

Pharmacodynamic Parameters and Toxicity of Netilmicin (6 Milligrams/Kilogram/Day) Given Once Daily or in Three Divided Doses to Cancer Patients with Urinary Tract Infection

P. VAN DER AUWERA,^{1*} F. MEUNIER,¹ S. IBRAHIM,² L. KAUFMAN,³ M. P. DERDE,³ AND P. M. TULKENS²

Service de Médecine et Laboratoire d'Investigation Clinique H.J. Tagnon, Clinique des Maladies Infectieuses et Laboratoire de Microbiologie, Institut Jules Bordet, Centre des Tumeurs de l'Université Libre de Bruxelles, Rue Héger-Bordet 1, 1000 Brussels,¹ and Laboratoire de Chimie Physiologique, International Institute of Cellular and Molecular Pathology, Université Catholique de Louvain,² and Farmaceutische Instituut, Vrije Universiteit Brussel,³ Brussels, Belgium

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The pharmacologic parameters and toxicity of netilmicin (6 mg/kg/day) given once daily (qd) or thrice daily (tid) for the treatment of urinary tract infections were studied in a randomized prospective study of 60 cancer patients. The overall efficacy was 96%. Nephrotoxicity, assessed by the measure of urinary excretion of phospholipids, was lower for the patients receiving the qd regimen than for those receiving the tid regimen. Elevation of serum creatinine (20% over baseline) occurred in one patient receiving the qd regimen and in three receiving the tid regimen. Cochleotoxicity, assessed by pure-tone audiometry (250 to 18,000 Hz) occurred in one patient receiving the qd regimen and none receiving the tid regimen. Concentrations in sera were measured on days 1 and 5. No significant accumulation was observed in either group. Median serum bactericidal titers, expressed as reciprocal values (percentage of the sera with a titer ≥ 8), were measured against 25 test organisms in samples collected 6 h after the administration of netilmicin and were, for the qd group, 16 (82%) against members of the family *Enterobacteriaceae* and <2 (8%) against *Pseudomonas aeruginosa*, and for the tid group, 4 (57%) against members of the *Enterobacteriaceae* and <2 (0%) against *P. aeruginosa*. The rate of killing in serum was rapid (2 to 3 log in 2 h against *P. aeruginosa*; 3 to 5 log in 2 h against members of the *Enterobacteriaceae*) and correlated with the sampling time and hence the concentration in serum of netilmicin. The duration of the postantibiotic effect in serum depended also on the strain and the sampling time of the serum.

Gram-negative infections are a major cause of morbidity in cancer patients, especially when they are neutropenic. Treatment of these infections consists of the administration of a broad-spectrum β -lactam antibiotic often in combination with an aminoglycoside. The use of combination therapy is advocated for infections due to *Pseudomonas aeruginosa* and is particularly important when microorganisms resistant to the β -lactam antibiotic are responsible for the infection. A favorable outcome in patients with gram-negative bacillary bacteremia or pneumonia treated with aminoglycosides is related directly to the initial peak concentration in serum (20, 21) and to achieving a ratio of initial peak serum concentration to the MIC for the offending pathogen of greater than 6:1 (19). These observations confirm previous studies on the predictive value of the serum bactericidal titer on the outcome of gram-negative bacillary bacteremia (26, 33). In addition, a prolonged postantibiotic effect (PAE) has been observed with aminoglycosides in vitro and in experimental infections (31, 32). The main side effects of aminoglycosides are ototoxicity and nephrotoxicity (4, 8, 17, 28). Studies in animals (1, 11, 16, 23, 34) have suggested that both nephrotoxicity and ototoxicity are lower when the daily dose is given as a single administration rather than in two or three divided doses over 24 h. A prospective study comparing netilmicin given once daily (qd) versus thrice daily (tid) at a daily dose of 6.6 mg/kg in combination with ampicillin and tinidazole in the treatment of pelvic inflammatory disease

showed a reduced level of renal alteration (as detected by phospholipid urinary excretion) and cochleotoxicity in the qd regimen as compared with the tid regimen (28). (As recommended by Jablonski [13], we have used throughout this paper the abbreviations qd and tid to refer to the administration of the daily dose in one administration per day [quaque die] or divided in three equal administrations at 8-h intervals [ter in die].)

Preliminary studies have suggested a similar clinical efficacy for aminoglycosides whether given qd, twice daily, or tid (9, 18).

The purpose of the present investigation was to further evaluate the tolerance of netilmicin given qd versus tid in cancer patients suffering from urinary tract infections who were treated with netilmicin alone. It should be noted that this is the only clinical situation in which aminoglycosides are given alone. In addition, blood samples were obtained from these patients to derive several pharmacodynamic parameters which may be predictive of efficacy in treating gram-negative bacillary bacteremia and bronchopneumonia, including the serum bactericidal titers, the rate of killing in serum (7), and the duration of the PAE in serum (30).

MATERIALS AND METHODS

The protocol for this study was reviewed and approved by the Ethical Committee of the Institut Jules Bordet, Brussels, Belgium. All patients gave informed consent.

Patients. Patients with documented urinary tract infection (two different urinary samples with >5 neutrophils per

* Corresponding author.

high-power field and $\geq 10^5$ CFU of bacteria per ml) due to ampicillin- and co-trimoxazole-resistant but netilmicin-susceptible microorganisms were eligible to participate in the study and were included after giving informed consent. Renal failure (serum creatinine, >2.0 mg/dl) was the only exclusion criterion.

Administration of the antibiotic. The patients were randomized to receive netilmicin at 6 mg/kg/day for 7 days either as a single qd injection or tid. Netilmicin was given by a 30-min intravenous infusion in 250 ml of 5% glucose in water. Some patients were treated intramuscularly (four qd, eight tid). For patients receiving intravenous treatment, blood samples were obtained on days 1 and 5 immediately after the end of infusion and at 30 min, 60 min, 120 min, and 6 h thereafter. Serum was separated from clotted blood and immediately stored at -80°C until used.

Evaluation of efficacy. Urine cultures were performed twice weekly. A minimal follow-up culture at 3 weeks was obtained. Cure was defined as eradication of the infection during treatment with no further urinary tract infection due to the same organism during the follow-up period. Relapse was considered to be when the same bacterial species with the same antibiotic susceptibility pattern as the initial isolate was recovered from at least two different urine samples in the presence of pyuria during the follow-up period. Reinfection was considered to be when another bacterial species or the same species but with a different antibiotic susceptibility pattern from the initial isolate was recovered during the follow-up period.

Patients were considered unevaluable for efficacy (i) in the event of protocol violation (e.g., netilmicin-resistant microorganism, addition of another antimicrobial agent, no follow-up culture), (ii) in cases of major intolerance requiring treatment interruption, or (iii) in the event of death during treatment that was unrelated to the netilmicin treatment.

Evaluation of toxicity. (i) **Nephrotoxicity.** Renal dysfunction was assessed by measuring serum creatinine. The criterion for toxicity was a 20% elevation in creatinine over baseline or an increase above 1.5 mg/dl (132.6 $\mu\text{mol/liter}$). Alteration of renal cortex was assessed by the measurement of urinary phospholipids. Urinary phospholipid excretion has been shown to correlate with aminoglycoside-induced tubular alterations in animals (12, 14) and was already used in a previous evaluation of the safety of netilmicin given qd (29). Assay of total phospholipids and of phosphatidyl-inositol was made on samples of urine collected over 24 h and treated as previously described, and results were expressed as nanomoles per milligram of creatinine. Creatinine was assayed by the Jaffe method.

(ii) **Cochleotoxicity.** Pure low-tone (bone and air) audiometry (Amplaid model A150 audiometer) and high-tone (air) audiometry (Interacoustics model AS10 audiometer) were performed before, during, and after treatment. Only cooperating patients (providing reproducible answers at each tone tested, within 5 dB) were tested. Furthermore, audiograms were considered to be evaluable only if pretreatment profiles showed thresholds above -30 dB at 8,000 Hz and above -70 dB at 12,000 Hz. Criteria for toxicity was a decrease in the threshold of 15 dB on two adjacent frequencies (3) over the whole range of frequencies investigated (250 to 18,000 Hz).

Pharmacodynamic evaluation. (i) **Test strains.** Five strains each of *P. aeruginosa*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Serratia marcescens*, and *Escherichia coli* were selected. The 25 strains were recent blood isolates from cancer patients of the Institut Jules Bordet.

(ii) **Susceptibility testing.** MICs and MBCs were measured

for all strains by microtiter serial dilution in Mueller-Hinton broth supplemented with CaCl_2 and MgSO_4 (50 and 20 mg/liter, respectively). The final inoculum in each well was 10^6 CFU/ml. MBC determinations were done by subculturing 10 μl of each well on drug-free agar. The criterion for MBC was a 99.9% killing of the original inoculum (22).

(iii) **SBA.** Serum bactericidal activity (SBA) against the test strains was determined for each serum sample taken at 6 h on days 1 and 5. Serum titration was done in a microtiter system, with a 1:1 mixture of calcium-and-magnesium-supplemented Mueller-Hinton broth and normal human serum as the diluent (24). Inoculum concentration and sampling for bactericidal determination were the same as described above. Results were expressed as the median reciprocal SBA for each microbial species at a given time and drug regimen and as a percentage of serum samples with a reciprocal SBA of ≥ 8 .

(iv) **Serum assays.** Netilmicin was assayed by using a fluorescence-polarization immunoassay (TDx; Abbott sa, Louvain-la-Neuve, Belgium). This assay has a sensitivity of 0.5 $\mu\text{g/ml}$ and variabilities of 7.5% at 5 $\mu\text{g/ml}$ and 8.2% at 10 $\mu\text{g/ml}$. Serum samples with a netilmicin concentration greater than 15 $\mu\text{g/ml}$ were diluted with control serum prior to assay.

(v) **Rate of killing in serum samples.** All serum samples obtained at 6 h (on days 1 and 5) after the end of infusion were pooled for each different regimen and stored at -80°C , and then each pool was tested for the killing rate (30). After the pooled samples were diluted 1:2 in Mueller-Hinton broth (final volume, 2 ml), supplemented with Ca and Mg or not, time-kill curves were graphed for five strains of each species (total, 25 strains). The initial bacterial inoculum was 10^6 CFU/ml at time zero. All tubes were placed on a rotary shaker at 37°C and agitated throughout the experiment. Sampling was done at time zero and at 2, 4, 6, and 24 h by using a 10- μl calibrated loop. Suitable 10:10 dilutions were made and plated by being spread on Mueller-Hinton agar, and colonies were counted after overnight incubation.

(vi) **PAE in serum.** To test for the PAE in serum, the dilution method in broth of Craig et al. was used (5). Aliquots of each serum sample obtained at 1 and 6 h after administration were pooled. Killing rates were measured as described above by using one strain from each species and drug susceptibility pattern and tested in duplicate in tubes containing 1 ml plus 10 μl of broth containing the bacterial inoculum. The starting inoculum was 10^7 CFU/ml. After 30 min of contact with netilmicin-containing serum samples or fresh pooled human sera for the control tube, the tubes were diluted 1,000-fold with antibiotic-free fresh medium. The growth was monitored afterwards by sampling at time zero and at 30 min and 2, 4, and 24 h after dilution. The PAE was expressed as the difference between the time required for the test tubes exposed to the antibiotic to reach 1 log CFU/ml above that obtained after 1,000-fold dilution and the time required for the control tube to reach a similar level of inoculum.

Statistics. All tests were done by using Statview 512+ software (v.1.1, Abacus Concepts, 1986) run on a Macintosh SE30 computer (Apple, Inc.).

Comparisons of proportions were done by Fisher's exact test. Comparisons of the serum bactericidal titers obtained with the two regimens was done by using the Wilcoxon matched-pairs test. The potential accumulation of netilmicin during treatment was assessed by analysis of variance. Student's paired test was used to compare the rates of killing derived from the killing curves in serum samples. Student's

TABLE 1. Characteristics of the patients

Patient group	Total no. of patients (M/F) ^a	Age range (yr) ^b	Wt range (kg) ^b	Dose range (mg) ^b	No. of patients			No. of patients treated ^d			
					With indwelling catheter	With underlying urinary tract disease	Duration of treatment (range in days) ^c	For 7 days (%)	i.m. all course	i.m. partially	Previously with aminoglycoside
Netilmicin qd	30 (10/20)	43–89 (66.4)	38–96 (61)	230–576 (365)	12	7	2–9 (7)	26 (86.7)	4		1
Netilmicin tid	30 (11/19)	45–79 (66.2)	45–95 (67)	90–190 (136)	18	9	1–10 (7)	24 (80.0)	6	2	2

^a M, male; F, female.

^b Mean shown in parentheses.

^c Median shown in parentheses.

^d i.m., Intramuscularly.

unpaired test was used to compare the values of phospholipid excretion. Two-tailed tests were done to assess statistical significance. Statistical significance was considered for *P* values of ≤ 0.05 .

RESULTS

Clinical study. (i) Patients. A total of 60 patients were enrolled in the study; 30 patients received netilmicin qd, and 30 patients received netilmicin tid. All patients had cancer, and 16 had underlying urinary tract disease (Table 1). Thirty patients had urinary indwelling catheters at the time of randomization. The two groups were comparable for sex distribution, age, weight, and duration of the treatment. Among the 60 patients, 3 had previously been exposed to an aminoglycoside within 1 year.

The incidences of previous (within 10 days) or concurrent administration of potentially nephrotoxic drugs, including anticancer chemotherapy (five qd, four tid), diuretics (three qd, two tid), and nonsteroidal anti-inflammatory drugs (four qd, six tid), were similar in the two groups. Previous antibiotic administration (within 10 days) had occurred in three tid patients (teicoplanin, cefazolin, co-trimoxazole) and none of the qd patients.

(ii) Efficacy. Table 2 shows that, among the evaluable patients, the efficacy was equivalent in both groups, with an overall efficacy of 96%. Two patients in the tid group had an unfavorable response, one with *E. coli* and one with *K. pneumoniae*. Table 3 details the patients who were not considered to be evaluable for efficacy. Table 4 details the patients whose treatment failed and those who relapsed or were reinfected.

(iii) Tolerance. Tolerance was in general excellent. One patient from each group had nausea during the infusion of netilmicin. Four patients had a 20% elevation of serum creatinine over baseline, one in the qd group and three in the tid group (Table 5). This difference was not statistically significant. Elevation of serum creatinine above 1.5 mg/dl (132.6 μ mol/liter) was observed in only one patient from the tid group. Significant cochleotoxicity was observed in one patient in the qd group.

To allow a comparison with the study of Tulkens et al. (29), patients with any alteration of ≥ 15 dB in the pure-tone air audiogram were considered to have possible netilmicin-related cochleotoxicity. For the qd regimen, the following decreases were observed in audiograms for the left (L) and right (R) ears of the patients (total netilmicin dose for the patient shown in parentheses): L, 9 to 10 KHz, 15 to 20 dB (1,610 mg); R, 0.25 to 0.5 KHz, 15 to 25 dB (1,610 mg); L, 1.5

KHz, 35 dB (2,160 mg); L, 16 KHz, 35 dB (2,520 mg); R, 8 KHz, 20 dB (3,080 mg). For the tid regimen, the following decreases were observed: R, 8 KHz, 20 dB (3,024 mg); L, 1.5 KHz, 15 dB (3,402 mg).

Figure 1 shows the evolution in total urinary phospholipids during treatment. The initial elevation (value on day 4 to 7 minus value on day 0 to 1) was higher in the tid group than in the qd group ($P = 0.09$), but both groups showed similar elevations on day 7. Upon treatment interruption, the total urinary daily excretion of phospholipid returned to pretreatment values, but this return seemed to be faster for patients treated qd. A similar pattern was observed for the urinary excretion of phosphatidylinositol (data not shown).

(iv) Concentration of netilmicin in serum. Figure 2 shows the concentrations of netilmicin in serum in both groups. No significant accumulation from day 1 to day 5 was observed by using an analysis of variance test. The concentrations in serum obtained in patients from the qd group were three times higher than those measured in patients from the tid group.

Pharmacodynamic evaluation. (i) In vitro susceptibility of

TABLE 2. Evaluation of efficacy

Characteristic	No. of patients	
	Netilmicin qd	Netilmicin tid
Evaluable for efficacy	26	22
Microbiological response		
Eradication	26	20
Failure rate		
No eradication	0/26	1/22
Relapse	0/26	1/22
Reinfection	3/26	4/22
Microorganisms isolated		
Two organisms isolated	3	3
<i>Escherichia coli</i>	19	10 (1) ^a
<i>Proteus mirabilis</i>	4	3
<i>Proteus vulgaris</i>		1
<i>Klebsiella pneumoniae</i>	3	6 (1) ^a
<i>Enterobacter cloacae</i>		1
<i>Pseudomonas aeruginosa</i>	2	4
<i>Citrobacter freundii</i>	1	
Outcome		
Death during treatment	1	2
Death within 1 mo	2	5

^a Number in parentheses indicates number of patients who experienced relapse.

TABLE 3. Patients inevaluable for efficacy

Patient group	Age (yr) ^a	Day of withdrawal	Dose (mg)/no. of doses	Reason for withdrawal
Netilmicin qd	64 (F)	2	325/2	Severe nausea and moderate diarrhea probably related to netilmicin Blood sampling impossible (no venous access); serum creatinine rose from 1.2 to 1.4 mg/dl <i>P. aeruginosa</i> resistant to netilmicin Death from cardiac arrhythmia (unrelated to netilmicin injection)
	74 (M)	4	420/4	
	66 (M)	4	260/4	
	76 (F)	3	270/3	
Netilmicin tid	68 (F)	2	140/3	Severe nausea probably related to netilmicin From day 2 to 4; ceftazidime administration Neutropenia, empiric treatment with ceftazidime plus amikacin Ampicillin given from day 3; death on day 15 unrelated to netilmicin No follow-up culture Cefepime given from day 2 empirically for bronchopneumonia; day 5, serum creatinine rose from 1 to 1.5 mg/dl; day 6, death unrelated to netilmicin Ampicillin plus metronidazole given from day 2 for purulent lymphadenopathy found at surgery (day 1) Death from superinfection on day 4: septicemia due to <i>Salmonella</i> (unknown origin)
	66 (F)	2	120/30	
	59 (F)	1	120/2	
	62 (M)	3	134/21	
	68 (M)	7	180/21	
	79 (M)	2	150/18	
	68 (M)	2	125/21	
	57 (F)	3	176/8	

^a F, Female; M, male.

the test strains. The following test strains were used (five for each species) (netilmicin MIC and MBC ranges shown in milligrams per liter): *E. cloacae* (MIC, 0.1 to 1.6; MBC, 0.1 to 12.5), *K. pneumoniae* (MIC, ≤ 0.05 to 0.2; MBC, ≤ 0.05 to 0.2), *S. marcescens* (MIC, ≤ 0.05 to 1.6; MBC, 0.1 to 6.2), *E. coli* (MIC, 0.2 to 0.8; MBC, 0.4 to 6.2), *P. aeruginosa* (MIC, 6.2 to 25; MBC, 6.2 to 50).

(ii) **Serum bactericidal titers.** Table 6 shows that the mean reciprocal titers obtained in patients from the qd group were usually two to three times higher than those obtained in patients from the tid group on day 1. Moreover, there was an excellent correlation between predicted (concentration in serum divided by the MBC) and measured reciprocal serum bactericidal titers (Spearman coefficient of correlation r , 0.891; $P < 0.001$). Low titers were observed with the strains of *P. aeruginosa*.

(iii) **Rate of killing in serum.** Figure 3 shows the killing rates measured by using the serum samples collected at 6 h

and the test strains. Killing curves for *E. coli* (not shown) were almost identical to those obtained for *K. pneumoniae*. The killing rates observed for members of the *Enterobacteriaceae* were high and were not different whether netilmicin was given qd or tid. Against *P. aeruginosa* and *S. marcescens*, the qd administration resulted in a higher killing rate than the tid administration. Regrowth was observed with the strains of *P. aeruginosa* for both types of administration. This regrowth occurred in 60% of the tests (serum-strain combination) irrespective of the mode of administration. For the regrowing colonies, a two- to eightfold increase in the MIC of netilmicin occurred.

(iv) **PAE.** Table 7 shows the PAE duration in serum for two strains of each species tested in duplicate. Reproducibility of the measurement of duration of the PAE was strain dependent and was good for *E. coli*, *E. cloacae*, and *K. pneumoniae* (≤ 0.5 h between duplicates), moderate for *P. aeruginosa* (0.5 to 1 h between duplicates), and poor for *S.*

TABLE 4. Failures, relapses, and reinfections

Parameter	Patient group	Patient no.	Isolate	
			Initial	Reinfection (day)
Failure within treatment (no eradication)	tid	4	<i>Klebsiella pneumoniae</i>	
Relapse after eradication	tid	1	<i>Escherichia coli</i>	Day 4
Reinfection after treatment	qd	18	<i>Proteus mirabilis</i>	<i>Pseudomonas aeruginosa</i> susceptible to netilmicin (day 20)
		25	<i>Escherichia coli</i>	<i>Salmonella epidermidis</i> susceptible to netilmicin (day 5)
		44	<i>Pseudomonas aeruginosa</i> ^a	<i>Pseudomonas aeruginosa</i> resistant to netilmicin after temporary sterilization ^a (day 9)
	tid	16	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i> resistant to netilmicin (day 10)
		22	<i>Klebsiella pneumoniae</i> and <i>Proteus mirabilis</i>	<i>Pseudomonas aeruginosa</i> resistant to netilmicin
	42	<i>Escherichia coli</i> ^a	<i>Escherichia coli</i> resistant to netilmicin after temporary sterilization ^a (day 6)	
	47	<i>Proteus mirabilis</i>	<i>Enterobacter faecalis</i> (day 7)	

^a Pairs of strains (initial, reinfection) were not typed.

TABLE 5. Evaluation of toxicity

Patient group	No. of patients showing toxicity/ total no. of patients evaluable			
	Serum creatinine		Cochleotoxicity (≥ 15 dB loss)	
	20% increase	1.5 mg/dl	Two frequencies	Any single frequency (air)
Netilmicin qd	1/26 ^a (3.8)	0	1/14 ^b	4/14 ^c
Netilmicin tid	3/22 ^a (13.6)	1 ^d	0/8 ^b	2/8 ^c

^{a,b,c} Values with identical superscripts were not significantly different by Fisher's exact test.

^d Actual value, 2.2 mg/dl.

marcescens (≥ 1 h between duplicates). Duration of the PAE was dependent on the strain and time of sampling (by analysis of variance, $P \leq 0.01$), with the exception of *P. aeruginosa* for which the duration of the PAE was short.

DISCUSSION

Administration qd of an aminoglycoside may represent a significant improvement in the management of patients, provided that efficacy is not decreased and toxicity is not increased. The primary purpose of this study was to assess tolerance and pharmacodynamic parameters of netilmicin administered as qd or tid regimens. Comparison of the efficacy of netilmicin given qd or tid was not the primary purpose of the present study, since the number of patients in each group was not sufficient to detect a significant difference with an acceptable β error. However, within the limits of the study, the overall efficacy of the two regimens was high, with a rate of failures, relapses, and reinfections of 9 patients of 48 evaluable patients (18.7%) for a follow-up period of at least 3 weeks. Furthermore, most of the patients included in the study had asymptomatic urinary tract infections, half of which were associated with an indwelling catheter. Only one of the patients who could be evaluated showed significant cochleotoxicity as defined by an altera-

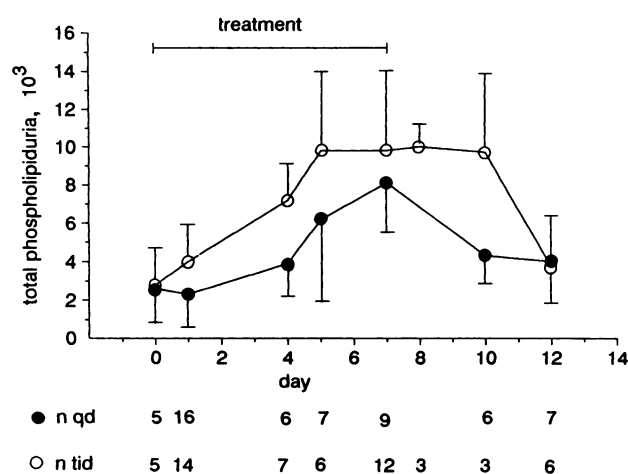


FIG. 1. Total phospholipid urinary excretion in patients receiving netilmicin (n) qd and tid during treatment and posttreatment periods. Results are means \pm standard deviation; the numbers indicate the numbers of patients examined for each point. The rate of increase of phospholipiduria between day 0 or 1 and day 4 or 5 is greater in the tid group than in the qd group ($P = 0.09$).

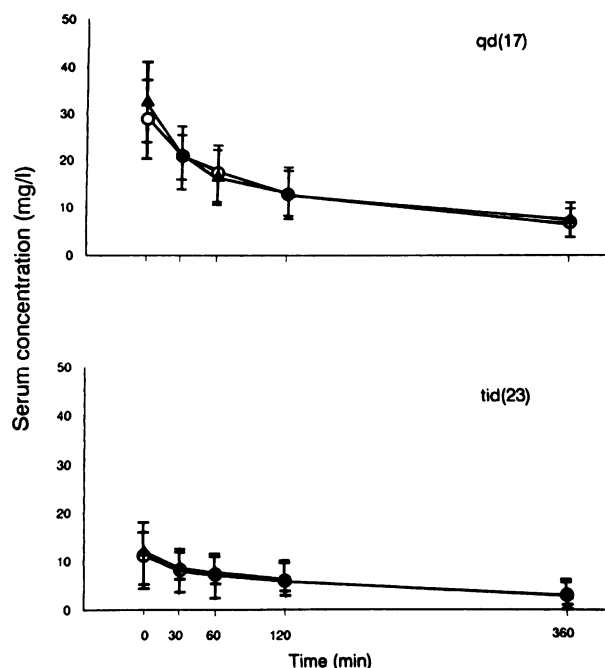


FIG. 2. Concentrations in sera of patients receiving netilmicin at 6 mg/kg/day as a single dose (qd; 17 patients) or in three divided doses (tid; 23 patients). Results are means \pm standard deviations. Symbols: \circ , day 1 of treatment; \blacktriangle , day 5 of treatment.

tion of at least 15 dB over two adjacent frequencies. This patient had received netilmicin qd for a total dose of 1,610 mg. Cochleotoxicity of aminoglycosides has seldom been studied in cancer patients since uncooperating patients cannot be included and the baseline audiogram is often already altered as a result of age or previous exposure to known or unknown ototoxic drugs, including diuretics, chemotherapy, and aminoglycosides, and to radiation therapy in the case of patients with head and neck cancer or brain metastasis. The toxicity of netilmicin given qd observed in this study is, however, not greater than that reported in a retrospective study of netilmicin given according to conventional schedules (4.4% at low frequency; 10) or in global analysis of a large number of clinical trials conducted with netilmicin up to 1983 (15). In a study of netilmicin in the treatment of

TABLE 6. SBA reciprocal titers in patients receiving netilmicin (6 mg/kg/day) either qd or tid^a

Dose regimen	Organism (no. of strains)	Day 1		Day 5	
		SBA median	% With titer >8	SBA median	% With titer >8
qd	<i>E. cloacae</i> (5)	32	96	16	100
	<i>K. pneumoniae</i> (5)	32	96	32	100
	<i>S. marcescens</i> (5)	8	64	8	68
	<i>E. coli</i> (5)	16	88	16	96
	<i>P. aeruginosa</i> (5)	<2	8	<2	4
tid	<i>E. cloacae</i> (5)	8	60	16	100
	<i>K. pneumoniae</i> (5)	8	85	32	100
	<i>S. marcescens</i> (5)	2	25	4	40
	<i>E. coli</i> (5)	4	35	16	60
	<i>P. aeruginosa</i> (5)	<2	0	<2	0

^a Serum samples were obtained 6 h after dosing on days 1 and 5.

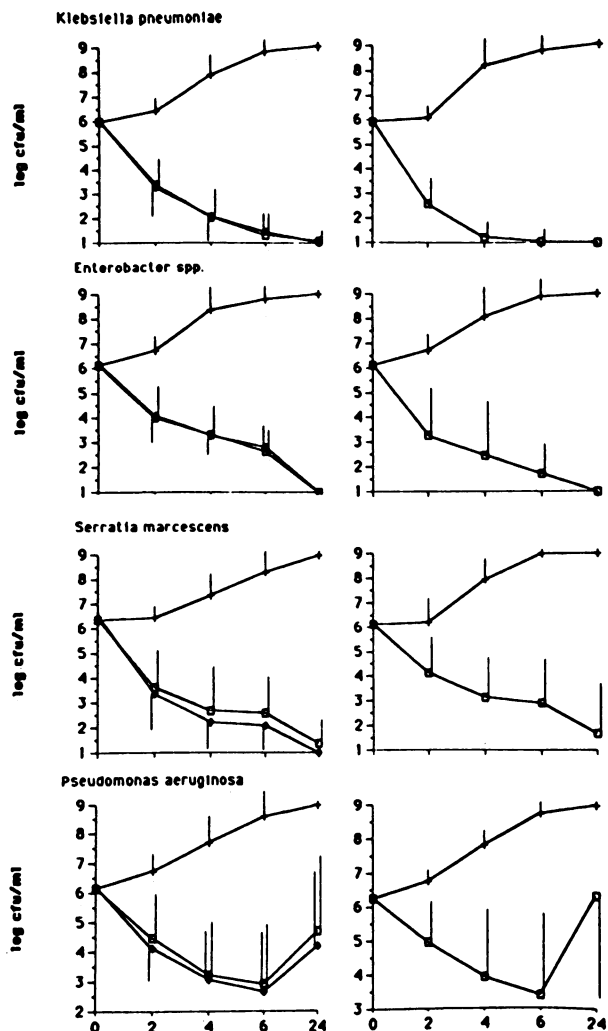


FIG. 3. Killing curves in serum samples of five strains each of *K. pneumoniae*, *Enterobacter* spp., *S. marcescens*, and *P. aeruginosa*. Results are expressed as the mean; vertical bars represent the standard deviations on viable counts. Curves on the left represent a pool of sera obtained 6 h after administration from patients who received netilmicin, 6 mg/kg, qd. Curves on the right represent a pool of sera obtained 6 h after administration from patients receiving the same daily dose tid. Symbols: +, control in serum without antibiotic; □, day 1 of treatment; ◆, day 5 of treatment.

pelvic inflammatory disease in young women, Tulkens et al. (29) observed no cochleotoxicity of netilmicin given qd in the low-frequency range and only 1 patient (of 19) with cochleotoxicity in the high-frequency range (3). By using the same criterion as Tulkens et al. (29) (i.e., any decrease of ≥ 15 dB), the occurrence of cochleotoxicity in the present study was not significantly different in the two groups. Nephrotoxicity, as assessed by a 20% increase in serum creatinine, was rare, occurring in 4 of 48 evaluable patients (8.3%). Of these, however, three were in the tid group and only one was in the qd group. Moreover, only one patient, in the tid group, had an elevation considered to be clinically significant. Recently, Ter Braak et al. (27) compared netilmicin given qd with netilmicin given tid in combination with ceftriaxone in 141 patients and reported an incidence of nephrotoxicity of 15 and 17%, respectively, when using more stringent criteria

TABLE 7. Duration of the PAE in serum^a

Strain	Time after dosing (h) on day 1	PAE duration ^b (h) for patients given netilmicin	
		qd	tid
<i>E. coli</i>	1	3.3, 3.5; ≥ 22 , ≥ 22	0.6, 1.2; ≥ 22 , ≥ 22
	6	0.3, 0.5; 3.5, 5	0.8, 1.3; 0.8, 1.6
<i>E. cloacae</i>	1	2.8, 3.2; 23, ≥ 24	1.5, 1.8; 3.5, ≥ 4
	6	2.3, 2.8; ≥ 4 , ≥ 4	0.5, 0.9; 1.9, 2.3
<i>K. pneumoniae</i>	1	4, 4.5; ≥ 24 , ≥ 24	0.25, 0.7; ≥ 21 , ≥ 21
	6	0, 0.5; 2.7, 3	3.3, 3.5; 5, 5.5
<i>S. marcescens</i>	1	≥ 2 , ≥ 2 ; 17, ≥ 20	0.3, 2; ≥ 19 , ≥ 19
	6	0, 3; ≥ 20 , ≥ 20	0.5, 3; ≥ 19 , ≥ 19
<i>P. aeruginosa</i>	1	0, 0.8; 1.5, 2.4	0, 0; 1.3, 2.2
	6	0, 0; 3, 4	0, 0; 2.4, 3.3

^a Two strains of each species were tested in duplicate. Bacteria were incubated for 30 min in pooled sera.

^b Strain 1; strain 2.

(i.e., 50% increase of serum creatinine over baseline and exceeding a concentration of 100 μ mol/liter). In a second study of netilmicin given qd versus netilmicin given tid (plus tinidazole) in 197 patients with intra-abdominal infection (6), nephrotoxicity (50% increase of serum creatinine over baseline) occurred in 10% of the patients regardless of the regimen. The measurement of urinary phospholipids is a sensitive method to detect aminoglycoside-induced tubular damage (12, 14, 29) and directly correlates with the earliest cellular alteration induced by these drugs (for a review, see reference 28). By using this method, the qd regimen seemed to induce a later and lower increase in phospholipid excretion than the tid regimen. In addition, the return to pretreatment baseline seemed to be more rapid with the qd regimen. Tissue accumulation of netilmicin was not observed as assessed by the comparison of the concentrations in serum at the beginning and end of treatment, further suggesting that the high peak concentrations in serum of netilmicin administered qd did not result in more renal accumulation than did the conventional schedule (for a discussion, see reference 25).

Similarly to what was observed in a previous study with amikacin given at doses of 15 and 30 mg/kg, the serum bacteriostatic activity and SBA titers correlated well with the expected values calculated from the concentration of netilmicin in serum and its MICs and MBCs (30). The rates of killing in serum samples collected at 6 h were increased by increasing the netilmicin concentration, although the difference was only significant for *S. marcescens* and *P. aeruginosa*. This rate was already high, and any increase would not be clinically significant. Regrowth was frequently observed with *P. aeruginosa* and was associated with an increase in the MICs of netilmicin and amikacin, suggesting a decreased transport across the inner membrane (small-colony variants). Similar findings were observed by Blaser et al. (2) in an in vitro pharmacokinetic model when the peak inhibitory concentration/MIC ratio remained below 10.

The duration of the PAE after 30 min of exposure to netilmicin-containing serum was longer in specimens obtained at the peak than in those obtained 6 h after administration. The longer PAEs were obtained with the 6-mg/kg dose. The duration of PAE depended on the strain tested; several strains were inhibited for an overnight period (15 h), whereas others were inhibited for only a few hours or less. Longer PAEs could be obtained by increasing the duration of exposure. Since the serum has been diluted twice in our in

vitro test, we can assume that in vivo the duration of exposure to a similar concentration is 1 to 2 h, and this is more likely to result in very prolonged PAE, allowing the use of netilmicin qd.

In conclusion, netilmicin at a 6-mg/kg dose provided higher peak levels in serum, serum bacteriostatic activity titers, and SBA titers and longer duration of the PAE than netilmicin at 2-mg/kg doses, and clinical efficacies were similar for the large dose given once every 24 h and the low dose given every 8 h. The two regimens caused similar cochleotoxicity and nephrotoxicity, whereas a comparison of the total urinary phospholipid excretion of the two regimens suggested that the qd regimen caused fewer cellular alterations in the kidney than the tid regimen.

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