Netilmicin in Gram-Negative Bacterial Infections

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Netilmicin, a new semisynthetic aminoglycoside, was used in the treatment of 42 patients with serious gram-negative bacterial infections. Of the 40 evaluable patients, 24 (60%) were cured, and 8 (20%) had a favorable clinical response, for a total clinical response rate of 80%. Eight patients failed to respond; of these, three had undrained abscesses and two had severe granulocytopenia. Three of the patients who failed had organisms in which resistance to netilmicin developed during therapy, and in two of these three netilmicin was the only aminoglycoside to which resistance developed. Of the 37 patients evaluable for toxicity, 8 (22%) developed renal insufficiency. Two patients had mild but persistent elevation in serum creatinine. Three patients had nephrotoxicity while on gentamicin in the past. Pre- and posttherapy audiograms were done on 26 patients; none had hearing loss. Four patients had mild, transient asymptomatic elevations in alkaline phosphatase. The pretreatment clinical isolates were tested for in vitro susceptibility. The median minimal inhibitory concentration of netilmicin, gentamicin, and tobramycin ranged between 0.5 and 2 μg/ml. The median minimal inhibitory concentration of amikacin was approximately twofold higher. No clear in vitro superiority of one aminoglycoside over another was observed.

Netilmicin, a newly developed semisynthetic analog of sisomicin, is active against a wide variety of aerobic gram-negative bacteria. Although its antimicrobial activity in vitro is similar to the other aminoglycosides (2, 4-6, 9, 10, 13, 17), it appears to be less nephrotoxic and ototoxic than these congeners in experimental animals (1, 8, 11). We undertook the present study to evaluate the efficacy and toxicity of netilmicin in infections in humans. This report is based on our experience with 42 patients.

MATERIALS AND METHODS

Patient studies. All patients were hospitalized at the Tufts-New England Medical Center. Patients selected for admission to the study had evidence of gram-negative bacterial infection which required an aminoglycoside for therapy, the only exclusions being children and pregnant women. Informed consent was obtained from each patient or next of kin before netilmicin treatment. Criteria for bacteremia included positive blood cultures with fever ≥38.3°C, hypotension, or chills. A separate category was suspected sepsis in which fever, hypotension, or chills were present but no growth of gram-negative bacteria was documented from blood cultures. Criteria for the diagnosis of pneumonia included roentgenological evidence of an infiltrate in addition to the presence of leukocytes and gram-negative organisms in secretions obtained by transtracheal aspiration or aspiration from an endotracheal tube. Criteria for urinary tract infection were cultures of 10^6 organisms per ml from voided urine specimens and fever (≥38.3°C) or dysuria, frequency, urgency, or pyuria. Criteria for peritonitis were fever, abdominal tenderness (usually with rebound), and polymorphonuclear leukocytes in ascitic fluid. Criteria for wound infection were suppuration and erythema.

Blood for culture was collected from all patients before, during, and after therapy. Patients with urinary tract infections had quantitative urine cultures obtained before, during, and after therapy; isolates were considered significant if counts were ≥10^7/ml of urine. Other specimens for studies were aspirates of soft tissues or wounds in appropriate situations.

Other antibiotics were sometimes employed as part of the therapeutic regimen, especially during mixed aerobic-anaerobic infection. If the other agent was active against all pathogens isolated, then the patient was not evaluated for efficacy (see below) but was evaluated for toxicity.

Drug dosage. Netilmicin therapy was initiated in doses of 2 mg/kg of lean body weight every 8 h. The drug was given intravenously. Some patients with severe infections received initial dosages of 2.5 mg/kg of lean body weight. Patients with renal impairment had the dosage adjusted in accordance with nomograms established for gentamicin; serum levels of netilmicin were used to determine subsequent doses. We sought to achieve a peak of 5 to 10 μg/ml and a trough of 1 to 2 μg/ml.

Serum levels. Serum levels of netilmicin were measured by the agar well method with Staphylococcus epidermidis (ATCC 27826) as the assay organism. Levels were determined on day 2 and every 3 to 4 days
thereafter while the patient was on therapy. Specimens were considered appropriately timed if blood for peak values was drawn at 30 min and that for trough values was drawn at 7.5 h after intravenous administration.

Toxicity. Hemoglobin, hematocrit, leukocyte, and differential leukocyte counts, urinalysis, serum creatinine, and liver function studies were obtained before therapy, at least every 3 days during treatment, and for several days after therapy.

Patients were evaluated for nephrotoxicity if they received the study drug for at least 72 h and were not being dialyzed at the time of initiation into the study. Nephrotoxicity was defined as an increase in serum creatinine of 0.4 mg/dl or greater if the initial serum creatinine was less than 2.0 mg/dl, or an increase of 0.8 mg/dl or greater if the initial creatinine was greater than 2.0 mg/dl.

Patients were evaluated for auditory toxicity if they were responsive and had received the drug for at least 72 h. Toxicity was defined as a decrease of greater than 15 decibels in audiological acuity in the range of 250 to 8,000 Hz. Electronystagmography was performed when vertigo or dizziness developed.

Efficacy. Efficacy was evaluated when netilmicin was given for at least 72 h, either alone or without another concurrent or sequential antibiotic to which the pathogen was susceptible. The criteria adopted were those of Smith et al. (15). Cure was defined as the absence of signs systemically (e.g., leukocytosis) or locally (lung, wound, or urinary tract) for 48 h and absence of fever for 4 days after stopping therapy. Patients who met the "cure" criteria but did receive another effective antibiotic after discontinuation of netilmicin were included in the "response" category.

Criteria for response were any two of the following: (i) decrease in maximal daily temperature of ≥1.7°C rectally or increase to normal if previously hypothermic; (ii) decrease of ≥15% in leukocyte count if elevated, or return to 5,000 to 10,000/ml if previously leukopenic; (iii) elimination of the pathogen from the local site of infection; and (iv) disappearance of signs locally.

Criteria for failure were death or persistence of signs systemically and locally.

Criteria for colonization were appearance of a new pathogen or an increase in numbers of other potential pathogens. Superinfection was defined as the appearance of new symptoms and signs of infection in association with the isolation of a new pathogen.

In vitro susceptibility testing and microbiological studies. All initial isolates from patient specimens were obtained by the Tufts-New England Medical Center Bacteriology Laboratory. We tested for in vitro susceptibility to netilmicin, gentamicin, tobramycin, and amikacin. Nonfermentative gram-negative bacilli were identified by the methods of Pickett and Petersen (14). Other gram-negative bacilli were identified by the method of Edwards and Ewing (3).

Antimicrobial susceptibility of the isolates was initially tested by standardized disk diffusion methods. Minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations were subsequently tested by broth dilution with microtiter techniques. The test medium was Mueller-Hinton broth; the inoculum was approximately 10^5 colony-forming units per ml of a 6-h broth culture; 0.05 ml of this inoculum was delivered to microtiter wells and the plates were incubated overnight in air at 37°C. The MIC was the lowest concentration of antibiotic showing no macroscopic growth. The minimal bactericidal concentration was performed with a microtiter 2000 inoculator (Cooke) and was defined as the lowest concentration showing no colonies on sub culturing 0.001 ml from the microtiter wells to antibiotic free Mueller-Hinton agar.

RESULTS

Forty-two patients, of whom 27 were men, were admitted to the study; 40 could be evaluated for efficacy. The mean age was 48.7 years (±16.7). Table 1 lists the sites of infections. Six (14%) of the infections were accompanied by bacteremia. Twenty-three patients were postsurgical. In four of these patients, mediastinitis and sternal osteomyelitis developed. Six patients had pre-existing renal disease; two of these had undergone renal transplantation, and three had accompanying urinary obstruction. Three patients had accompanying hematological disorders, and five patients had received immunosuppressive drugs. Two patients had neurological disease which led to recurrent aspiration pneumonia. Two infections were the result of trauma, one infection was associated with intravenous drug abuse, and one infection was related to cirrhosis.

Fever was present at the initiation of therapy in 35 of 42 patients; the mean peak rectal temperature was 38.6°C (standard deviation ± 0.9). Of the seven patients without fever, three had urinary tract infections and four had postoperative abscesses (sternal osteomyelitis). Leukocytosis occurred in 28 patients, and hypotension occurred in 2 patients.

Bacteriological studies in the 42 infections yielded a total of 60 organisms (Table 2); 13 patients had multiple pathogens. Escherichia coli, Pseudomonas aeruginosa, Klebsiella

<table>
<thead>
<tr>
<th>Table 1. Site of infection (42 patients)</th>
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</thead>
<tbody>
<tr>
<td>Infection site</td>
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<tr>
<td>-------------------------</td>
</tr>
<tr>
<td>Postoperative wound</td>
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<tr>
<td>Urinary tract</td>
</tr>
<tr>
<td>Pleuropulmonary</td>
</tr>
<tr>
<td>Intraabdominal abscess</td>
</tr>
<tr>
<td>Biliary</td>
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<tr>
<td>Soft tissue</td>
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<tr>
<td>Documented sepsis</td>
</tr>
<tr>
<td>Suspected sepsis</td>
</tr>
<tr>
<td>Bone and joint</td>
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<tr>
<td>Total</td>
</tr>
</tbody>
</table>

* Nine patients had two sites infected.
**TABLE 2. In vitro susceptibility of gram-negative bacilli isolated from 42 patients before netilmicin therapy**

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Total isolates (blood)</th>
<th>Median MIC (µg/ml) (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Netilmicin</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>11 (2)</td>
<td>1 (0.06–2)</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>9</td>
<td>1 (0.25–2)</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>7 (1)</td>
<td>1 (0.06–1)</td>
</tr>
<tr>
<td><em>Enterobacter sp.</em></td>
<td>6 (2)</td>
<td>0.5 (0.03–1)</td>
</tr>
<tr>
<td><em>P. mirabilis</em></td>
<td>4</td>
<td>1 (1–2)</td>
</tr>
<tr>
<td><em>P. Morganii</em></td>
<td>3 (1)</td>
<td>0.25 (0.03–1)</td>
</tr>
</tbody>
</table>

Total 60 (6)*

* There were 18 other pathogens isolated, including the following: enterococcus, 5; anaerobes, 5; *S. aureus*, 3; *Serratia marcescens*, 2; *Acinetobacter anitratus*, 2; *Proteus rettgeri*, 1; *Hemophilus influenzae*, 1, and *α*-streptococcus, 1. Thirteen patients had mixed infections.

**TABLE 3. Development of resistance to netilmicin during therapy in four patients**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Pretreatment MIC (µg/ml)</th>
<th>Post-treatment MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. aeruginosa</em></td>
<td>4</td>
<td>32</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>0.5</td>
<td>4</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td><em>P. mirabilis</em></td>
<td>2</td>
<td>8</td>
</tr>
</tbody>
</table>

**pneumoniae**, and *Enterobacter* species were the most frequent gram-negative bacilli encountered. Enterococci and multiple anaerobic organisms were isolated frequently in addition to the aerobic gram-negative bacilli.

Serum levels of netilmicin were obtained during treatment in most patients. Of 42 patients, 27 had appropriately timed serum specimens obtained within 72 h of the initiation of therapy. The geometric mean peak level 0.5 h after intravenous injection was 5.91 µg/ml (range, 2.9 to 18.8). The geometric mean trough level was 1.75 µg/ml (range, 1.0 to 8.3).

**Clinical evaluation.** Netilmicin treatment could be evaluated for efficacy in 40 of 42 gram-negative infections. With regard to side effects, 37 patients could be assessed for nephrotoxicity, and 26 could be assessed for ototoxicity. Of the 40 evaluable patients, 24 (60%) patients satisfied the criteria for cure. Eight of 40 (20%) patients achieved a response by our criteria; 3 of the 8 responders were changed to potentially less toxic therapy; and the remaining 5 patients did not have adequate bacteriological studies, although there was an apparent clinical response. There were 8 (20%) failures. Among these failures, three were due to the persistence of undrained abscesses, two patients were granulocytopenic, and one patient had Legionnaire's disease upon retrospective serological determination. (He was thought to have recurrent aspiration pneumonia at the time of the study.)

**Netilmicin resistance.** Four patients were infected by organisms in which resistance to netilmicin developed during therapy. Three of these patients were therapeutic failures. The organisms and their change in MICs are listed in Table 3. One organism was repeatedly isolated from blood, two were isolated from wound abscesses, and one was from an ileal loop. Although three of the isolates were from mixed infections, in no instance did these infections contain a pretreatment strain which was resistant to netilmicin; thus, there was no evidence of interbacterial transfer of resistance. The onset of resistance was first noted between days 18 and 28 of therapy in three patients, and after 3 days in the fourth.

Three of the four netilmicin-resistant strains retained susceptibility to the other aminoglycosides. The fourth individual was a neutropenic patient in whom a strain of *K. pneumoniae* became resistant to gentamicin and tobramycin after 3 days of netilmicin therapy; resistance to amikacin and netilmicin supervened on day 18 of therapy.

Two patients developed a superinfection in conjunction with therapy, due in each instance to new pathogens. One developed *Candida* and *P. aeruginosa* in a chest wound, whereas the other developed *P. aeruginosa* and *S. aureus* pneumonia. The urine of another patient became colonized with *Torulopsis glabrata*.

**Toxicity evaluation.** The major toxicity was that affecting the kidney. Eight of 37 (21%) patients displayed deteriorating renal function. Analysis of risk factors in these patients revealed that six had received prior gentamicin, three had had prior nephrotoxicity from gentamicin, and one patient had a solitary kidney. The median interval from the onset of therapy to the first rise in creatinine was 12.1 days. The degree of renal impairment was mild in six, but severe in two patients who had rising serum creatinine just before death and acute tubular necrosis at
autopsy. The mean peak creatinine was 2.6 mg/dl (±1.1). Of the six patients with nephrotoxicity who lived, three returned to normal creatinine and two had a mild residual elevation (1.3 and 1.9 mg/dl); one patient was lost to follow-up. The patients who developed nephrotoxicity tended to receive netilmicin for a longer time period (mean of 12 days) when compared with the rest of the study patients (mean treatment of 10 days; not statistically significant by unpaired t test); they also received a larger total dose of netilmicin (5,410 versus 3,811 mg; not statistically significant by unpaired t test). The development of nephrotoxicity was accompanied by both high peak and trough levels of netilmicin. However, serum levels from these patients had been in the desired range before the onset of nephrotoxicity. None of 26 patients developed audiological toxicity.

Four patients developed mild alkaline phosphatase abnormalities. All patients remained asymptomatic, and these values returned to normal during the course of hospitalization. Three of the four patients developed this abnormality in the early postoperative period; one had a subhepatic abscess, and two patients were receiving intravenous oxacillin.

In vitro study. The median MICs of netilmicin, gentamicin, and tobramycin for pretreatment isolates of most gram-negative bacteria ranged from 0.5 to 2 μg/ml, and the values for individual strains were ≤4 μg/ml in all but two instances (Table 2). Both involved strains for which the MIC of tobramycin was 8 μg/ml. Thus, there was no clear superiority in vitro of one drug over another with regard to netilmicin, gentamicin, or tobramycin. With regard to amikacin, median MICs for initial isolates ranged for 2 to 4 μg/ml and were as high as 16 μg/ml in three instances.

DISCUSSION

Netilmicin appears to be an effective agent in the therapy of serious gram-negative infections. Our cure rate was 60%. An additional eight patients had a response, making an overall rate of efficacy of 80%. This rate is similar to that in a controlled trial of amikacin and gentamicin (15), as well as other recently reported series (7, 12, 16).

The majority of failures in the present study could be attributed to surgically unresectable abscesses and persistent unremitting pancytopenia; however, three of the patients who failed had organisms which developed resistance to netilmicin during therapy. In two instances netilmicin was the only aminoglycoside to which resistance developed. We did not perform serotyping or other tests to prove that the resistant isolates were derived from the original susceptible strains; however, the fact that the organism could be cultivated throughout therapy in each instance suggests that these were not suprainfections. The development of resistance in almost 10% of isolates may have important therapeutic implications.

Serum levels of netilmicin were in the therapeutic range in 20 of 27 (75%) patients within the first 72 h of therapy; however, wide variability among patients was apparent in both peak and trough values. We attribute this variability to the changing physiological status of postoperative patients, with fluctuating levels of hydration, renal function, and cardiovascular function. These levels are comparable to those reported by others (7, 12, 16). In seriously ill patients, a dose of 2.5 mg/kg every 8 h would appear to afford adequate levels more regularly than 2 mg/kg. Because the resulting levels in the serum are rather unpredictable, it may be prudent to measure them shortly after the initiation of therapy.

The use of netilmicin was accompanied by appreciable nephrotoxicity. Eight of 37 (21%) patients developed some decrease in renal function. This rate of nephrotoxicity is somewhat higher than that seen with amikacin or gentamicin (10%) (15), but is not substantially different from that previously reported with netilmicin (16%) (12) in a study in which the definition of nephrotoxicity was the same as ours. The patients that developed nephrotoxicity were treated for a longer period of time and received a greater total dose than the patients who did not develop this adverse effect. Furthermore, six of these eight patients had received aminoglycoside antibiotics in the past, and three of them had developed renal impairment during those prior courses of therapy. Such patients may constitute a high risk group. The degree of nephrotoxicity was generally mild; however, two patients died from overwhelming sepsis and had acute tubular necrosis at autopsy. Although the sepsis undoubtedly contributed to their renal failure, both patients had rising serum creatinine concentrations before the development of hypotension and subsequent death. Of the remaining six patients, three returned to normal renal function, two patients had mild residual decrease in renal function, and one was lost to follow-up.

Despite the significant nephrotoxicity, none of 26 patients developed any auditory or vestibular toxicity. This compares favorably with experience with other aminoglycosides in which one might have expected a 5 to 10% risk of ototoxicity (15), but differs from a recent report by Trestman et al. (16). Although a precise definition of ototoxicity was not enumerated in this
report, the decreased hearing reported would fit our definition of toxicity.

Mild alkaline phosphatase abnormalities were noted in four of our patients without other changes in liver function. All four patients were postoperative, and these mild changes were regarded as nonspecific. In three, the abnormalities could be explained by the presence of liver disease or the concomitant use of oxacillin. In no instance did the alkaline phosphatase abnormality persist. As noted by Panwalker et al., who found alkaline phosphatase elevations in 40% of their patients, the etiology and significance of this finding deserves further study (12).

The susceptibility studies in our laboratory showed no clear superiority in vitro of one aminoglycoside over another for the small number of isolates examined.

The results of this study indicate that netilmicin is an effective therapeutic agent. Although the incidence of nephrotoxicity was moderate and that of ototoxicity was low, controlled trials will be necessary to accurately compare its propensity to produce these adverse effects with that of other aminoglycosides.

LITERATURE CITED