MATERIALS AND METHODS

1. Subject

The Research Ethics Committee of the Faculty of Medicine, Chiang Mai University has approved the study protocol and written informed consent was obtained from parent of the neonates before starting the study. The neonates (postnatal age between > 0 to 28 days) who admitted to the Department of Pediatric, Faculty of Medicine, Chiang Mai University during October 15th, 2003 to February 29th, 2004 were eligible for the study if they had been diagnosed or suspected of having bacterial infection from Gentamicin-sensitive Gram-negative bacteria. Bacterial infection was defined based on maternal risk factors for sepsis and/or clinical signs and symptoms consistent with infection. Gentamicin was indicated in the neonates as part of their antibiotic regimen. Neonates should have normal renal function and neonates with elevated serum creatinine level, oliguria, polycystic kidney disease, renal agenesis or allergic to gentamicin were excluded from the study.

2. Study Design

The study was a prospective nonrandomized open label phase I clinical trial. The neonatal patients were divided into 4 groups based on their gestational age (GA $\leq$ 29 weeks, 30-33 weeks, 34-37 weeks and $\geq$ 38 weeks for group I, II, III and IV, respectively). The study drug gentamicin was given to all neonatal patients. The dosage of administered gentamicin was based on their gestational age and the duration of treatment was based on clinician judgement. The peak-trough concentrations were drawn and the pharmacokinetic parameters were calculated. Demographic data included sex, gestational age (GA), post natal age, birth weight, length, body surface area (BSA), weight at beginning of therapy and 5-minute Apgar scores of the neonates were recorded as well as their pertinent neonatal laboratory, clinical data such as blood and/or cerebrospinal fluid cultures, hemoglobin, hematocrit, differential white blood count, serum creatinine, blood urea nitrogen (BUN), presence of PDA and concurrent medication.

3. Drug and Method of Drug Administration

Drug : Gentamicin 10 mg/ml in 2 ml (Thai Meiji, Thailand).

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Based on the 2002 guidelines in Neofax by Young and Mangum, neonatal patients were divided into four groups according to their gestational age (GA). The dose and dosing interval of gentamicin were administered as follow.

Group	GA (weeks)	Dose (mg/kg/dose)	Duration (hrs)
1	≤ 29	5	48
2	30-33	4.5	48
3	34-37	4	36
4	≥ 38	4	24

After calculated the dose of gentamicin, the drug was diluted with 3 ml 5% dextrose in water, given by a slow intravenous infusion by a syringe pump over 30 minutes. Thereafter, the intravenous line was flushed with 1-1.5 ml of normal saline upon completion of the infusion. The accurate dose of gentamicin and the time of drug administration were recorded in the case record form.

4. Blood Sampling and Sampling Time

One millilitre (1 ml) of blood samples (clot blood) was obtained from vein or umbilical artery catheter (UAC) to determined gentamicin concentrations, as well as renal function indexes (BUN and Cr). Gentamicin peak concentrations were drawn 30 minutes after completion of a 30 minutes intravenous infusion and the trough concentrations were drawn within 30 minutes before the next dose. The initial peak concentration was drawn after the 1^{st} dose, which represented the loading dose, and subsequently after the 3^{rd} and the 6^{th} doses which represented maintenance doses. The blood for BUN and Cr were also determined on the 1^{st}, 3^{rd} and 6^{th} doses. The trough concentrations were drawn before the 3^{rd} and the 6^{th} doses. The initial and subsequent peak-trough concentrations were used to calculate and adjust for the subsequent appropriate dosing of gentamicin.

Blood samples were allowed to clot at room temperature and centrifuged for 5 minutes at 1,500 rpm to separate the serum which were immediately kept at –20 °c until assay. The actual sampling times were recorded in the case record form.

5. Determination of serum gentamicin concentrations

Serum gentamicin samples were analyzed for gentamicin concentration by FPIA technique using the Abbott TDx clinical analyzer (Abbott Laboratory, North Chicago, IL, U.S.A). The FPIA procedure is an automated method for drug level monitoring routinely performed at the Clinical Pharmacology Unit, Department of Pharmacology, Faculty of Medicine, Chiang Mai University. The assay was conducted according to the manufacturer's protocol without modification, and 3 controls (low 1 ug/ml, medium 4 ug/ml and high 8 ug/ml) were run with each carousel of serum samples. The coefficient of variation between measurement was less than 15 % (n = 16) for the gentamicin levels of 0.85-8.80 ug/ml and the results were shown in Table 8.

Table 8. Coefficient of variation of 3 control concentrations of gentamicin.

Standard concentration (ug/ml)	Reading (ug/ml)	Coefficient of variation (%)
0.85 – 1.15	1.00 ± 0.12*	11.77
3.6 – 4.4	3.98 ± 0.28*	7.04
7.2 – 8.8	7.89 ± 0.50*	6.29

* Data present as Mean ± SD

6. Efficacy and Safety criteria

Therapeutic peak concentrations of 4 - 12 ug/ml represented efficacy.

Trough concentrations of less than 2 ug/ml represented safety.

Serum BUN and creatinine of 12.9 - 25.8 mg/dl and 0.34 - 0.6 mg/dl, respectively, represented normal renal function. [29]

7. Calculation

7.1 BSA was calculated by the use of the equation of Du Bois and Du Bois.[30]

$$\textbf{BSA } (m^2) = (W^{0.425} \times H^{0.725}) \times 0.007184$$

Where: W is weight of patient.

H is height of patient.

7.2 Adjusted dose and interval[18]

7.2.1 If peak concentration is lower than 4 ug/ml or higher than 12 ug/ml, despite of trough concentration is lower than 2 ug/ml. The dose should be adjusted by the use of the following equation.

$$C_{pss,max} = \frac{\frac{(S)(F)(Dose / t_{in})}{CL}(1 - e^{-K_e t_{in}})}{(1 - e^{-K_e T})}(e^{-K_e t_2})$$

Where: Cpss,max is the maximum plasma drug concentration produced by a given dose of drug during the dosing interval at steady state (ug/ml)

S is salt form of gentamicin (= 1)

F is bioavailability of gentamicin (=1)

t_{in} is duration of infusion (hr)

K_e is Elimination rate constant (hr^{-1})

t_2 is the time during the end of infusion and blood sampling for peak concentration (hr)

T is dosing interval (hr)

CL is gentamicin clearance (L/kg/hr)

7.2.2 If trough concentration is higher than 2 ug/ml, despite of peak concentration is about 4 to 12 ug/ml, the duration of should be by the use of the following equation.

$$Cpss,min = \frac{\frac{(S)(F)(Dose / t_{in})}{CL}(1 - e^{-Ke\ tin})}{(1 - e^{-KeT})}(e^{-Ke\ (T - tin)})$$

Where: Cpss,min is the minimum plasma drug concentration produced by a given dose of drug during the dosing interval at steady state (ug/ml)

7.3 Pharmacokinetic parameter

7.3.1 Elimination rate constant (K_e; hr^{-1})

$$K_e = [\ln (Cp_2 / Ct_1)]/(t_{t1} - t_{p2})$$

Where: Cp_2 is peak concentration when sampling after the third dose (ug/ml)

Ct_1 is trough concentration when sampling before the third dose (ug/ml)

t_{t1} is the time to sampling before the third dose (hr)

t_{p2} is the time to sampling after the third dose (hr)

7.3.2 Clearance (CL; L/kg/hr)

$$CL = \frac{\frac{(S)(F)(Dose / t_{in})}{Cpmax}(1 - e^{-Ke\ tin})}{(1 - e^{-KeT})}(e^{-Ket2})$$

7.3.3 Volume of distribution (V_d; L/kg)

$$V_d = Cl / K_e$$

7.3.4 Half-life ($t_{1/2}$; hr)

$$t_{1/2} = 0.693 / K_e$$

8. Statistic analysis

Demographic data of neonatal patients, peak concentrations, trough concentrations and pharmacokinetic parameters of gentamicin in each gestational age group were described using means, standard deviation and range. Statistical analysis for the difference of mean of gentamicin pharmacokinetic parameters between groups of patients was determines by Analysis of Variance (ANOVA). Correlation of gentamicin pharmacokinetic parameters and demographic data were evaluated by linear regression analysis. Statistical analysis for the difference of mean of serum creatinine and BUN were analyzed by Student Paired t test. SPSS version10 was used for the statistical calculations.