

Bioequivalence Study of a Single 150-mg Dose of Two Intramuscular Netilmicin Products in Thai Healthy Volunteers

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ABSTRACT

Objective: Netilmicin, a broad-spectrum semisynthetic aminoglycoside antibiotic, is used in the treatment of a wide variety of bacterial infections. The purpose of this study is to compare the bioavailability of two products of 150 mg netilmicin by intramuscular administration in Thai healthy volunteers: The generic drug is Nelin® (test; Biolab Co., Ltd., Thailand) will be called “Test” and Netromycin® (reference; Schering-Plough Ltd., USA) will be called “Reference”.

Methods: A single-dose, 2-treatment, 2-period, 2-sequence, randomized crossover study was conducted in 24 healthy Thai volunteers. Each volunteer received a 150 mg intramuscular injection of the reference or test drugs. Blood samples were collected before dosing and at various time points up to 48 hours after dosing and analyzed for netilmicin concentrations using a validated liquid chromatography-mass spectrometry (LC-MS/MS) method. The pharmacokinetic parameters including C_{max} , AUC_{0-4} , $AUC_{0-\infty}$, T_{max} and $t_{1/2}$ were analyzed using the non-compartmental model. Drug safety and tolerability were assessed.

Results: Twenty-four volunteers completed both treatment periods. The geometric mean ratios (test/reference) between the two products of netilmicin were 100.30% (90% CI, 88.02%-114.28%) for C_{max} , 94.94% (90% CI, 88.94%-101.34%) for AUC_{0-4} , and 94.88% (90% CI, 88.85%-101.32%) for $AUC_{0-\infty}$. There was no significant difference of the T_{max} values between the two studied products ($P>0.05$). No adverse events related to the study drugs were found.

Conclusion: The two products of intramuscular netilmicin are bioequivalent. Both products are well tolerated.

Keywords: Bioequivalence, netilmicin, intramuscular injection

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Netilmicin, the 1-N-ethyl sisomicin derivative obtained from *Micromonospora inyoensis*, is a semisynthetic aminoglycoside. It is capable of killing a wide range of bacteria and possesses bactericidal activity against gram-negative bacilli resistant to gentamicin, sisomicin and tobramycin, including strains that produce aminoglycoside-adenylating enzymes.¹ It often provides synergistic effect with other antibiotics, such as beta-lactams, that contributes significantly to the empirical antibiotic treatment. Moreover, it is considered the least ototoxic and nephrotoxic aminoglycoside, particularly, when administered intramuscularly.^{2,3} Hence, netilmicin has been widely used for the treat-

ment of severe infections caused by gram-negative bacteria.⁴ A once-daily therapy of aminoglycosides including netilmicin (5-7 mg/kg every 24 h) has been suggested to provide therapeutic efficacy and possibly less toxicity if compared with multiple doses.^{5,6} Aminoglycosides including netilmicin are polycationic and possess high affinities for nucleic acids. They are capable of inhibiting bacterial protein synthesis by irreversibly binding to the 30S ribosomal subunit and the 16S rRNA.⁷ Pharmacokinetic characteristics of netilmicin are considered similar to those reported for the other aminoglycosides. It is not absorbed from the gastrointestinal tract and is therefore only administered by intravenous (i.v.) or intramuscular (i.m.) injection. In patients with normal renal function, the peak concentrations (C_{max}) in serum of 3.76, 5.6, 9.8 and 11 $\mu\text{g/ml}$ were observed after intramuscular (i.m.) administration

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of a 1-, 2-, 4- and 5-mg/kg dose, respectively.⁸⁻¹⁰ The time to achieve $C_{\max}$ ($T_{\max}$) ranged from 30 to 60 minutes. The volume of distribution of netilmicin is approximately 17 to 28% of body weight and it binds to serum proteins to a small extent.¹¹ While there is no evidence of biotransformation of netilmicin following parenteral administration, it undergoes some degree of tubular reabsorption, which appears to correlate with nephrotoxicity.² The serum half-life and elimination of netilmicin depends on renal function and dosage should thus be adjusted in patients with impaired renal function.^{12,13} In normal subjects, approximately 69-85% of the dose of netilmicin after i.m. or i.v. administration was excreted unchanged in the urine within 24 hours with the elimination half-life ($t_{1/2}$) of 1.3-3.5 hours.^{11,14}

Bioequivalence is defined by the US FDA as "the absence of a significance in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study".¹⁵ Comparable rate and extent of drug absorption that can be determined by pharmacokinetic parameters indicating systemic exposure are proposed to reflect comparable therapeutic effects.¹⁶

This study aims to assess the bioequivalence of two products of 150 mg intramuscular netilmicin: a generic drug, Nelin[®] (Biolab Co., Ltd., Thailand) will be called 'Test' and Netromycin[®] (Schering-Plough Ltd., USA) will be called 'Reference' in Thai healthy volunteers to determine whether the two products of netilmicin are similar in pharmacokinetics and adverse effects.

MATERIALS AND METHODS

Study drugs

Nelin[®] provided by Biolab Co., Limited (Thailand) and Netromycin[®] from Schering-Plough Ltd. (USA) were used as the test and reference products, respectively. Both products were prepared as netilmicin sulfate equivalent to netilmicin base 150 mg in a concentration of 100 mg/ml of solution (1.5 mL/vial).

Study population

The study was carried out at the Siriraj Clinical Research Center, Siriraj Hospital, Bangkok, Thailand. The study protocol was approved by the Ethics Committee of the Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand, and was performed in accordance with the Declaration of Helsinki Principles as outlined in the Guidelines for Good Clinical Practice (GCP). All subjects were given a detailed description of the study and written informed consent was obtained prior to the enrollment.

The twenty-four healthy male and female volunteers between the ages of 18-45 years with a body mass index between 18-24 kg/m² were assessed to be in good physical condition by a complete medical screening including a medical history, physical examination and laboratory screening test for hematologic and blood biochemistry parameters. Eligible volunteers did not smoke for at least 6 months before study entry. Female volunteers who were pregnant or lactating were not

eligible for participation. Subjects with a history of hypersensitivity to any ingredients in the netilmicin products or other aminoglycosides, or using any medication and alcohol consumption for a 14-day period prior to the study were excluded. Subjects who had a history of cardiovascular, hepatic, renal, gastrointestinal or hematologic diseases were excluded from the study.

Study design

The study was a randomized, single-dose, two-treatment, two-period and two-sequence crossover with at least one week washout period. Subjects were randomly allocated to two groups by the sequence of product administered [Test-Reference (TR) and Reference-Test (RT) group]. Each subject received a 150 mg netilmicin of the test or reference product by intramuscular injection at different sides of the dorsogluteal muscle for each period. The subjects were under continuous medical observation by a physician throughout the study and were discharged from the research ward after collecting the 24-h sample and returned for the 48-h sample. After a minimum of 1 week washout period, the subjects were crossed-over to the next treatment in the same procedure as conducted in the 1st period.

Sample collection

Blood samples (6 ml) were collected prior to drug administration (0 h) and at 0.25, 0.5, 1, 1.5, 2, 2.5, 4, 6, 8, 12, 24, and 48 h after dosing. The samples were collected into vacutainers for clotted blood samples. Serum was separated by centrifugation and then stored at -70°C until analysis.

Netilmicin analysis by LC-MS/MS

A liquid chromatography-tandem mass spectrometry (LC-MS/MS) method with positive ion electrospray ionization using multiple reaction monitoring (MRM) was developed and validated for the determination of serum concentrations of netilmicin. Serum netilmicin and pioglitazone hydrochloride as an internal standard (Dr. Reddy's Laboratories Ltd., India) were extracted by liquid-liquid extraction technique. A 10 μ l aliquot of internal standard (pioglitazone hydrochloride, 200 ng/ml) was added to a 100 μ l of serum sample or quality control sample and was mixed with 1,000 μ l of extract mixture, methyl-t-butyl ether and methanol (1:1, v/v). The mixture was then adjusted to pH 1.8 with a 50 μ l of 50% trichloroacetic acid and mixed at ambient temperature for 10 min and centrifuged at 12418 g for 10 min. All of the organic phase was evaporated to dryness under nitrogen gas and the residual was redissolved in acetonitrile:deionized water (20:80 v/v). A 20 μ l measure of each extracted sample was injected into the LC-MS/MS system.

The LC-MS/MS system consisted of an Alliance 2795 HPLC system (Waters Corp., Milford, MA, USA) and Quattro Micro-tandem mass spectrometer (Micro-mass UK Ltd., Manchester, UK). The analytical column was a Luna C18 (150 x 2 mm, 3 μ m material, Phenomenex Inc., Torrance, CA, USA). The mobile phase consisted of acetonitrile and 0.1% formic acid (21:79 v/v). The flow rate was 0.2 ml/min and the run time was 12 min. For the MS, the positive electrospray ionization source was operated using N₂ as a cone gas flow and desolvation gas flow, the flow rates were 20 l/h and 500 l/h and the desolvation and source tem-

peratures were 350°C and 120°C, respectively. Data acquisition and analysis were carried out using a Mass-Lynx version 4.0 software (Waters Corp.). The m/z transition for netilmicin was monitored from 476.24 to 229.19 and 476.24 to 191.20 and for the internal standard from 356.98 to 134.05. The retention times of netilmicin and internal standard were 3.96 and 8.18 min, respectively.

The validation of this method was performed as recommended by the USFDA (2001).¹⁷ Linearity calibration curves were established ranging from 0.5 to 20 µg/ml for netilmicin ($r^2 > 0.999$). The lower limits of quantification for netilmicin extracted from serum samples were 0.5 µg/ml. The mean recovery ranged from 79.37% to 100.56% at concentrations of 1.5, 9, and 18 µg/ml. Netilmicin (at concentrations of 1.5, 9, and 18 µg/ml) were also prepared to evaluate the within-run and between-run precision and accuracy of the assay. For within-run precision, the percent coefficients of variation (%CV) for netilmicin ranged from 2.381% to 10.796%. The %CVs for the between-run precision of the assay for netilmicin ranged from 3.839% to 5.614%. The percentage of recovery (%recovery) as a measure of within-run accuracy for netilmicin ranged from 93.425% to 103.322%. For between-run accuracy, the corresponding %recovery values for netilmicin ranged from 96.965% to 99.936%.

Pharmacokinetic and statistical analysis

Pharmacokinetic parameters of netilmicin were analyzed using a non-compartmental method. The area under the plasma concentration-time curve (AUC) was calculated using WinNonlin edition version 3.1. To evaluate the bioequivalence between the test and reference products, the maximum serum concentration (C_{max}), AUC_{0-t} (AUC from time 0 to the last measurable concentration) and $AUC_{0-\infty}$ (AUC from time 0 extrapolated to infinity) of netilmicin were considered to be the primary pharmacokinetic parameters. Values for C_{max} and T_{max} (time to maximal plasma concentration) of netilmicin were directly determined from the observed concentration-time data. The AUC_{0-t} was calculated using the log-linear trapezoidal rule and $AUC_{0-\infty}$ was calculated as $AUC_{0-t} + C_t/\lambda_z$, where C_t is the last measurable drug concentration and λ_z is the elimination rate constant obtained as the slope of the linear regression of the log-transformed concentration-time in the terminal phase. The terminal elimination half-life ($t_{1/2}$) was calculated using the equation $t_{1/2} = 0.693/\lambda_z$. ANOVA was used to evaluate the effects of products, period, sequence and subject within sequence on C_{max} , AUC_{0-t} and $AUC_{0-\infty}$. A nonparametric statistical method (Friedman test) was used to assess the differences in median T_{max} values between the 2 products at the 5% significance level. The bioequivalence between the two products was accepted if the 90% geometric confidence intervals of the ratio (test/reference) of least square means from the ANOVA of the log-transformed C_{max} , AUC_{0-t} and $AUC_{0-\infty}$ were within 80.00% to 125.00%.

Tolerability assessment

Physical examination and measurement of vital signs were performed at baseline prior to each study drug administration and were repeated at 24 and 48 h of blood sampling. Adverse events were monitored throughout the study and recorded by physicians.

RESULTS

Study population

Twenty four healthy Thai adults considered eligible for the study enrollment were randomly divided into 2 groups [Test-Reference (TR) and Reference-Test (RT)] according to the sequence of drug administration. All volunteers completed both study periods. Table 1 demonstrates the demographic characteristics, which were not statistically different between groups. There were 6 males and 6 females for the TR group ($n=12$) and 5 males and 7 females for the RT group ($n=12$), respectively. The mean $\pm$ SD of age, weight, height, and BMI were 21.8 ± 3.5 years, 57.8 ± 7.3 kg, 165.6 ± 7.9 cm, and 21.1 ± 1.7 kg/m² for the TR group ($n=12$); and 21.5 ± 2.0 years, 60.3 ± 10.4 kg, 167.9 ± 8.5 cm, and 21.2 ± 2.1 kg/m² for the RT group ($n=12$), respectively.

Bioequivalence analysis

The accuracy and precision of the analytical method (LC-MS/MS) in our study was determined by within-run and between-run analyses showing that %CVs did not exceed 15%.¹⁸ Fig 1 shows the mean plasma concentration-time profiles for netilmicin after intramuscular injection of the test and reference products. Detection of serum netilmicin levels at the first sampling time (15 min) in all volunteers indicated that it was rapidly absorbed following intramuscular injection. Additionally, the plasma concentrations of netilmicin declined to levels below the limit of detection by 24 h for both products. The pharmacokinetic parameters for netilmicin and bioequivalence analysis are summarized in Table 2. The geometric mean ratios (test/reference) for C_{max} , AUC_{0-t} and $AUC_{0-\infty}$ of netilmicin were 100.30%, 94.94% and 94.88%, respectively. The 90% CIs for C_{max} , AUC_{0-t} and $AUC_{0-\infty}$ were 88.02-114.28%, 88.94-101.34% and 88.85-101.32%, respectively, and were well within the bioequivalence acceptable range (80.00-125.00%).¹⁵ Moreover, T_{max} was not statistically different between the two products. Hence, these results demonstrated that the test product was bioequivalent to the reference product in terms of rate and extent of absorption.

Tolerability

Both products of netilmicin were well tolerated and no serious adverse events were reported. A total of 3 adverse drug events were observed in 3 volunteers over the 2 study periods. One of these events occurred after receiving the reference product and two after receiving

TABLE 1. Demographic data.

Characteristics	Volunteers	
	TR Group (n=12)	RT Group (n=12)
Sex		
Male	6	5
Female	6	7
Age (years $\pm$ SD)	21.8 ± 3.5	21.5 ± 2.0
Body weight (kg. $\pm$ SD)	57.8 ± 7.3	60.3 ± 10.4
Height (cm. $\pm$ SD)	165.6 ± 7.9	167.9 ± 8.5
BMI (kg./m ² $\pm$ SD)	21.1 ± 1.7	21.2 ± 2.1

T = Test product; R = Reference product; BMI= Body Mass Index

TABLE 2. Pharmacokinetic parameters of netilmicin and bioequivalence analysis for the Test (Nelin[®]) and Reference (Netromycin[®]) products. Values are geometric mean (SE) of $n = 24$ unless otherwise indicated.

Parameter	Test	Netilmicin	
		Reference	% Geometric mean ratio, Test/Reference (90% CI)
$C_{\max}$ ($\mu\text{g/ml}$)	16.9 (1.13)	16.8 (1.08)	100.30 (88.02-114.28)
AUC_{0-1} ($\mu\text{g.h/ml}$)	61.1 (3.32)	64.4 (3.60)	94.94 (88.94-101.34)
$\text{AUC}_{0-\infty}$ ($\mu\text{g.h/ml}$)	64.6 (3.50)	68.0 (3.81)	94.88 (88.85-101.32)
$T_{\max}^{(h)}$	1.23 (0.09)	1.31 (0.108)	-
median	1.50 (0.50-2.00)	1.25 (0.50-2.50)	-
$t_{1/2}$ (h)	3.15 (0.61)	3.19 (0.46)	-
λ_z (h^{-1})	0.22 (0.03)	0.218 (0.03)	-

AUC = area under plasma concentration-time curve; $C_{\max}$ = maximal plasma concentration; $T_{\max}$ = time for the maximal plasma concentration [data expressed as median (range)]; $t_{1/2}$ = half-life; λ_z = elimination rate constant.

the test products. The adverse events were skin abrasion, common cold and diarrhea and not related to the study drugs.

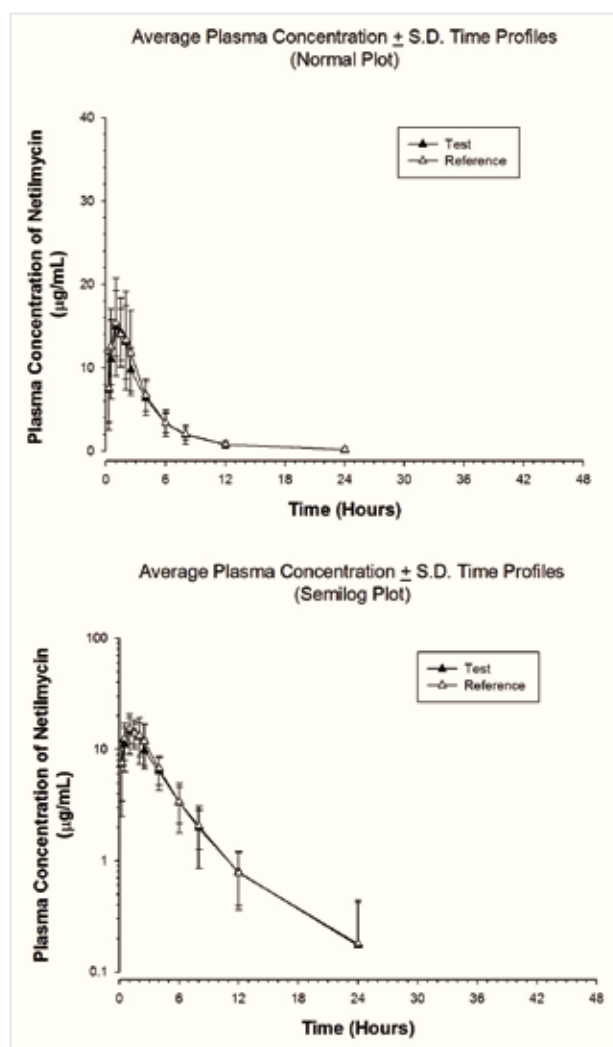


Fig 1. Mean plasma concentration of netilmicin after intramuscular injection of Nelin[®] (Test) and Netromycin[®] (Reference) in 24 healthy volunteers; normal plot (above) and semilog plot (below).

DISCUSSION

Severe infections caused by gram-negative bacteria remain a significant problem in Thailand. Netilmicin, a semisynthetic aminoglycoside antibiotic, has been an attractive option to treat a wide range of infections due to its effectiveness against gram-negative bacteria including gentamicin-resistant strains with evidence of less ototoxicity and nephrotoxicity¹⁹. Hence, the bioequivalence analysis of the generic product of netilmicin is important to assure the clinicians that the generic product has comparable pharmacokinetics and adverse

effects with the reference product.

A randomized, two treatments, two periods, two sequences crossover design in 24 healthy volunteers in our study was considered appropriate for the bioequivalence evaluation of the generic and reference products. Netilmicin, not subject to gastrointestinal absorption, was administered intramuscularly and a comparison of pharmacokinetic parameters of the products was determined. Following a singledose intramuscular injection of netilmicin sulfate (Netromycin[®]), our study demonstrated that $C_{\max}$ of $16.8 \pm 1.08 \mu\text{g/ml}$ were achieved within 1.25 h. The serum concentrations of netilmicin in plasma were observed to decrease rapidly with a terminal elimination in a half-life ($t_{1/2}$) of approximately 3.19 h. While the $T_{\max}$ and $t_{1/2}$ obtained in our study were in accordance with those previously reported, the relatively high values for $C_{\max}$ were demonstrated in this study^{9,11}.

AUC, $C_{\max}$ and $T_{\max}$ are important pharmacokinetic variables for bioavailability analysis in order to evaluate the bioequivalence of the test drug and the reference drug. The extent of absorption is indicated by AUC and rate of absorption by $C_{\max}$ and $T_{\max}$. Two drug products are considered therapeutically equivalent when they are bioequivalent in terms of rate and extent of absorption¹⁶. Our study demonstrated that the 90% CIs for $C_{\max}$, AUC_{0-1} and $\text{AUC}_{0-\infty}$ of the two netilmicin products administered intramuscularly were all within the bioequivalence acceptance range of 80.00% to 125.00%. Furthermore, the powers of the test obtained from this study for these parameters were more than 80%. Thus, the two products (Netromycin[®] and Nelin[®]) of netilmicin are considered bioequivalent with respect to the rate and extent of absorption following intramuscular injection in healthy volunteers. In addition, the excellent tolerability profile of both products was confirmed in the studied population.

In conclusion, the test (Nelin[®]) and reference (Netromycin[®]) products of intramuscular netilmicin (100 mg/ml, 1.5 ml) are bioequivalent.

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