin were performed by the cup-plate technique (2), with a strain of *Staphylococcus aureus* resistant to penicillin and clindamycin as the test microorganism and Mueller-Hinton agar.

Clinical studies. The clinical studies were carried out on three dosage groups of ten patients each. All were hospitalized at the Institut Jules Bordet. Depending on which group they were in, these patients received a daily dose of 4.5, 6.0, and 7.5 mg of netilmicin per kg.

Most patients had metastatic carcinoma. The types of infections varied among the different groups and were considered to be caused primarily by gram-negative bacilli (*Enterobacteriaceae, P. aeruginosa*), all of which were susceptible in vitro to netilmicin. Occasionally, other bacteria were isolated from the sites of infection (most often *Streptococcus* sp. or *Bacteroides* sp.), and penicillin or clindamycin was used in those patients in addition to netilmicin. No patient in the present series received concomitant therapy with antibiotics other than those mentioned above, except that one patient received cephalothin in addition to netilmicin for an infection caused by *P. aeruginosa*. Another patient received methicillin instead of penicillin.

It should be stressed that most infections in this series were complicated by underlying factors. Urinary tract infections occurred in patients with obstructive uropathies or tumors of the urinary tract; all these patients received surgical attention to relieve the obstruction of the urinary tract and achieve better drainage. However, as might be expected, major anatomical alterations resulting from extensive tumors or previous surgery could not always be corrected. Wound infections usually complicated postoperative lesions or tumors, and patients with pulmonary infections usually had tracheostomy or bronchial carcinoma.

Infection was considered to be present when a potential pathogen was seen along with abundant polymorphonuclear neutrophils on Gram-stained films and was recovered repeatedly as the only or predominant microorganism from urine (more than 100,000 organisms per ml were required), tracheal secretions, or pus from wounds, in addition to clinical signs such as fever, hyperleukocytosis, leukocyturia, pulmonary infiltrates, etc., suggesting real infection rather than mere bacterial colonization.

Clinical cure was considered to exist when the initial clinical signs and symptoms related to the infection disappeared or markedly improved during therapy. Bacteriological cure was considered to have been obtained when the offending microorganism had been eradicated. Bacterial colonization was defined as the presence of large numbers of a potential pathogen, resistant to netilmicin, in cultures of sputum, urine (counts of $\geq$100,000 microorganisms per ml), or wounds after therapy with netilmicin was started but without any clinical signs of infection.

In most patients in this study, cultures of the tracheobronchial secretions, urine, and wounds (when present) were carried out before and on days 3 and 6 of treatment, as well as after its discontinuation. Similarly, complete hematological evaluation, examination of the urinary sediment, and determination of blood urea nitrogen (BUN), creatinine, alkaline phosphatase, bilirubin, and glutamic oxalacetic and glutamic pyruvic transaminases were performed before, during, and after therapy. Creatinine clearances were obtained in many patients included in the present series. Surveillance of the auditory and vestibular functions was accomplished by daily questioning of the patients on hearing, vertigo, dizziness, and headache. Romberg's test, Rinne's test (tuning fork), and the watch test were carried out daily in most patients. Audiograms were obtained in most patients before, during, and after therapy with netilmicin.

RESULTS

As indicated in Fig. 1, among 163 strains of *Klebsiella* species, 50% showed MICs for the five aminoglycosides between 0.1 and 0.2 μg/ml. These differences are small; on the other hand, the MICs necessary to kill 90% of strains (MIC_{90}) for *Klebsiella* were lower for netilmicin, tobramycin, and amikacin (MIC between 0.2 and 0.6 μg/ml) than for sisomicin (MIC_{90} = 6 μg/ml) and for gentamicin (MIC_{90} = 20 μg/ml). This figure shows also that more than 90% of the strains of *Klebsiella* were susceptible to netilmicin, sisomicin, tobramycin, and amikacin, whereas only 75% were susceptible to gentamicin.

The results obtained with the 21 strains of *Enterobacter* (Fig. 2) indicate a difference among the antibiotics tested here. Netilmicin, tobramycin, and amikacin were very active: 100% of the strains were inhibited by 0.7 μg of netilmicin or tobramycin per ml and by 3 μg of amikacin per ml. On the other hand, sisomicin and gentamicin showed decreased activity: 85% of the strains of *Enterobacter* had a sisomicin

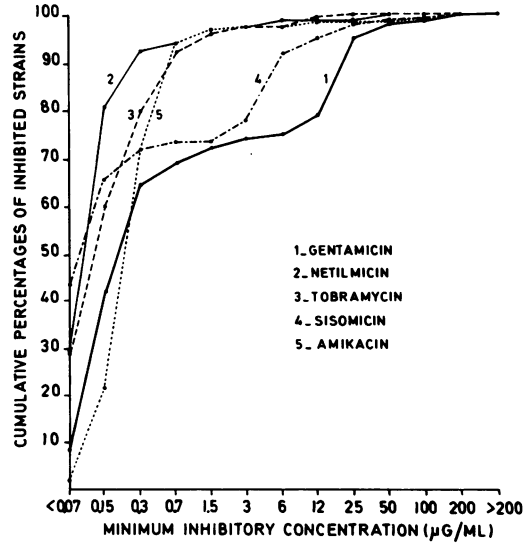

FIG. 1. *Susceptibility of 163 strains of Klebsiella sp. to netilmicin and to gentamicin, tobramycin, sisomicin, and amikacin.*

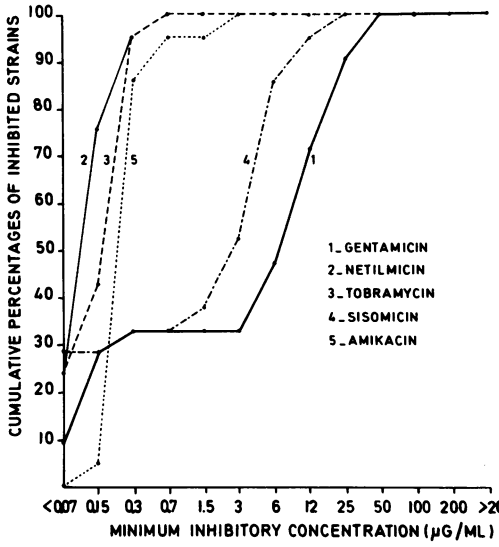

FIG. 2. *Susceptibility of 21 strains of Enterobacter sp. to netilmicin and to gentamicin, tobramycin, sisomicin, and amikacin.*

MIC of 6 μg/ml and 47% had a gentamicin MIC of 6 μg/ml.

Figure 3 shows the susceptibility of 58 strains of *P. mirabilis* to the five aminoglycosides. These microorganisms were very susceptible to the drugs tested here: 100% of the *Proteus* strains were inhibited by 0.7 μg of sisomicin, tobramycin, gentamicin, and netilmicin per ml, and 3 μg of amikacin per ml also inhibited 100% of these microorganisms.

Figure 4 compares the activity of the five aminoglycosides on 178 strains of *E. coli*; all the antibiotics tested appeared to be effective: 90% of the microorganisms showed MICs of ≤1 μg/ml. There was no major difference among them; however, netilmicin seemed slightly more active than the others. Figure 5 represents the results for *P. aeruginosa* (94 strains). The curves obtained for sisomicin and tobramycin are displaced to the left as compared with those obtained for gentamicin, amikacin, and netilmicin. However, all five aminoglycosides can be considered as active, since 95% of the strains showed MICs of less than 6 μg/ml; 95% of the *P. aeruginosa* strains had sisomicin and tobramycin MICs equal to 0.7 μg/ml, and 65% of the strains showed MICs lower than 0.07 μg/ml.

The cross-resistance to the various aminoglycosides was examined for 546 strains (Table 1). Forty-four strains (8%) were found to be resistant to gentamicin. No strain was resistant to any of the other aminoglycosides unless it was resistant to gentamicin as well. Among these resistant strains, 20 (45.4%) were *Klebsiella* sp.; as a matter of fact, 20 (12.2%) of 163 strains of *Klebsiella* were resistant to gentamicin. The proportion of gentamicin-resistant microorganisms was also high among *Enterobacter* sp. (38.1%) and *Serratia marcescens* strains (26.3%). No strain of *Proteus* was found to be resistant to gentamicin.

Most strains (90.9%) resistant to gentamicin were also resistant to sisomicin. Resistance to

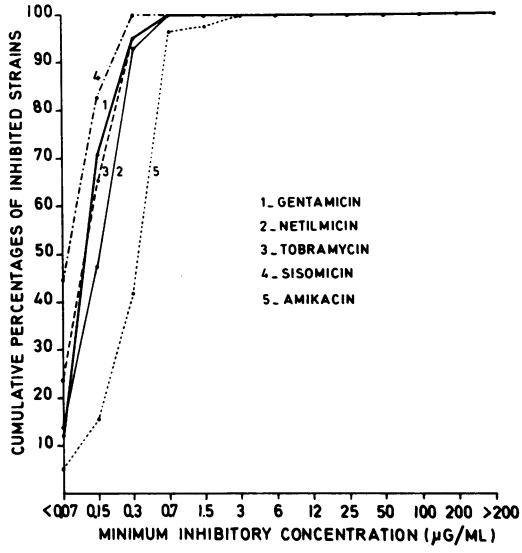

FIG. 3. *Susceptibility of 58 strains of Proteus mirabilis to netilmicin and to gentamicin, tobramycin, sisomicin, and amikacin.*

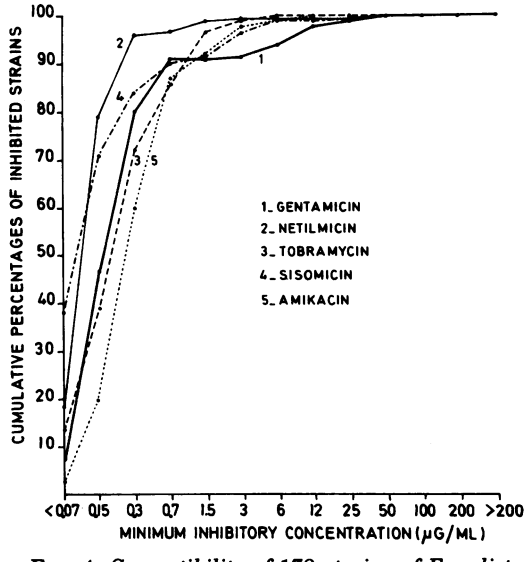

FIG. 4. *Susceptibility of 178 strains of E. coli to netilmicin and to gentamicin, tobramycin, sisomicin, and amikacin.*

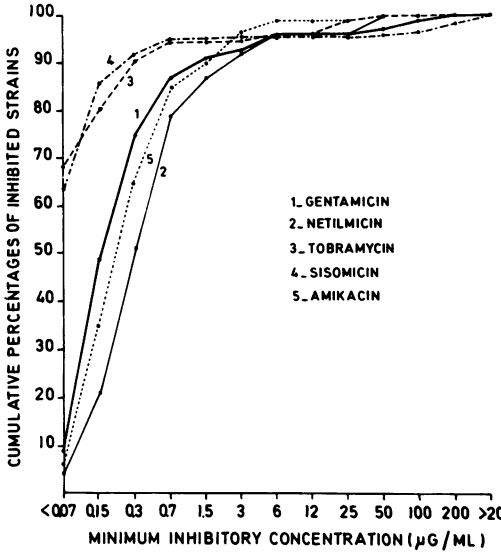

FIG. 5. *Susceptibility of 94 strains of P. aeruginosa to netilmicin and to gentamicin, tobramycin, sisomicin, and amikacin.*

gentamicin and to one or several other aminoglycosides was found for approximately 1% of the strains; three strains (0.5%) were resistant to all the aminoglycosides tested here.

A favorable clinical response was observed in 21 (70%) of the patients included in this series; as summarized in Table 2, no clear relationship between the dosage and the frequency of favorable responses could be observed. The offending pathogen was eliminated from the site of the infection in 16 (53%) patients, more often (7/10) when the highest dose of netilmicin had been given than with the lowest dose (4/10). The colonization of the original site of infection by a resistant microorganism occurred three times in this series: the microorganisms responsible for it were *S. marcescens*, *Herrellea* sp., and *Bacteroides* sp. and were not responsible for a new clinical infection.

The type of infection did not influence the frequency with which a favorable response was observed. Infection arising in surgical wounds responded favorably in 12 of 16 patients (75%); with urinary tract infections and bronchopulmonary infections, the favorable response was observed in 2 of 3 and in 3 of 4 patients, respectively. Infections associated with bacteremia responded in 5 of 11 patients (45%). No clear relationship between the dosage and the outcome could be observed in these various subgroups, but it is recognized that the numbers of patients in these subgroups are very small.

All the infections due to *Klebsiella-Entero-*

TABLE 1. *Cross-resistance among 44 gentamicin-resistant strains*

Species	Antibiotics to which microorganisms were resistant[a]														
	G	G+S	G+T	G+N	G+A	G+S+T	G+S+N	G+S+A	G+T+N	G+T+A	G+T+ N+A	G+S+T +N+A
Escherichia coli (178)	7	6	0	0	0	0	0	0	0	0	0	0
Klebsiella sp. (163)	20	19	3	3	3	2	2	2	3	3	3	2
Enterobacter sp. (21)	8	8	0	0	0	0	0	0	0	0	0	0
Serratia marcescens (19)	5	4	0	2	1	0	1	0	0	0	0	0
Proteus sp. (58)	0	0	0	0	0	0	0	0	0	0	0	0
Pseudomonas aeruginosa (44)	2	2	2	2	1	2	2	1	2	1	1	1
Providencia (13)	2	1	1	2	0	1	2	0	1	0	0	0
Total (%)	44 (8.0)	40 (7.3)	6 (1.1)	9 (1.6)	5 (0.9)	5 (0.9)	7 (1.3)	3 (0.5)	6 (1.1)	4 (0.7)	4 (0.7)	3 (0.5)

[a] G, Gentamicin; S, sisomicin; T, tobramycin; N, netilmicin; A, amikacin.

TABLE 2. *Relationship between dosage and frequency of clinical and microbiological responses and that of adverse effects*

Criterion	Daily dose of netilmicin (mg/kg)			Total (%)
	4.5	6.0	7.5	
No. of patients	10	10	10	30 (100)
Favorable clinical response	7	6	8	21 (70)
Elimination of pathogen	4	5	7	16 (53)
Bacterial colonization	1	1	1	3 (10)
Adverse effects				
Granular casts in urine	3	4	7	14
Rise of BUN and/or creatinine	2	2	5	9
Discontinuation for possible toxicity	0	1	2	3

bacter strains responded favorably (six of six), as did six of seven infections due to *Proteus* sp. Infections due to *E. coli* responded in four of nine patients; those caused by *P. aeruginosa* and *S. marscecens* were each observed in three patients and responded well in two patients in each group.

Concerning eradication of the pathogen from the site of the infection, netilmicin seemed also more effective in cases due to *Klebsiella-Enterobacter* and *Proteus* strains; six of six and five of seven pathogenic organisms, respectively, were eradicated.

Adverse effects that might have been associated with the administration of netilmicin were observed in 14 (46%) patients in this series. All these adverse effects were related to the kidneys. Granular casts appeared in the urine of 14 patients; in 9, this finding was associated with a rise above normal values of the BUN or the serum creatinine level or both. Therapy with netilmicin was discontinued in three of these patients, and another patient died during therapy, presumably as a consequence of severe pulmonary infection caused by *P. mirabilis*; to what extent the renal failure in that patient contributed to the fatal outcome is difficult to assess. Similarly, it is difficult to delineate the respective roles of netilmicin and severe infection in the appearance of acute renal dysfunction in that patient. In the other patients, the renal function impairment was mild and transitory: abnormal findings in the urinary sediment and abnormal values of the BUN and serum creatinine levels returned to normal after discontinuation of the drug. As indicated in Table 2, there was a possible relationship between the dosage of netilmicin and the occurrence of renal function impairment.

Since most strains involved in the infections studied here were very susceptible to netilmicin (MIC, ≤ 1 μg/ml), no relation between the susceptibility in vitro and the clinical outcome could be found. The bactericidal activity was higher in the group receiving 7.5 mg of netilmicin per kg as compared with the other groups; the geometric mean for the peak level was 1/8 and 1/4 for the trough level; the corresponding values were 1/4 and 1/2 in the two other groups. No clear relationship could be found between the bactericidal activity of the serum and the clinical outcome: in patients who failed to respond as well as in those who had a favorable outcome, the mean peak bactericidal activity of the serum against the offending pathogen was 1/8.

The serum levels of netilmicin are indicated in Fig. 6. These levels were similar for the two groups of patients receiving the lower doses of netilmicin. Respectively, the mean peak and trough levels in micrograms per milliliter were 6.1 ± 0.48 (standard error) and 1.9 ± 0.29, for the patients receiving 4.5 mg/kg, and 6.2 ± 0.58 and 2.0 ± 0.3 in those treated with 6.0 mg/kg. No significant difference could be found between the serum levels obtained early and those obtained later during therapy. In the patients who received 7.5 mg/kg, the blood levels of netilmicin were much higher. The peak and trough levels were 9.8 ± 0.9 and 4.5 ± 0.76 if all the available values were taken into account. The corresponding means calculated from values obtained during the first 2 days of therapy, when no significant renal function impairment was present, were 9.3 ± 1.1 and 3.6 ± 0.7.

DISCUSSION

Infections caused by gram-negative pathogens have become increasingly important as a cause of severe infection in the past decade, and the effectiveness of aminoglycosides against these microorganisms should be studied, since there has been a significant increase in resistance to these antimicrobial agents. Gentamicin remains very active against *P. mirabilis* and *E. coli* strains, but some *Klebsiella* and *Enterobacter* are becoming gentamicin resistant, es-

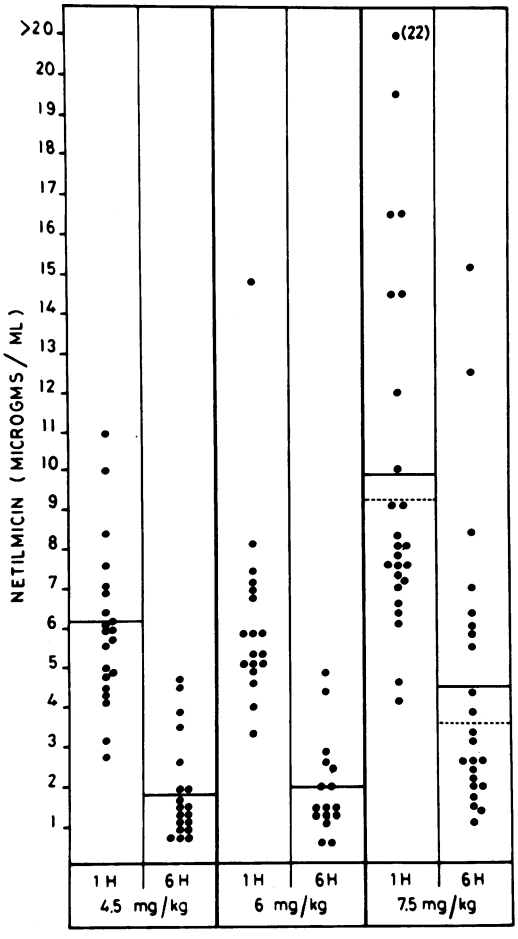

FIG. 6. *Blood levels of netilmicin observed 1 and 6 h after the injection of 1.5, 2.0, and 2.5 mg of netilmicin per kg. Horizontal solid bars represent means; interrupted bars represent means calculated from values observed during the first 2 days of therapy.*

pecially in hospitals where this antibiotic is often used.

Tobramycin, in our study, seems more active than gentamicin against *Enterobacter* and *P. aeruginosa*; it is particularly effective against species of *P. aeruginosa* that are resistant to gentamicin. Sisomicin is a gentamicin derivative, and our results show that sisomicin may be more active than gentamicin against some strains, but that there is a high incidence of cross-resistance between gentamicin and sisomicin. Amikacin, a new aminoglycoside antibiotic similar to kanamycin, is less active on a weight basis than tobramycin or gentamicin, but it might have a therapeutic advantage on the basis of higher blood levels. Amikacin was the most active drug when compared with the other aminoglycosides.

Netilmicin is another new semisynthetic aminoglycoside antibiotic; it is a semisynthetic derivative of sisomicin. Our data suggest that netilmicin is more active against *Klebsiella* and *Enterobacter* than the other aminoglycosides are but, on the other hand, netilmicin is less active against *P. aeruginosa* when compared with sisomicin or tobramycin. Moreover, among the 44 strains resistant to gentamicin in our study, 9 strains were also resistant to netilmicin.

Our in vitro studies confirm recent data from studies that compared the newer aminoglycosides, including netilmicin, against a variety of clinical isolates (3, 5, 11). The activity of netilmicin, sisomicin, gentamicin, tobramycin, and amikacin against most strains of *Enterobacteriaceae* and *P. aeruginosa* is, in general, quite similar with respect to potency by weight and achievable blood levels. Available controlled studies have failed so far to demonstrate a clinical advantage of one of these drugs over the other, provided that the infections treated were caused by pathogens susceptible in vitro to the antibiotic that was being administered (9, 14). A major role in clinical practice, however, is to be expected from the newer aminoglycosides, namely, tobramycin, netilmicin, and amikacin, in the management of infections caused by gentamicin-resistant strains. Studies by other investigators indicate that both netilmicin and amikacin show a high degree of activity against gentamicin-resistant strains (12).

A favorable clinical response was observed in 70% of the patients treated with netilmicin in this series; in another recent study, the total favorable response rate was 77% for amikacin and 78% for gentamicin (14). Sisomicin has been effective in 66% of wound infections in a population of patients similar to the one studied here (8), and in a controlled study we did not find any difference between gentamicin and tobramycin in terms of clinical effectiveness (9).

This is an overall evaluation, since the frequency of favorable response is related to the severity of the underlying disease and to the site of infection; nevertheless, it appears that netilmicin has a clinical efficacy that is approximately similar to the other newer aminoglycosides, provided that the microorganism responsible for the infection treated is susceptible in vitro. Feld and co-workers did not find any difference between amikacin and tobramycin in the treatment of infection in patients with cancer. The overall rate of favorable response was 67% for tobramycin and 69% for amikacin in that study (4).

Netilmicin should probably be used for the treatment of infection at a dosage between 4.5

and 6.0 mg/kg, which is the dosage usually recommended for gentamicin and tobramycin. At a higher dosage (7.5 mg/kg), the frequency of untoward reactions (renal function impairment) was higher than that observed at the lower dosages: granular casts appeared in the urine of 7 of 10 patients, and a rise of the BUN and/or the serum creatinine level was observed in 5/10 patients.

The bactericidal activity of the serum of patients receiving antibiotics is considered as a useful guide for predicting a favorable clinical outcome (7). This has not been verified in the present study; however, it should be emphasized that the number of patients studied here was small. In addition, the antibacterial activity of the serum may not be as valuable for aminoglycosides as for other antibiotics in predicting the clinical outcome, since local factors such as the pH and binding to purulent material (1) may influence the antimicrobial effectiveness of this type of antibiotic at the site of the infection.

To conclude, our study suggests that netilmicin is probably as effective as gentamicin and the other newer aminoglycosides in the management of bacterial infections caused by susceptible microorganisms. Its role in the management of infections caused by gentamicin-resistant organisms will probably prove to be important, since cross-resistance between gentamicin and netilmicin does not always occur. Nephrotoxicity due to aminoglycosides has been reported to be dose related in experimental animals, and comparative studies have shown netilmicin to be less nephrotoxic than gentamicin. In the present study, granular casts or increased levels of serum creatinine or BUN were observed in several of the patients who received 7.5 mg of netilmicin per kg per day. Although these changes were usually mild and transient, they should be watched for and, if found, should suggest appropriate dosage adjustments.

The relatively low margin between the effective and the toxic dose of netilmicin and other aminoglycosides makes it mandatory to explore their effectiveness when used as a part of a combination with a penicillin or a cephalosporin. Many aminoglycoside antibiotics are synergistic against various *Enterobacteriaceae* and *P. aeruginosa* when used in combination with penicillins or cephalosporins (10). Some studies suggest that this might be the case for netilmicin as well (13). Since the in vitro synergism between antibiotics probably has a clinical significance in the management of gram-negative infections (6), further in vitro and clinical studies of the effectiveness of netilmicin combined with penicillins or cephalosporins are probably warranted.

ACKNOWLEDGMENTS

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