เภสัชจลนศาสตร์ของยาอะมิกาซินในทารกแรกเกิดคลอดก่อนกำหนด ที่โรงพยาบาลพระมงกุฎเกล้า

พันตรีหญิง ชนกพร บุญทริกศิริ

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PHARMACOKINETICS OF AMIKACIN IN PREMATURE NEONATES AT PRAMONGKUTKLAO HOSPITAL

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ชนกพร บุญทริกศิริ : เภสัชจลนศาสตร์ของยาอะมิกาซินในทารกแรกเกิดคลอดก่อนกำหนดที่โรงพยาบาลพระมงกุฎเกล้า (PHARMACOKINETICS OF AMIKACIN IN PREMATURE NEONATES AT PHRAMONGKUTKLAO HOSPITAL) อ. ที่ปรึกษา : ดร.พรอนงค์ อร่ามวิทย์ อ. ที่ปรึกษาร่วม : พ.ท.พรพัฒน์ รัศมีมารีย์ 92 หน้า ISBN 974-17-1558-7

ทารกแรกเกิดคลอดก่อนกำหนดที่สงสัยว่ามีภาวะติดเชื้อ จำนวน 37 ราย ณ หอผู้ป่วยวิกฤตทารกแรกเกิด โรงพยาบาลพระมงกุฎเกล้า ได้รับยาอะมิกาซินเพื่อรักษาแบบคาดการณ์ โดยทารกที่มีอายุในครรภ์น้อยกว่าหรือเท่ากับ 30 สัปดาห์ จะได้ยา 18 มิลลิกรัม/กิโลกรัม/ครั้ง ทุก 48 ชั่วโมง ทารกที่อายุในครรภ์ 31 - 33 สัปดาห์ จะได้ 16 มิลลิกรัม/กิโลกรัม/ครั้ง ทุก 48 ชั่วโมง และทารกที่อายุในครรภ์ 34 - 36 สัปดาห์ จะได้ยา 15 มิลลิกรัม./กิโลกรัม/ครั้ง ทุก 24 ชั่วโมง โดยให้ยาทางหลอดเลือดดำแบบต่อเนื่องเป็นเวลา 30 นาที และวัดระดับยาในเลือดที่ภาวะคงที่ 2 ค่า คือเมื่อสิ้นสุดการให้ยาแล้ว 30 นาที เพื่อเป็นระดับยาสูงสุดและที่ชั่วโมงที่ 18 หรือ 36 หลังให้ยาเพื่อใช้คำนวณหาระดับยาต่ำสุดและค่าตัวแปรทางเภสัช-จลนศาสตร์

ผลการศึกษาพบว่าระดับยาเฉลี่ยสูงสุดในเลือดที่ภาวะคงที่ของทารกแรกเกิดที่มีอายุในครรภ์ $\leq$ 30 สัปดาห์, 31 - 33 สัปดาห์ และ 34 - 36 สัปดาห์ เท่ากับ 28.49 $\pm$ 8.63, 24.22 $\pm$ 5.99 และ 24.91 $\pm$ 5.73 ไมโครกรัม/มิลลิลิตร ตามลำดับ และระดับยาเฉลี่ยต่ำสุดที่ภาวะคงที่เท่ากับ 1.65 $\pm$ 1.28, 1.13 $\pm$ 2.11 และ 2.01 $\pm$ 1.12 ไมโครกรัม/มิลลิลิตร ระดับยาสูงสุดและต่ำสุดนี้ไม่แตกต่างกันระหว่างกลุ่มอย่างมีนัยสำคัญทางสถิติ ($p>0.05$) ระดับยาเฉลี่ยสูงสุดนี้อยู่ในช่วงของการรักษาที่เหมาะสมตามที่กำหนดไว้ใน Neofax 2001 คือ 20 - 30 ไมโครกรัม/มิลลิลิตร และระดับยาต่ำสุดไม่เกินช่วงที่เหมาะสม คือไม่เกิน 2 - 5 ไมโครกรัม/มิลลิลิตร ค่าคงที่ในการขจัดออกของยา และ ค่าการขจัดออกของยา ในทารกมีความแตกต่างกันระหว่างกลุ่มและสัมพันธ์กับอายุในครรภ์มารดาอย่างมีนัยสำคัญ ($p<0.05$) ในกรณีค่าครึ่งชีวิตของยา หากไม่คำนึงถึงค่าที่เบี่ยงเบนผิดปกติเมื่อเทียบกับประชากรในกลุ่มเดียวกัน ซึ่งมีเพียง 1 ราย จะพบว่ามีความแตกต่างกันระหว่างกลุ่มและมีความสัมพันธ์กับอายุในครรภ์มารดาอย่างมีนัยสำคัญทางสถิติเช่นเดียวกัน แต่ค่าปริมาตรการกระจายตัวของยาของแต่ละกลุ่มไม่แตกต่างกันและไม่สัมพันธ์กับอายุในครรภ์มารดาอย่างมีนัยสำคัญทางสถิติ การศึกษานี้สรุปได้ว่าค่าตัวแปรทางเภสัชจลนศาสตร์ของยาอะมิกาซินในทารกไทยแรกเกิดที่คลอดก่อนกำหนดไม่แตกต่างจากผลการศึกษาในต่างประเทศ

ภาควิชา.....เภสัชกรรม.....ลายมือชื่อนิสิต.....

สาขาวิชา.....เภสัชกรรมคลินิก.....ลายมือชื่ออาจารย์ที่ปรึกษา.....

ปีการศึกษา.....2545.....ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

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CHANOKPORN BOONTHARIKSIRI : PHARMACOKINETICS OF AMIKACIN IN PREMATURE NEONATES AT PHRAMONGKUTKLAO HOSPITAL. THESIS ADVISOR : PORNANONG ARAMWIT, Pharm D., Ph. D. THESIS CO-ADVISOR : LIEUTENANT COLONEL PHORNPHAT RASAMIMARI, MD. 93 PP. ISBN 974-17-1558-7

Thirty-seven premature neonates with suspected infection at Neonatal Intensive Care Unit (NICU), Phramongkutklao Hospital have been given amikacin as an empirical treatment. Dosage of amikacin are 18 mg/kg every 48 hours, 16 mg/kg every 48 hours and 15 mg/kg every 24 hours for neonates with gestational age not greater than 30 weeks, 31 - 33 weeks and 34 - 36 weeks, respectively. Medications have been administered by intravenous infusion for 30 minutes. For each neonates, two amikacin serum levels have been obtained at steady state. Peak concentration has been measured at 30 minutes after complete infusion. Second concentration has been measured at either 18^{th} hours or 36^{th} hours after administration depend on dosing interval of each patient. Trough concentration has been derived from calculation. Pharmacokinetic parameters of amikacin also obtained from calculation.

Mean peak concentration at steady state of neonates with gestational age not greater than 30 weeks, 31-33 weeks and 34-36 weeks are 28.49 $\pm$ 8.63, 24.22 $\pm$ 5.99 and 24.91 $\pm$ 5.73 μg/ml, respectively. Mean trough concentration at steady state are 1.65 $\pm$ 1.28, 1.13 $\pm$ 2.11 and 2.01 $\pm$ 1.12 μg/ml, respectively. Mean peak and trough concentration are not significantly different between groups ($p>0.05$). Mean peak concentration of every groups are in desired therapeutic range (20-30 μg/ml) as mentioned in Neofax 2001. Mean trough concentration of every groups are not greater than desired range (2-5 μg/ml). Elimination rate constant and clearance of amikacin are significantly different between groups and are also good proportional correlation with gestational age ($p<0.05$). If extreme value has been eliminated (from 1 subject), the elimination half life is also significantly different between groups and inversely correlate with gestational age. However, volume of distribution is not significantly different between groups and no correlation with gestational age. In conclusion, pharmacokinetic parameters in Thai premature neonates are similar to data previously published from other countries.

Department……Pharmacy……..…….Student's signature……

Field of study…Clinical Pharmacy….Advisor's signature……...

Academic year...2002…..……....Co-advisor's signature……..

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Major Chanokporn Boonthariksiri

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ABBREVIATIONS

%	=	percent
°C	=	degree celsius
Amp	=	ampicillin
APGS	=	apgar score
BW	=	birth weight
Cl	=	clearance
cm	=	centrimetre
CrCl	=	creatinine clearance
CSF	=	cerebrospinal fluid
CXR	=	chest x-ray
ESR	=	eosinophil sedimentation rate
GA	=	gestational age
gm	=	gram
Ht	=	height
hr	=	hour
IM	=	intramuscular
IT	=	intrathecal
IV	=	intravenous
K_e	=	elimination rate constant
kg	=	kilogram
l	=	litre
mg	=	milligram
min	=	minute
ml	=	millilitre
PNA	=	postnatal age
PROM	=	prolong rupture of membrane
RDS	=	respiratory distress syndrome
rpm	=	round per minute
SCr	=	serum creatinine
$t_{1/2}$	=	elimination half life

μg	=	microgram
V_d	=	volume of distribution

CHAPTER I

INTRODUCTION

Background and Rationale

Infection is a major cause of morbidity and mortality in neonatal period.[1,2] Yurdakok M. has reported that the mortality rate of infected neonates is about 10 - 50%.[2] Because signs and symptoms of infection are not specific, antibiotics, as an empirical treatment, need to be started as soon as an infection is suspected. The treatment must be safe since only a small proportion of these neonates has real infection. The predominant infection organisms are group B streptococci, *Listeria monocytogenes* and *Escherichia coli*. The latter one is gram negative bacteria and is one of the most suspicious organisms. Aminoglycosides are widely used as the first - line antibiotics either alone or combine with beta-lactams or third generation cephalosporins for neonatal infection.[3] The most popular regimen is a combination of gentamicin and ampicillin to cover both gram negative and gram positive, respectively. However, amikacin has been identified as an effective agent against resistant strains. Because of their narrow therapeutic index, potential complications such as nephrotoxicity or ototoxicity may arise. It has been suggested that therapeutic drug monitoring is necessary to avoid their toxicities.

Gatta et al and Botha et al have shown that aminoglycosides have wide inter and intra-patient pharmacokinetic variabilities.[4,5] Pharmacokinetic parameters have been determined by many factors such as gestational age, weight, disease conditions and renal function.[4, 6-11] Despite recommendations that all patients receiving aminoglycosides should have their dosing regimens prospectively individualized by determination of appropriate pharmacokinetic parameters, it is almost impossible to have individuals dosage regimens in every hospitals.[12] Accordingly, it is important to develop alternate approaches to individualize therapy for various populations.

Several studies conducted in neonatal population have shown that adult dosage regimens are not well adapted to neonates due to the differences in body composition and the development of renal function.[6,12,13] They also have suggested that aminoglycosides for the treatment of infection in neonates should be administered at larger dose and longer dosing interval to obtain the same therapeutic serum level as adults because of the larger volume of distribution and smaller renal clearance of neonates.[12] Studies about renal function in premature neonates have revealed that the development of renal function is associated with gestational age and postnatal age.[14] From these data, it is recommended that dosage regimens in neonates should be based on gestational age, birth weight and also postnatal age. Recently, several studies have attempted to create a dosing chart for neonates at birth, but there is no sufficient evidence to define the optimal dosage regimen.[15,16] Despite of this fact, Neofax, worldwide handbook used among the pediatricians, has set the guideline providing dosage regimens for aminoglycosides. Dosage regimens in this guideline have been developed from previously published pharmacokinetic parameters investigated in neonates from many countries, which might not be suitable for Thai neonates. There are a few studies regarding the use of gentamicin in neonates in Thailand, however, no study is conducted about the use of amikacin in preterm infants.[17] Since amikacin is widely used and compared to gentamicin, its dosing intervals recommended in Neofax have been modified in Phramongkutklao Hospital's Neonatal Intensive Care Unit due to the inconvenience of the regimen.

This study has been conducted to obtain the serum amikacin levels, relationship between serum levels and efficacy together with toxicity such as nephrotoxicity and pharmacokinetic parameters of amikacin dosage regimens used at Phramongkutklao's Neonatal Intensive Care Unit. Moreover, the correlation between pharmacokinetic data and patient's demographics or renal function indexes such as serum creatinine and creatinine clearance have also been studied.

Objectives

1. To measure the amikacin serum levels resulted from the dosage regimens used at Phramongkutklao's Neonatal Intensive Care Unit and the percentage of premature neonates achieved therapeutic, overtherapeutic and subtherapeutic levels of amikacin.
2. To identify pharmacokinetic parameters of amikacin in Thai premature neonates such as elimination rate constant, amikacin clearance, volume of distribution and half-life.
3. To examine the correlation between amikacin serum levels and efficacy and nephrotoxicity of amikacin dosage regimens used at Phramongkutklao's Neonatal Intensive Care Unit.
4. To examine the correlation between pharmacokinetic parameters and patient's demographics such as gestational age, birth weight and serum creatinine.
5. To examine the correlation between amikacin clearance and serum creatinine and creatinine clearance.

Benefits

1. Obtain the amikacin serum levels resulting from the amikacin dosage regimen used at Phramongkutklao's Neonatal Intensive Care Unit.
2. Obtain the pharmacokinetic parameters of amikacin in Thai premature neonates.
3. Obtain the correlation between amikacin serum levels, efficacy and toxicity of the dosage regimens.
4. Obtain the correlation between pharmacokinetic parameters of amikacin and demographic characteristics of the neonates.
5. Obtain the correlation between amikacin clearance and renal function indexes.
6. Obtain the fundamental information guiding for future research.

CHAPTER II

RELATED LITERATURE REVIEW

I. Review of amikacin

A. Physical and chemical properties

Amikacin (BB-K8) is a semisynthetic aminoglycoside derived from kanamycin A which is isolated from *Streptomycetes kanamyceticus* and then acetylated with L(-)-γ-amino-α- hydroxybutyric acid at the C-1 amino group of the 2-deoxystreptamine moeity.[18,19] It appears as a white, crystalline powder and is sparingly soluble in water. The molecular formula is $C_{22}H_{47}N_5O_{21}S_2$ and the chemical structure is shown below.

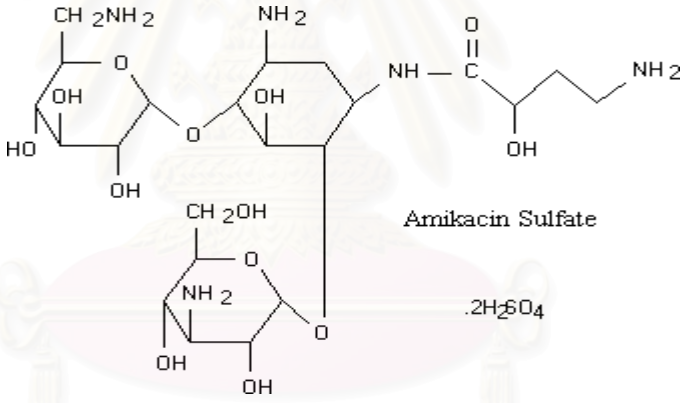


Figure 1.1 The structure of amikacin sulfate

Due to poor oral absorption, amikacin is commercially available only as sulfate salt injection which is formed *in situ* during the manufacturing process. Amikacin injection is a sterile clear, colorless to light straw-colored solution. Sulfuric acid is added during the manufacture of the injection to adjust the pH to 3.5 - 5.5. The vial also contains 50 mg sodium citrate B.P. and 4.8 mg sodium metabisulfite B.P. as antioxidants. No preservative is added into the formulation.

Amikacin sulfate injection should be stored at a temperature less than 40 °C, preferably between 15 - 30 °C because of the degradation to BB-K11 and BB-K29 at high temperature. Also, freezing should be avoided. At room temperature, amikacin sulfate injection is stable for at least 2 years following the date of manufacture.[20]

Amikacin sulfate is stable for 24 hours at room temperature at the concentrations of 0.25 and 5 mg/ml in most intravenous infusion fluids, including 0.9% sodium chloride or 5% dextrose solution. Amikacin sulfate solutions at concentration of 0.25 and 5 mg/ml in D-5-W, 0.45% or 0.9% NSS, lactate ringer are stable for 24 hours at room temperature after being refrigerated at 4°C for 60 days or frozen for 30 days at –15°C, thawed and stored at 25°C . The manufacturers have stated that amikacin sulfate injection should not be mixed with other drugs.[20]

B. Pharmacokinetics

The pharmacokinetics of amikacin are similar to those of the other aminoglycosides.

1. Absorption

a. Oral

Amikacin is poorly absorbed through gastrointestinal tract. The drug is well absorbed following parenteral administration.

b. Intramuscular (IM)

Intramuscular administration of amikacin is cited as a possible route of delivery. Following IM administration of a single dose amikacin at 7.5 mg/kg in adults with normal renal function, peak plasma amikacin concentrations of 17-25 μg/ml are attained within 45 minutes to 2 hours. Plasma concentrations of the drug have been averaged to be 2.1 μg/ml at 10 hours.[20]

Howard et al have shown that the average peak plasma levels after a single IM dose of 7.5 mg/kg to newborns attained at 30 minutes is about 17-20 μg/ml which is more quickly than in adults.[21] These values are consistent with the results reported by Sandermann et al.[22]

However, there may be a variability of drug adsorption via IM route for each individual. Diabetic and critically ill patients such as hypertension associated with gram-negative shock condition would have less blood perfusion at IM site resulting in the reduction of blood perfusion at route of administration of amikacin.[23]

c. Intravenous (IV)

IV infusion is preferred administration route for amikacin. Peak plasma concentration obtained from IV route is higher than IM route. After a single dose administration of amikacin by IV infusion over 1 hour at 7.5 mg/kg, the average of peak plasma concentrations of the drug are 38 μg/ml immediately after the infusion, 5.5 μg/ml at 4 hours, and 1.3 μg/ml at 8 hours, respectively.

d. Intrathecal (IT)

Intrathecal is an alternative route chosen to administer the drug in case of cerebrospinal infections since the distribution in cerebrospinal fluid (CSF) after IM or IV injection might be at subtherapeutic level. In one adult patient with meningitis, IT administration of 4 mg of amikacin daily in conjunction with IM administration of 15 mg/kg daily for 2 weeks resulted in CSF concentrations of the drug ranging from 7- 40 μg/ml 12 hours after an intrathecal dose and 1-19 μg/ml 24 hours after an intrathecal dose.[20]

2. Distribution

Amikacin mainly distributes into extracellular fluids after parenteral administration. It has been found in bone, heart, gall bladder, and lung tissue. Amikacin also well distributes into bile, sputum, bronchial secretions, interstitial, pleural, and synovial fluids. It has been found in the cerebrospinal fluid, vitreous fluid, prostrate and brain at subtherapeutic concentration as well.[20]

Amikacin level in bronchial secretion is about 14-66 % of plasma level.[9] Endotracheal administration results in higher bronchial levels compared to systemic administration. However, the differences in clinical outcome have not been consistent. This medication can also cross the placenta but only a small amount distribute into breast milk. Since the teratogenicity of amikacin in

fetus has not been well established, this medication should be avoided during pregnancy.

Because of its polar structure, amikacin is poorly penetrated through biological membrane as a result of low intracellular concentrations, with the exception of the proximal tubule. Amikacin concentration at renal cortical tissue is 10-50 times higher than plasma levels.[23,24] In rats, the renal cortical concentrations are higher after continuous infusion than after three IV injections at 8 hour interval. Plasma protein binding of amikacin is low (<30%) resulting in high volume of distribution which is 0.25 l/kg in adults or 25 % of lean body weight. It increases in burn and severe infection patients but decreases in obese patients. Volume of distribution in infants and neonates is greater than adults. It is about 0.3-0.4 l/kg in infants, 0.4-0.5 l/kg in fullterm neonates and 0.5 - 0.6 l/kg in preterm neonates, respectively.[25]

3. Elimination

In adults with normal renal function, 94-98% of a single IM or IV dose of amikacin is excreted unchanged by glomerular filtration within 24 hours. There are some renal tubular reabsorption. The reduction of plasma concentration can be divided into 3 phases; α, β and γ. Alpha (α), the rapid phase, is the distributed phase which half life is about 5-15 minutes and this phase completes in 25-75 minutes. The second phase (β) is the renal elimination and the elimination rate constant varies with renal function. Urine concentrations may be 30-100 times higher than those found in serum. The average urine concentration of amikacin in adults with normal renal function at 6 hours is about 563 μg/ml and 832 μg/ml after a single IM injection of 250 mg and 500 mg, respectively. Complete recovery of the dose in urine requires approximately 10-20 days in patients with normal renal function, and terminal elimination half-lives of greater than 100 hours have been reported in adults with normal renal function following repeated IM or IV administration of the drug.[20]

Small amounts of drug have been found in the bile and may represent an additional route of elimination.

The plasma elimination half – life of amikacin is usually 2-3 hours in adults with normal renal function and is reported to range from 30-86 hours in adults with severe renal impairment. The plasma elimination half-life of amikacin is reported to be 4-5 hours for full term infants, 7 days of age or older, and 7-8 hours in low birth weight infants, 1-3 days of age.[22]

4. Factors effect pharmacokinetics of aminoglycoside

a. Age

Pharmacokinetics of aminoglycosides are age-dependent. Volume of distribution is higher in neonates than in adults because neonates have more percentage of extracellular fluid. In contrast, elimination rate constant is lower in neonates compared to adults because of the low renal function in neonates.[12]

b. Diseases

Patients with different underlying disease have different pharmacokinetics profile. Fdez de Gatta et al have shown that patients with trauma have higher clearance of amikacin than patients with sepsis or pneumonia.[4] Volume of distribution also varies on conditions. Patients with edema or burn have higher volume of distribution while obese patients show lower volume of distribution.[15,20,26]

c. Renal function

Since aminoglycosides is excreted unchange by glomerular filtration, patients with renal impairment or renal failure have a significant lower elimination rate constant compared to patients with normal renal function. Elimination half-life is also significantly different between both groups. In adults with normal renal function, the mean half life of amikacin is

about 2 hours. In adults with impaired renal function, half life ranged from 3.59 hours to 82 hours.[11]

C. Pharmacology

1. Mechanism of action

Amikacin is a bactericidal antibiotic. Bacterial elimination is concentration dependent. The higher the concentration, the greater the rate at which bacteria is eliminated. The antibacterial properties of aminoglycosides are believed to be the result from inhibition of bacterial protein synthesis through irreversible binding to the 30S bacterial ribosome. The initial site of action is the outer bacterial membrane. The cationic antibiotic molecules create fissures in the outer cell membrane, resulting in leakage of intracellular contents and enhanced antibiotic uptake. This rapid action at the outer membrane probably accounts for most of the bactericidal activity.[27] Energy is needed for aminoglycoside uptake into bacterial cell. Anaerobes have less energy available for this uptake resulting in less activity against anaerobes.

2. Susceptibility

Amikacin is mainly active against gram negative bacteria such as *Pseudomonas* spp., *Escherichia coli*, *Proteus* spp., *Providencia* spp., *Klebsiella* spp., *Enterobacter* spp., *Serratia* spp. and *Acinetobacter* spp. and some strains of gram positive bacteria as shown in table 2.1.

Table 2.1 Susceptibility of organisms to aminoglycosides[28]

Organism	Organism generally susceptible to aminoglycosides					
	Amikacin	Genta-micin	Kanamycin	Netilmycin	Strepto-mycin	Tobra-mycin
Gram- positive						
Mycobacterium tuberculosis	✓				✓[1]	
Staphylocci	✓[2]	✓[2]		✓[2]		✓
S. aureus	✓	✓	✓[2]	✓[2]		✓
S. epidermedis	✓		✓			
Streptococci					✓[1]	
S. faecalis		✓[1]		✓[1]	✓[1]	✓[1]
Gram- negative						
Acinetobacter spp.	✓		✓	✓		
Brucella spp.					✓	
Citobacter spp.	✓	✓	✓	✓	✓	✓
Enterobacter spp.	✓	✓	✓	✓	✓	✓
Escherichia coli	✓	✓	✓	✓	✓	✓
Hemophillus influenzae	✓		✓		✓[1]	
Hemophillus ducreyi					✓	
Klebsiella spp.	✓	✓	✓	✓	✓[1]	✓
Morganella morganii						✓
Neisseria spp.	✓		✓	✓	✓	
Proteus spp.	✓[3]	✓[3]	✓[3]	✓[3]	✓[3]	✓[3]
Providencia spp.	✓	✓		✓	✓	✓
Pseudomonas spp.	✓			✓		
P. aeruginosa	✓	✓[1]		✓	✓	✓
Salmonella spp.	✓	✓	✓	✓	✓	✓
Serratia spp.	✓	✓	✓	✓	✓	✓
Shigella spp.	✓	✓	✓	✓	✓	✓
Yersinia (Pasteurella) pestis	✓	✓	✓	✓	✓	✓

✓ = Generally susceptible

✓[1] = Usually used concomitantly with other anti-infective agents

✓[2] = Penicillinase-producing and non penicillinase producing

✓[3] = Indole-positive and indole-negative

3. Resistance

There are three mechanisms of the resistance to aminoglycosides which are changing in drug permeability, drug's structure-modifying enzymes production and changing in binding sites on ribosome. The most common mechanism is inactivating - enzymes production. Because of structural differences, amikacin is a poor substrate for three of four enzymes that inactivated gentamicin and for two of three that inactivate tobramycin.[29] Therefore, a large proportion of the gram negative aerobes that are resistant to gentamicin and tobramycin are sensitive to amikacin. Amikacin is inactivated by acetylation at the amino group by acetyltransferases, adenylation reaction at the hydroxyl group by adenyltransferases and phosphorelation reaction at hydroxyl group by phosphotransferases. Because these reactions change amikacin's structure, the ability of the drug to bind with ribosomes has been reduced. Most resistance to amikacin is found among strains of *Acinetobacter* spp., *Providencia* spp., *Flavobacter* spp. and strains of *Pseudomonas* spp. other than *Pseudomonas aeruginosa*.[27]

4. Indications

Amikacin injection is indicated in the short-term treatment of serious infections caused by susceptible strains of gram negative bacteria, including *Pseudomonas* spp., *Escherichia coli*, *Proteus* spp. (Indole-positive and indole negative), *Providencia* spp., *Klebsiella* spp., *Enterobacter* spp., *Serratia* spp. and *Acinetobacter* spp.[27,28] Even some gram positive organisms are sensitive to amikacin, the medication sometimes should be restricted to second-line therapy.

Amikacin injection can be prescribed for the treatment of neonatal sepsis when sensitivity testing indicates that other aminoglycosides cannot be used. In neonatal sepsis, concomitant therapy with penicillin type drug may be indicated because of the possibility of infections due to gram positive organisms such as streptococci or pneumococci.[28] Amikacin can be used

effectively in the treatment of bacteremia, septicemia including neonatal sepsis and serious infections of the respiratory tract, bone and joints, skin and skin structures (including those resulting from bone), central nervous system, intra-abdominal organs, post-operative infections and complicated and recurrent urinary tract infections, when caused by susceptible organisms.[28] Moreover, amikacin has unlabeled use as a part of a multiple-drug regimen for *Mycobacterium avium* complex, a common infection in AIDS patients.[25,27]

5. Contraindications

Amikacin injection is contraindicated in patients with a known history of hypersensitivity to amikacin, any constituents of the injection. This medication should be also avoided in patients with subclinical renal or eighth nerve damage induced by prior administration of nephrotoxic and/or ototoxic agents, as the toxicity may possibly be additive.

Aminoglycosides can cross the placenta and there have been several reports of total irreversible, bilateral congenital deafness in children whose mothers received streptomycin during pregnancy.[30] Although serious side effects to the fetus or newborns have not been reported in the treatment of pregnant woman with other aminoglycosides, the potential for harm exists so that they are contraindicated in pregnancy. It is not known whether amikacin is excreted in breast milk and whether its harmful effect on fetus. It is recommended that if nursing mothers must be treated with amikacin, the infants should not be breast-fed during maternal therapy.

6. Dosage and Administration

a. Adults, children and older infants

In general, recommended dose is 15 mg/kg/day divided into 2 or 3 equal doses every 8 or 12 hours by IM or IV, up to maximum of 1.5 gm daily in adults.[28] Use the patient's ideal body weight for dosage calculation.

b. Infants and premature neonates

In neonates with normal renal function, it is suggested to initiate treatment with a loading dose of 10 mg/kg followed by 7.5 mg/kg every 12 hours. The maximum total daily dose should not exceed 15 mg/kg. Solution infusions via the IV route should be given over 1 to 2-hour periods.

Insufficient clinical use has enabled to firm dosage guidelines to be established for the use of amikacin in premature infants.

c. Elderly

As amikacin is excreted by kidney, dosage should be adjusted in elderly according to renal function.

d. Single daily dose

Since the serum level of antimicrobial agents must be maintained above the minimum inhibitory concentration (MIC) for the target organisms at all times during the course of therapy, traditionally, aminoglycosides are given in multiple daily doses. The validity of this assumption has been strongly challenged during the past decade. The high peak extended interval dosing of aminoglycoside called once daily dosing has been first proposed by Labovitz in 1974 and then rediscovered by several groups.[9, 15, 31-37] By the year 2000, it has been estimated that 80% of the teaching hospitals worldwide have already practiced with this dosing regimens. The rationale for the pulse dosing is based on the following observations :

a) Aminoglycosides exhibit a significant post-antibacterial effect (PAE) against aerobic gram negative bacteria both *in vitro* and *in vivo*.[38] The duration of this effect depends on several factors, such as the chief one is the height of the preceding aminoglycoside peak. The PAE phenomenon

suggests that the aminoglycoside serum level may be allowed to fall below the MIC of the pathogen without compromising antimicrobial efficacy.

b) As mentioned earlier, aminoglycosides have concentration dependent bactericidal properties.[39] The multiple daily dosing regimen usually results in relatively low peak/MIC ratio (<5), but when the same total daily dose is given as a single bolus, much higher ratios are obtained (>10). Adult and pediatric patients receiving single daily doses of amikacin has shown significantly higher cure rates than those receiving multiple daily doses.[34]

c) Aminoglycoside uptaked into renal tubule cells and the inner ear appears to be saturated at relatively low serum levels, suggesting that higher peaks do not necessarily results in a greater risk of toxicity. Besides, trough serum at or near zero may promote tissue drug disposition, shorten tissue exposure, and promote recovery. In addition to the well known risk factors such as age, volume depletion, liver disease, co-administration of certain drug, the duration of exposure to the aminoglycoside appears to be a more important determinant of toxicity than the serum aminoglycoside level. Although no definitive evidence has been indicated, animal and human studies strongly suggest that pulse dosing shows less nephrotoxic.

d) *In vitro* studies indicate that more frequent dosing of aminoglycoside tends to reduce its uptake into bacterial cell of aerobic gram negative bacilli. This phenomenon is called adaptive post-exposure resistance and has been observed as an apparent increase in the MIC_{90}. Longer

dosing intervals appear to shorten the time required for the MIC to revert to its original value.[40]

The main objectives of the pulse dosing strategy are to:

1. Achieve a high aminoglycoside peak (10 or more times higher than MIC) to maximize efficacy.
2. Allow a drug-free interval of 3-5 hours to minimize toxicity and permit the reversal of the adaptive post exposure resistance.

During the last few years, over hundred articles have been published on the topic of aminoglycoside dosing.[9,15,32-37] They obviously show that the pulse dosing regimen offers more benefits than multiple daily dosing.[9,15,32-37]

7. Duration

Uncomplicated infection due to sensitive organisms should respond to the treatment within 24 to 48 hours at the recommended dosage. If no improvement occurs within 3-5 days, the use of amikacin sulfate should be re-evaluated and considered as an alternative therapy. Failure of the treatment may be due to resistance of the organism or the presence of septic foci requiring surgical drainage. Whenever possible, especially in patients with impaired renal function, peak and trough of amikacin should be monitored.

8. Adverse Reactions

a. Nephrotoxicity

Aminoglycosides are highly polar drugs which few molecules or none can cross biological membrane. Besides, they are filtered by the glomerulus. After entering the luminal fluid of the proximal renal tubule, a small portion of the total filtered drug is reabsorbed and stored in the proximal tubular cells. Continuously after charge-mediated binding, the drug is taken up

into the cell in small invaginations of the cell membrane, by a mechanism called “carrier mediated pinocytosis”.[41] Within one hour after injection, the drug is translocated into apical cytoplasmic vacuoles.[42] These endocytic vesicles fuse with lysosomes, sequestering aminoglycosides inside those organelles in an unchanged form. Since pinocytosis is a continuing phenomenon, aminoglycosides tend to accumulate extensively inside the lysosomes. Once trapped in the lysosomes of proximal tubular cells, aminoglycosides inhibit lysosomal phospholipases and sphingomyelinase, resulting in lysosomal phospholipidosis.[43,44] As the threshold in cortical drug concentration is reached, the lysosomal phospholipidosis progresses and the overloaded lysosomes continue to swollen even in the absence of any further drug administration. This may result in the loss of integrity of the restricting membranes of lysosomes and release of large amount of aminoglycosides, lysosomal enzymes and phospholipids into the cytosol. The extralysosomal aminoglycosides can gain access to other organelles, disturbing their functional integrity which may lead to cell death.[45]

The nephrotoxicity of aminoglycosides is determined by two major variables : (a) the intrinsic potential of the drug to damage subcellular structure and (b) the amount of drug accumulation in the renal cortex.[46] Any factors increased the renal uptake of aminoglycosides are risk factors for nephrotoxicity. Potential risk factors for aminoglycoside nephrotoxicity are shown in Table 2.2

Table 2.2 Potential risk factors for aminoglycoside nephrotoxicity [27,47]

Drug related	Patient related
Dose	Age
Duration	
Dosage regimen	
Prolong high trough concentrations	
Prior aminoglycoside treatment	Prior renal insufficiency
	Hepatic insufficiency
Choice of drug	Critically ill patient
	Sodium-volume depletion
Associated drugs	Other causes
Diuretics	
Cyclosporin	
Cisplatin	
Amphotericin B	

The duration of exposure to proximal tubular cells of the drug is a critical factor since it determines the extent of drug uptake. Persistent exposure undoubtedly results in an increasing renal drug levels. Dosage regimen is also another important determinant of the extent of cortical aminoglycoside concentrations. Powell et al and Rybak et al show that the nephrotoxicity caused by gentamicin is more severe when the total daily dose is divided or given by continuous infusion than which it is given as a single bolus.[32,48] Controversy exists over the importance of trough concentration as risk factors for nephrotoxicity.[27,49]

The incidence of aminoglycoside-induced nephrotoxicity has been reported approximately 8-26% in adult group.[27] Newborns may be resistant than older patients to the toxicity of aminoglycosides. Various markers have been used to assess risk of nephrotoxicity, one of them is an excess aminoglycoside serum level. Potential markers for nephrotoxicity are urinary concentration of N-acetyl-β-glucosaminidase (NAG), β_2-microglobulin and increasing in serum creatinine. NAG is a lysosomal enzyme excreted in urine after the damage of the proximal tubule cell. β_2-microglobulin is a low-

molecular-weight protein filtered at the glomerulus and reabsorbed by the proximal tubule. Increased serum creatinine likely occurs relatively in the late stage of aminoglycoside toxicity but widely used to assess nephrotoxicity.[16,50]

b. Ototoxicity

The incidence of aminoglycoside-induced ototoxicity has been reported about 4.6-24 % in adults.[27,51] In patients treated at high doses or for periods longer than those recommended, ototoxicity, both auditory and vestibular can occur. Patients with renal damage have the highest risk of developing amikacin induced ototoxicity . The risk of hearing loss due to aminoglycosides increases with the degree of exposure to either high peak or high trough serum concentrations. Aminoglycoside-induced ototoxicity is usually irreversible and it may occur during therapy or up to 4–6 weeks after termination of treatment. A pre-treatment audiogram should be obtained and repeated during therapy in patients with renal impairment undergoing treatment for 7 days or more as well as in other patients being treated for 10 days. If tinnitus or subjective hearing loss develops, or if follow-up audiograms show significant loss of high frequency response, the use of amikacin therapy should be discontinued immediately.

Table 2.3 Potential risk factors for aminoglycoside ototoxicity [27,52]

Drug related	Patient related
Dose	Age
Duration	
Dosage regimen	
Prolong high trough concentrations	
Prior aminoglycoside treatment	Prior renal insufficiency
	Choice of drug
	Sodium-volume depletion
Associated drugs	Other causes
Diuretics	

c. Neuromuscular Blockade

Neuromuscular blockade is an unusual toxic reaction of aminoglycosides.[27] Aminoglycosides can produce varying degrees of neuromuscular blockade. Although the blockade induced by an aminoglycoside is generally dose related and self-limiting, it may rarely result in respiratory paralysis. Neuromuscular effects are most likely to occur when an aminoglycoside is given to patients with neuromuscular disease (e.g. myasthenia gravis) or hypocalcemia or to patients who received general anesthetics, neuromuscular blocking agents or massive transfusion of citrated blood.[27] If blockade occurs, calcium salts may reverse this phenomenon. Other manifestations of neurotoxicity may include numbness, skin tingling, muscle twitching and convulsions.

d. Other adverse reactions

Adverse reactions which have been reported on rare occasions are skin rash, drug fever, headache, paresthesia, tremor, hypotension, nausea, vomiting, hypomagnesemia, eosinophilia, arthralgia and anemia.[27]

9. Drug Interaction

Table 2.4 Drug interaction of aminoglycosides.[53]

Precipitant drug	Significant level	Onset	Severity	Efficacy	Management
Cephalosporins	2	Delayed	Moderate	Neprotoxicity may increase Bactericidal activity against certain microorganism may enhance	Monitor aminoglycoside levels and renal function closely. Reduce dosage or discontinue one or both drugs and use alternative drug if renal dysfunction occurs.
Penicillins	2	Delayed	Moderate	Certain penicillins may inactivate certain aminoglycoside	Do not mix them together in the same solution. Monitor aminoglycoside concentration and renal function. Adjusted dosage if needed.
Loop diuretics	4	Rapid	Major	Auditory toxicity may increase	Perform baseline periodic testing and monitoring avoid excessive dose. Reduce dose if needed in patient with renal insufficiency.

Significance rating	Severity	Documentation
1	Major	Suspected or >
2	Moderate	Suspected or >
3	Minor	Suspected or >
4	Major or moderate	Possible
5	Minor, any	Possible, Unlikely

Amikacin Studies in Neonates

Pharmacokinetics of amikacin widely vary in populations, especially in neonates. Howard and McCracken have found that a single intramuscular injection of 7.5 mg /kg amikacin to neonates resulted in a range of elimination half-life between 5 and 11 hours.[21] Kinetics of amikacin in neonates are different from adults. Serum half-life is about 6 hours in infants aged less than 7 days and decreases to 3 hours in 1 month aged patients.[22,54] Volume of distribution also decreases conversely against postnatal age.

Thereafter, Prober et al have proposed that there is a progressive decrease in mean peak and trough blood concentration with increasing chronological age.[55] This finding probably due to the improvement of renal function with increasing chronological age. This observation suggests that infants older than two weeks may require a higher total daily dose of amikacin to maintain peak blood concentrations at therapeutic range. The finding for an increasing dose has previously been emphasized by Sardemann et al.[22]

Assael et al have conducted a study regarding the effect of intrauterine maturation on the pharmacokinetics of amikacin in neonatal period using 29 preterm and term neonates.[8] They have found that the initial elimination half life and volume of distribution are significantly related to intrauterine maturation whereas no significant linear correlation has been found between clearance and gestational age.

Since serum concentration cannot be monitored in all clinical setting, population pharmacokinetic parameters of neonates have been developed for dosing calculation. In 1991, Peterson et al have evaluated a dosing chart based on population pharmacokinetic parameters for infants with various postnatal ages and birth weights.[56] Thirty-eight suspected infection neonates with postconceptional age 25-43 weeks have been evaluated by comparing the predicted versus observed steady state peak and trough of serum

amikacin concentrations. Desired therapeutic Css_{max} at 20-30 μg/ml has been produced in about 76.3 % of patients.

Padovani et al have studied the pharmacokinetics of amikacin in neonates, both term and preterm babies, who have been repetitively given amikacin by intramuscular route.[57] One-compartment model has been used and the results are similar to the values reported previously by Kenyon et al, except the volume of distribution, which is higher in this study.[12]

In further study, Botha et al used an alternative approach, nonlinear mixed effect model (NONMEM) to develop population parameters from 53 black neonates with mean gestational age of 35 weeks.[5] By using one compartment model, they have found that birth weight is an important determinant of both clearance and volume of distribution.

When concept of single daily dose regimen has approached with benefits of reducing aminoglycoside nephrotoxicity and ototoxicity, There is no data available for neonates so Langhendries et al have conducted a first study about the toxicity of once daily amikacin dosing schedule in neonates compare to the twice daily dosage regimen.[16] However, they have not been able to show clearly significant clinical benefits of once daily dosage regimen over the twice daily dosage regimen. Later on, Kotze et al have conducted a study in 1999 to assess the incidence of amikacin's toxicity on auditory and renal glomerular function by comparing doses of amikacin 15 mg/kg once daily and 7.5 mg/kg twice daily in full term neonates.[37] They have concluded that amikacin administered once or twice daily to full term neonates has been found to have similar toxicity. The once daily administration of amikacin may be recommended in fullterm neonates since it does not increase the risk of toxicity.

Additionally, Cervantes-Munguia et al have studied the scheme of amikacin treatment on premature neonates which is defined by gestational and postnatal age.[58] They have shown that amikacin serum peak and trough concentration have been maintained within recommended limits.

Even most studies have been conducted in foreign countries, Titipunkun has compared once and twice daily dosing of amikacin in Thai infants aged 3 months to 15 years.[59] She has concluded that once daily dosing may show benefit over twice daily because of the higher peak concentration with no toxicity.

There is no study about amikacin in Thai preterm neonates.

II. Review of neonatal sepsis

Neonatal sepsis is a life-threatening emergency. This term has been used to describe the systemic response to infection in the newborn infant. The incidence varies from less than one to eight cases per 1000 live births. It is divided into 2 major groups, early onset and late onset sepsis, according to the onset of infection.

A. Risk factors for neonatal sepsis [3,60]

1. Prolonged rupture of membrane (PROM) > 18 hours
2. Premature rupture of membrane
3. Chorioamnionitis
4. Maternal colonization with group B streptococcus
5. Maternal fever
6. Maternal urinary tract infection
7. Prematurity
8. Low birth weight
9. Foul smelling amniotic fluid
10. Male gender
11. Perinatal asphyxia
12. Combination of risk factors

Table 2.5 Risk factors for neonatal sepsis[3]

Condition	Incidence proven sepsis	Incidence proven and highly suspected sepsis
PROM[a] > 18-24 hrs	1%	1-2%
Maternal + GBS[b]	0.5-1%	1-2%
PROM and + GBS[b]	4-6%	7-11%
+GBS[a] and maternal fever	3-5%	6-10%
PROM and chorioamnionitis	3-8%	6-10%
PROM or + GBS and preterm	4-6%	7-11%
PROM and Apgar 5 min < 6	3-4%	6-10%
Male gender	risk ↑ 4 fold	risk ↑ 4 fold

PROM[a] = Prolong rupture of membrane, GBS[b] = Group B Streptococci

B. Clinical manifestations

Most infants present with nonspecific symptoms and signs include lethargy, thermal instability (hypothermia, fever), feeding intolerance, unexplained jaundice or unhealthy looking. Furthermore, severe systemic funding may be observed such as hypotonia, vomiting, abdominal distention, grunting, apnea, cyanotic spell, pure perfusion or shock, seizure, petichii or purpura. Respiratory distress in preterm should be considered as sepsis if risk factors have been detected.

C. Diagnostic Laboratory Tests

1. Nonspecific: Complete blood count (CBC), C-reactive protein (CRP), Erythrocyte sedimentation rate (ESR), Chest x-ray (CXR), Blood sugar (BS) etc.
2. Specific: Hemoculture (H/C), amniotic fluid, tracheal fluid, gastric content and CSF culture, Counter immunoelectrophoresis (CIE) or Latex particle agglutination (LPA).

Table 2.6 Performance of hematological tests and screens in the diagnosis of neonatal sepsis[3]

Tests	Sensitivity	Specificity	Positive Predictive	Negative Predictive
1. Neutropenia (Manroe or < 1750 /mm)	38-96	61-92	20-77	96-99
2. ↑ I/T (Manroe or > 0.2)	90-100	50-78	11-51	99-100
3. ↑ Total immature neutrophils	63-67	69-77	17-46	95
4. 1+2+3	94-100	NA	NA	100
5. Seven point hematologic score ≥	96	78	31	99
6. Total WBC < 5000	29	91	27	91
7. WBC vacuolization or toxic granulation	67-81	90-93	45-59	96-98
8. Platelet count < 150000	22-38	82-99	20-60	92-93

D. Treatments

1. Specific treatment: proper antibiotics by organisms but usually start with combination of ampicillin and aminoglycosides.
2. Supportive care: hydration, nutrition, thermal control etc.
3. Care for complications e.g. correct acid base imbalance, seizure etc.
4. Adjunctive therapy by enhancement of neonatal host defense
    a. Intravenous Immunoglobulin (IVIG)
    b. Granulocyte transfusion
    c. Cytokines: human granulocyte colony stimulating factor
    d. Immunomodulating agents

E. Prognosis

Mortality : 5-25 %

Morbidity : 20-30 % with meningitis

CHAPTER III

PATIENTS AND METHODS

This study has been conducted at the Neonatal Intensive Care Unit of Phramongkutklao Hospital in Bangkok from June 2002 to February 2003. The ethical review committee on human research, Phramongkutklao Hospital, has approved the study protocol on 3rd June, 2002.

I. Definitions

A. Preterm neonates

Premature neonates are defined when a baby is born with gestational age less than 37 weeks. Assessment of gestational age is only based on the measurement scores described by Ballard et al.[61]

B. Efficacy of Amikacin

Efficacy has been evaluated from clinical factors as follow :

1. Clinical Response
   - □ Favorable clinical responses are defined as : patients demonstrate improvement in alertness, feeding tolerance and respiration.
   - □ Unfavorable clinical responses are defined as : any signs and symptoms of clinical sepsis still persist.
2. Body Temperature
   - □ Favorable

     Return to normal temperature (36.5-37.5 °C) within 48 hours after therapy

- □ Unfavorable

  Body temperature is higher than 37.5°C or lower than 36.5°C after 48 hours of treatment

- □ Not evaluate

  There is no abnormal temperature at initial therapy

3. White Blood Cell Count

   - □ Favorable

     Normalization of white blood cell count (9.0-35.0x10^3 /mm^3).[62]

   - □ Unfavorable

     Abnormality of white blood cell count

   - □ Not evaluate

     There is no abnormal white blood cell count at initial therapy

4. Hemoculture

   - □ Favorable

     Organisms found at initiation of therapy are killed and there is no organism left after therapy.

   - □ Unfavorable

     Hemoculture still positive or there are new microorganisms and signs of infection.

   - □ Not evaluate

     There is no growth of organism after 2-7 days of culture.

C. Nephrotoxicity

- Toxic

  Increase in serum creatinine ($\geq$ 0.5 mg/dl) after discontinuation of therapy, compare to value obtained at day 1 of life.

- Non-toxic

  Increase in serum creatinine (< 0.5 mg/dl) after discontinuation of therapy, compare to value obtained at day 1 of life

- Not evaluate

  Not enough data to compare

II. Sample size

$$n = \frac{Z_\alpha^2 \sigma^2}{d^2}$$

Z_α = 1.96 (two-tailed, 95%)

σ = Standard deviation of peak concentration

d = Acceptable equivalent concentration

There is no previous study in premature neonates with the dose of 16 mg/kg or 18 mg/kg, but there was a study of amikacin 15 mg/kg in premature neonates by Kotze et al.[37] The peak concentration and standard deviation found in that study was 30.57$\pm$2.81 μg/ml. In this study, these values will be used to calculate the sample size. The range of ten percent variation from this peak concentration is considered acceptable.

$$n = \frac{(1.96)^2 (2.81)^2}{(0.10 \times 30.57)^2}$$

$$= \frac{3.84 \times 7.90}{9}$$

$$= 3.37$$

From this result, in each group should have at least 4 patients.

III. Subjects

Inclusion criteria Patients with the following characteristics are included into this study:

1. Born at Phramongkutklao Hospital
2. Premature neonates
3. Parents permit and sign the consent form
4. Patients are treated with amikacin either alone or in combination with other antibiotics due to suspected or documented infections

Exclusion criteria Patients with the following characteristics are excluded from this study:

1. Patients with severe asphyxia (Apgar score less than 2 at 5 minutes).
2. Patients have allergy to aminoglycosides.
3. Patients have renal impairment before the study (serum creatinine at the first day is greater than or equal to 1.5 mg/dl).
4. Patients with neutropenia (WBC < 1000 cell/mm^3, PMN 0-10 %).
5. Patients are treated with the drugs having nephrotoxicity before collecting blood samples.
6. Patients have multiple congenital abnormalities.
7. Suspected by physicians, patients may be unsafe when participate in the study.

IV. Materials

A. TDx Amikacin

1. No. 9508-01, Amikacin calibrators (Lot.no. 83330Q100)

Six vials of amikacin in 2.5 ml normal human serum at following concentrations have been prepared to be calibrators.

Vial	Amikacin concentration (μg/ml)
A	0.0
B	3.0
C	10.0
D	20.0
E	35.0
F	50.0

Preservative: Sodium azide

2. No. 9508-10, Amikacin controls

Three vials of amikacin in 2.5 ml normal human serum at following concentrations have also been prepared in order to be amikacin controls.

Vial	Target conc. (μg/ml)	Range (μg/ml)
L	5.0	4.25 - 5.75
M	15.0	13.50 - 16.50
H	30.0	27.00 - 33.00

Preservative: Sodium azide

3. No.9508, Amikacin reagent pack (Lot.no. 79399Q100)

The reagent pack consists of three vials as following:

Vial	Contents
S	< 1% Amikacin antiserum (sheep) in buffer with protein stabilizer (4.0ml) Preservative: Sodium azide
T	< 0.01% Amikacin fluorescein tracer in buffer containing surfactant and protein stabilizer (3.5 ml) Preservative: Sodium azide
P	Pretreatment solution. Surfactant in buffer containing protein stabilizer (2.5 ml). Preservative : Sodium azide

4. No.9519, X SYSTEMS dilution buffer

Bovine gamma globulin in phosphate buffer is used as buffer solution and has been prepared with sodium azide.

B. Apparatus

1. Automated Fluorescence Polarization Analyzer (Diagnostic Division , Abbott Laboratories, Inc., Irving, TDx, U.S.A. Serial No.18581-96).
2. Centrifuge (Spectrafuge 16M, Labnet Inc., USA).
3. Freezer (Ice Pack Freezer, Yommaraj Co.Ltd., Thailand (-40 to 40°C)).

V. Methods

A. Dosage and Administration

Neonates met the inclusion criteria were treated with amikacin dosage regimens following through the guideline adapted from Neofax 2001 guideline (Table 3.2) by intravenous infusion at constant rate for 30 minutes.

Table 3.1 Dosage regimens of amikacin in Neofax 2001

Gestational age (wks)	Dosage (mg/kg)	Interval (hrs)
≤ 27	18	48
28-30	18	36
31-33	16	36
34-36	15	24

Table 3.2 Dosage regimens of amikacin using in this study

Gestational age (wks)	Dosage (mg/kg)	Interval (hrs)
≤30	18	48
31-33	16	48
34-36	15	24

B. Sample Collection

The 0.5-1 ml of blood sample were drawn to measure amikacin serum concentration at time of steady state. It takes approximately three to five half life after administration. From previous published data, half life of amikacin in preterm neonates is about 7 – 8 hours.[20] As a result, blood sample was drawn at the dose giving at the forty-eighth hours.

From the study conducted by Titipunkul, the trough concentration cannot be obtained at thirty minutes before the next dose in more than seventy percent of patients. As a consequence, the second serum concentration should be measured at twelve or eighteen hours after the administration. In the same way, Labaune et al have also collected blood sample at the eighteen hours after the administration.[15] In order to obtain information on the elimination and other pharmacokinetic parameters, this time of sampling is preferred than thirty minutes before the next dose. In this study, the second time of blood collection is eighteen hours or thirty-six hours after the administration in patients receiving drug every twenty four hours or every forty eight hours, respectively. In summary, blood sampling time was done as follow:

a. For patients receiving amikacin every 24 hours blood samples were collected for peak concentration within 30 minutes after the completion of the third dose and blood samples were drawn again at the eighteenth hours of the third dose in order to calculate trough concentration.

b. For patients receiving amikacin every 48 hours blood samples were collected for peak concentration within 30 minutes after the completion of the second dose and blood samples were drawn again at the thirty-sixth hours of the second dose in order to calculate trough concentration.

After blood clotting occurred, serum was separated by centrifugation at 3,000 rounds per minutes for 10-15 minutes at room temperature. They were analyzed by Fluorescence Polarization Immunoassay Techniques (FPIA). If the blood samples were not immediately analyzed, they were frozen at -8 to -70 $^{\circ}$C within 24 hours until assayed.

C. Analytical Method

Amikacin plasma levels were determined by immunoassay method using TDx Analyzer system, Abbott Laboratories based on fluorescence polarization technique. Coefficient of variation is less than 5 % at a concentration range between 5-30 μg/ml.

D. Data Collection

1. Demographics

Patients' background information related to the study from medical records were obtained including gestational age, postnatal age, sex, weight, height, diagnosis, clinical status, vital sign, laboratory data such as blood urea nitrogen, serum creatinine, complete blood count and culture sensitivity specimens.

2. Amikacin peak and trough levels

Peak concentration was measured from blood drawn at 30 minutes after the completion of infusion. Trough concentration was calculated from the equation 1.

Equation 1

$$C_t = C_p (e^{-kt})$$

C_p = Peak concentration (μg/ml.)

C_t = Trough concentration (μg/ml.)

t = Time interval between peak concentration and trough concentration (hr.)

3. Pharmacokinetic parameters

Pharmacokinetic parameters such as elimination rate constant and half life, volume of distribution, amikacin clearance were calculated by using equations 2-5.

Equations 2

$$K_e = \frac{\ln C_1/C_2}{\Delta t}$$

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

C_1 = Concentration at time t_1 (μg/ml)

C_2 = Concentration at time t_2 (μg/ml)

Δt = Time interval between t_1 and t_2 (hr)

Equations 3

$$V_d = \frac{FSD\,(e^{-kt})}{C_p\,(1-e^{-k\tau})}$$

V_d = Volume of distribution (l)

C_p = Peak concentration (μg/ml)

F = Bioavailability factor = 1

S = Salt Factor = 1

D = Dose (mg)

t = Time after the start of the infusion (hr)

τ = Dosing interval (hr)

Equation 4

$Cl = K_e \times V_d$

Cl = Clearance of amikacin (l/hr)

Equation 5

$t_{1/2} = 0.693/ K_e$ (hr)

4. Renal parameters

Creatinine clearances were calculated using equations of Schwartz et al as follow: [62]

CrCl = K x L / SCr

CrCl = Creatinine clearance (ml/min/1.73m^2)

K = Constant of proportionality that is age specific (for birth weight less than 2500 grams, K = 0.33)

L = Height (cm)

SCr = Serum creatinine concentration (mg/dl)

E. Data Analysis and Statistical

1. Demographic characters

Descriptive analysis was used to describe demographic characters of subjects.

2. Pharmacokinetics

a. The mean steady state peak and trough of amikacin concentrations between three gestational groups, group I, group II and group III, were compared using ANOVA.

b. Demonstrate the numbers and percentage of patients whose peak serum concentration reached therapeutic level (20-30 μg/ml), subtherapeutic level (< 20 μg/ml) and overtherapeutic level (>30 μg/ml).

c. Demonstrate the numbers and percentage of patients whose trough serum concentration were in the range 2-5 μg/ml, less than 2 μg/ml and over 5 μg/ml.

d. Pharmacokinetic parameters of three gestational groups, group I, group II and group III, were compared using ANOVA.

e. Correlation between pharmacokinetic parameters and demographic characteristics were analyzed by using linear regression.

f. Correlation between both serum creatinine and creatinine clearance with amikacin clearance were analyzed by using linear regression.

CHAPTER IV

RESULTS AND DISCUSSION

1. Characteristics of patients

According to the criteria, thirty-seven preterm neonates have been enrolled in this study. The characteristics of the patients, indications for treatment, dose, duration and concomitant antibiotic are presented in Table 4.1. There are 7 patients with gestational age not greater than 30 weeks (group I), 16 patients with gestational age of 31- 33 weeks (group II), and 14 patients with gestational age of 34-36 weeks (group III). There are 5 males (71.42%) and 2 females (28.57%) in group I, 7 males (43.75%) and 9 females (56.25%) in group II, 8 males (57.14%) and 6 females (42.86%) in group III. There is no significant difference in sex between gestational groups when analyzed by chisquare test ($p > 0.05$).

Comparison of the patients' characteristics between gestational groups are shown in Table 4.2 and 4.3. There are significant differences between groups with respect to gestational age (GA), birth weight (BW) and height (Ht) ($p < 0.001$). In contrast, there is no significant differences between groups according to Apgar score (APGS) at 1 minute, 5 minute and duration of therapy ($p > 0.05$). Postnatal age in smaller GA is shorter than in higher GA. However, there is no significant difference between mean postnatal age (PNA) of each group. For all babies, mean GA and BW are 32.38 ± 2.34 weeks and 1813.24 ± 494.58 grams. They are in range of 27 - 36 weeks and 920 - 3,030 grams, respectively.

Table 4.1 Demographics of patients in this study

No.	Sex	GA	BW	Ht.	APGS		PNA	Dose	Dosing interval	Calculate dosage regimen	Duration	Con.ATB
		(wks)	(gm)	(cm)	1 min	5 min	(days)	(mg/dose)	(hrs)	(mg/kg)	(days)	
1	M	32	1620	40	9	10	0.15	25	48	15.43	4	Amp.
2	F	32	1420	41	9	10	0.10	22	48	15.49	8	Amp.
3	F	33	1240	37	9	10	0.06	19	48	15.32	7	Amp.
4	F	34	2110	42	9	9	0.17	30	24	14.22	5	Amp.
5	M	29	1000	36	8	9	0.10	18	48	18.00	11	Amp.
6	M	29	1520	41	7	8	0.11	27	48	17.76	8	Amp.
7	F	33	2060	43	7	9	0.46	32	48	15.53	5	Amp.
8	M	30	1275	39	8	9	0.38	22	48	17.25	6	Amp.
9	F	27	1170	36	7	7	0.10	21	48	17.95	7	Amp.
10	M	35	2600[a]	45	5	8	0.23	40	24	15.38	7	Amp.
11	M	34	3030 [a]	45	4	7	0.81	45	24	14.85	8	Amp.
12	F	36	2165	44.5	5	8	0.01	32	24	14.78	6	Amp.
13	F	33	1400	38	9	10	0.04	22	48	15.71	6	Amp.
14	M	28	1080	37	4	7	0.08	18	48	16.67	7	Amp.
15	F	31	1500	39	4	8	0.06	24	48	16.00	6	Amp.
16	F	31	1600	41	9	10	0.06	25	48	15.63	8	Amp.
17	M	30	1560	43	3	7	0.13	28	48	17.95	5	Amp.
18	F	32	1860	42	8	9	0.05	30	48	16.13	5	Amp.
19	M	31	1410	42	8	9	0.52	22	48	15.60	6	Amp.
20	M	35	1770	41	7	8	0.04	27	24	15.25	5	Amp.
21	M	36	2310	46	8	10	0.42	33	24	14.29	5	Amp.
22	F	34	1500	41	7	10	0.13	22	24	14.67	5	Amp.
23	M	35	2020	41.5	8	9	0.56	30	24	14.85	4	Amp.
24	F	27	920	34	2	10	0.10	16	48	17.39	7	Amp.
25	F	35	2450	48	9	10	0.25	36	24	14.69	7	Amp.
26	M	32	1820	40	7	8	0.96	30	48	16.48	7	Amp.
27	M	32	1900	41	9	10	0.92	30	48	15.79	7	Amp.

Table 4.1 Demographics of patients in this study (Continued)

No.	Sex	GA	BW	Ht.	APGS		PNA	Dose	Dosing interval	Calculate dosage regimen	Duration	Con.ATB
		(wks)	(gm)	(cm)	1 min	5 min	(days)	(mg/dose)	(hrs)	(mg/kg)	(days)	
28	M	34	2080	47	9	10	0.31	30	24	14.42	6	Amp.
29	M	34	2550 [a]	48	8	9	0.31	38	24	14.90	7	Amp.
30	F	31	1895	40	5	5	0.06	30	48	15.83	6	Amp.
31	M	33	2510 [a]	47	9	10	0.07	40	48	15.94	4	Amp.
32	F	33	1600	43	7	9	0.21	25	48	15.63	5	Amp
33	M	32	1880	44	6	9	0.21	30	48	15.96	5	Amp
34	M	34	2100	44	7	9	0.35	30	24	14.29	7	Amp
35	F	34	2500 [a]	47	9	10	0.04	38	24	15.20	4	Amp
36	M	33	2020	44	1	6	0.1	32	48	15.84	5	Amp
37	F	34	1645	41.5	7	8	0.25	24	48	14.59	7	Amp

[a] patients with birth weight $\geq$ 2500 gm, however, the same equation has been used to calculate creatinine clearance since no specific equation has been identified by other researcher for this group of patient.

GA = gestational age, BW = birth weight, Ht = height, APGS = Apgar score, PNA = postnatal age
Con.ATB = concomitant antibiotic, Amp = ampicillin.

Table 4.2 Comparison of mean ± SD of the patients' characteristics between gestational groups

Characteristics	Mean ± SD (Range)				P Value*
	GA ≤30 (Gr I, n = 7)	GA = 31-33 (Gr II, n =16)	GA = 34-36 (Gr III, n = 14)	Total (n =37)	
Gender					
Male	5	7	8	20	0.452
Female	2	9	6	17	
GA (wks)	28.57±1.27 (27-30)	32.13±0.81 (31-33)	34.57±0.76 (34-36)	32.38±2.34 (27-36)	0.000
BW (grams)	1,217.86±248.02 (920-1,560)	1,733.44±321.57 (1,240-2,510)	2,202.14±409.97 (1,500-3,030)	1,813.24±494.58 (920-3,030)	0.000
Ht (cm)	38.00±3.16 (34-43)	41.44±2.56 (37-47)	44.39±2.61 (41-48)	41.91±3.51 (34-48)	0.000
APGS[a]					
1 min	5.57±2.51 (2-8)	7.25±2.29 (1-9)	7.29±1.64 (4-9)	6.95±2.16 (1-9)	0.176
5 min	8.14±1.21 (7-10)	8.88±1.50 (5-10)	8.93±1.00 (7-10)	8.76±1.28 (5-10)	0.378
Duration of amikacin therapy (days)	7.29±1.89 (5-11)	5.88±1.26 (4-8)	5.93±1.27 (4-8)	6.16±1.46 (4-11)	0.074

*P Value compare between three gestational groups analyzed by ANOVA except sex analyzed by chi-square test

APGS[a] = Apgar score

Table 4.3 Comparison of postnatal age between gestational groups

Gestational age (wks), (Group , n)	Postnatal age (hrs)			
	Mean[a]	Median	Range	SD
≤ 30 (Gr I, n = 7)	3.90	2.50	2.00-9.00	2.62
31-33 (Gr II, n = 16)	4.96	2.50	1.00-23.25	5.96
34-36 (Gr III, n = 14)	6.77	6.25	0.33-19.55	5.14

[a] P Value analyzed by ANOVA is 0.442, SD = standard deviation

II. Pharmacokinetics Data of Amikacin

The pharmacokinetic parameters of amikacin have been shown in Table 4.4. They are analyzed with assumption of one compartment model and first order kinetics since plasma concentrations have been obtained during the elimination phase which errors from calculation would be minimized.

Table 4.5 presents mean $\pm$ SD of peak and trough concentrations of each groups and of total patients. The mean $\pm$ SD of peak concentration (C_p) at steady state of group I, II, III are 28.49 $\pm$ 8.63, 24.22 $\pm$ 5.99, 24.91 $\pm$ 5.73 μg/ml, respectively. The means are calculated from the concentrations range from 15.45 - 41.40, 16.11 - 38.05 and 13.17 - 36.43 μg/ml, for group I, II, III, respectively. The mean $\pm$ SD of C_p have no significant differences between groups ($p > 0.05$). Mean $\pm$ SD of peak concentrations of all newborns is 25.29 $\pm$ 6.46 μg/ml which is in desired therapeutic range and similar to the value have been reported by Langhendries et al.[63] Numbers and percentage of patients whose amikacin peak concentrations are subtherapeutic, therapeutic and overtherapeutic levels have been shown in Table 4.6. Only about 54 % of all babies have desired therapeutic levels. The most percentages of patients achieving desired concentration have been found in group III. Subtherapeutic and overtherapeutic levels have been found in about 22% and 24% of total patients, respectively. The results have shown that five of eight patients who have subtherapeutic peak concentration are in group II. Notably, most patients in group I have overtherapeutic peak concentrations .

Eventhough the second drawing for amikacin serum level has been done at six to twelve hours before the next dose, six of sixteen patients in group II have serum drug concentration less than 0.8 μg/ml. This result shows that there is wide variability in this group. Mean $\pm$ SD of trough concentration (C_t) at steady state of group I, II, III are 1.75 $\pm$ 1.32, 1.16 $\pm$ 2.13, 2.21 $\pm$ 1.20 μg/ml, respectively. The means are calculated from the concentrations range from 0.35 - 3.63, 0.05 -7.17, 0.27- 4.54 μg/ml, for group I, II, III, respectively.

The mean $\pm$ SD of amikacin trough concentration (C_t) of total doses is 1.77 $\pm$ 1.60 μg/ml. There is no significant difference of C_t between groups ($p > 0.05$). Numbers and percentage of patients whose amikacin trough concentration are less than 2 mg/dl, between 2 and 5 μg/ml, and over 5 μg/ml have been shown in Table 4.7. None of these patients has C_t over 5 μg/ml except one patient in group II.

Table 4.4 Amikacin levels and pharmacokinetic parameters

No.	Dose (mg/dose)	Dose (mg/kg)	C_p (μg/ml)	C_t (μg/ml)	K_e (hr^{-1})	$t_{1/2}$ (hr)	V_{ds} (l)	V_d (l/kg)	Cls (l/hr)	Cl (l/hr/kg)
Gr I (GA <30 wks)										
5	18	18.00	20.15	3.63	0.0369	18.79	1.0376	1.0376	0.0383	0.0383
6	27	17.76	41.40	2.90	0.0572	12.12	0.6583	0.4331	0.0377	0.0248
8	22	17.25	30.59	1.94	0.0593	11.69	0.7196	0.5644	0.0427	0.0335
9	21	17.95	15.45	0.59	0.0703	9.86	1.3119	1.1213	0.0922	0.0788
14	18	16.67	27.57	0.35	0.0939	7.38	0.6010	0.5565	0.0565	0.0523
17	28	17.95	34.21	0.39	0.0963	7.20	0.7507	0.4812	0.0722	0.0463
24	16	17.39	30.05	2.43	0.0541	12.82	0.5451	0.5925	0.0294	0.0320
Gr II (GA 31-33 wks)										
1	25	15.43	18.87	7.17	0.0208	33.30	2.0538	1.2678	0.0428	0.0264
2	22	15.49	23.51	1.06	0.0667	10.39	0.9126	0.6427	0.0608	0.0428
3	19	15.32	31.09	0.79	0.079	8.78	0.5777	0.4659	0.0456	0.0368
7	32	15.53	23.37	<0.8						
13	22	15.71	16.11	<0.8						
15	24	16.00	16.66	0.3	0.0866	8.00	1.3469	0.8979	0.1167	0.0778
16	25	15.63	23.85	<0.8						
18	30	16.13	19.47	0.5	0.0789	8.78	1.4569	0.7833	0.1149	0.0618
19	22	15.60	21.26	<0.8						
26	30	16.48	29.28	<0.8						
27	30	15.79	38.05	<0.8						
30	30	15.83	31.30	0.24	0.1048	6.61	0.8689	0.4585	0.0910	0.0480
31	40	15.94	18.71	0.41	0.0823	8.72	1.9944	0.7946	0.1642	0.0654
32	25	15.63	25.04	0.30	0.0951	7.29	0.9296	0.5810	0.0883	0.0552
33	30	15.96	23.84	0.82	0.0725	9.56	1.1981	0.6373	0.0869	0.0462
36	32	15.84	27.20	0.05	0.1376	5.04	1.0260	0.5079	0.1412	0.0699
Gr III (GA 34-36 wks)										
4	30	14.22	27.41	2.57	0.1051	6.59	0.9997	0.4738	0.1051	0.0498
10	40	15.38	30.16	2.80	0.1057	6.56	1.2958	0.4984	0.1370	0.0527
11	45	14.85	21.60	0.59	0.1601	4.33	1.8141	0.5987	0.2906	0.0959
12	32	14.78	13.17	1.65	0.0924	7.50	2.4865	1.1485	0.2297	0.1061
20	27	15.25	22.83	0.27	0.1971	3.52	0.9797	0.5535	0.1931	0.1091
21	33	14.29	24.42	2.10	0.109	6.36	1.3075	0.5660	0.1425	0.0617
22	22	14.67	21.64	1.57	0.1166	5.94	0.9633	0.6422	0.1124	0.0749
23	30	14.85	16.29	2.72	0.0796	8.71	1.9962	0.9882	0.1590	0.0787

Table 4.3 Amikacin levels and pharmacokinetic parameters (Continued)

No.	Dose (mg/dose)	Dose (mg/kg)	C_p (μg/ml)	C_t (μg/ml)	K_e (hr^{-1})	$t_{1/2}$ (hr)	V_{ds} (l)	V_d (l/kg)	Cls (l/hr)	Cl (l/hr/kg)
Gr III (GA 34-36 wks) (Continued)										
25	36	14.69	26.6	2.65	0.1024	6.77	1.336	0.5453	0.137	0.0559
28	30	14.42	28.03	3.47	0.0929	7.46	1.093	0.5255	0.1015	0.0488
29	38	14.90	26.56	1.24	0.1362	5.09	1.2977	0.5089	0.1767	0.0693
34	30	14.29	26.8	4.82	0.0725	9.56	1.1981	0.6373	0.0869	0.0462
35	38	15.20	26.81	1.29	0.1346	5.14	1.3425	0.537	0.1813	0.0725
37	24	14.59	36.43	3.61	0.1028	6.74	0.6498	0.395	0.0668	0.0406

C_p = peak concentration at 30 minutes after complete infusion, C_t = trough concentration at 30 minutes before next dose, K_e = elimination rate constant, $t_{1/2}$ = elimination half life, V_{ds} = volume of distribution, V_d = volume of distribution per body weight, Cls = clearance, Cl = clearance per body weight

Table 4.5 Comparison of amikacin dose and serum concentration of amikacin in three gestational groups

Parameters	Mean ± SD (Range)				P Value*
	Group I (n=7)	Group II (n=16)	Group III (n=14)	Total (n = 37)	
Calculate dosage regimen (mg/kg)	17.57±0.49 (16.67-18.00)	15.77±0.29 (15.32-16.48)	14.74±0.37 (14.22-15.38)	15.72±1.08 (14.22-18.00)	0.000
C_p (μg/ml)	28.49±8.63 (15.45-41.40)	24.22±5.99 (16.11-38.05)	24.91±5.73 (13.17-36.43)	25.29±6.46 (13.17-41.40)	0.343
C_t (μg/ml)	1.75±1.32 (0.35-3.63)	1.16±2.13[a] (0.05-7.17)	2.21±1.20 (0.27-4.54)	1.77±1.60 (0.05-7.17)	0.290

[a] calculate from n = 10 , P Value * analyzed by using ANOVA ,

C_p = peak concentration, C_t = trough concentration

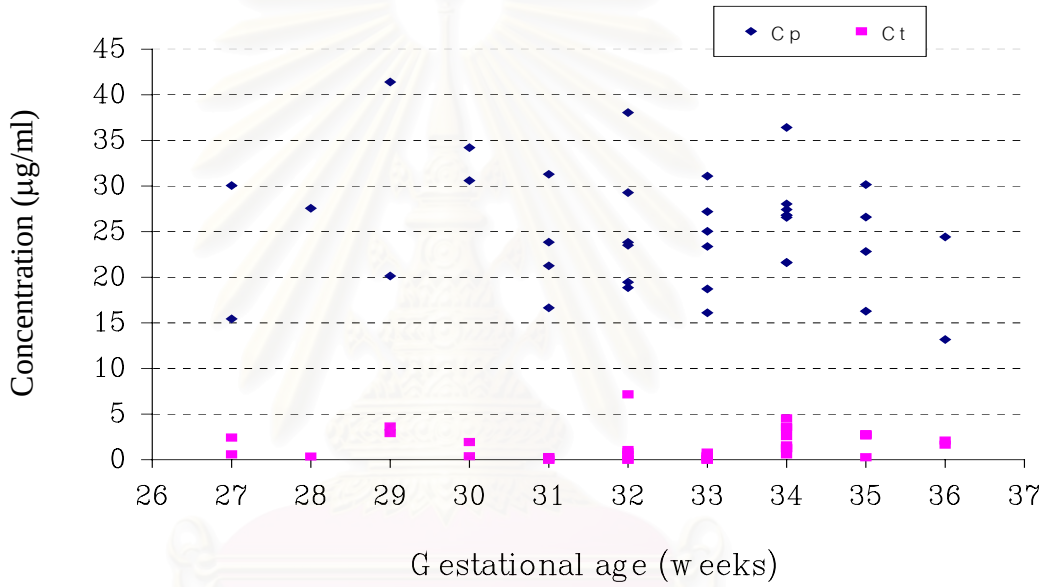
Table 4.6 Peak concentrations in different gestational age groups

Peak serum level	Number of the patients (%)			
	Group I (n=7)	Group II (n=16)	Group III (n=14)	Total (n = 37)
<20 μg/ml	1 (14.29)	5 (31.25)	2 (14.28)	8 (21.62)
20-30 μg/ml	2 (28.57)	8 (50.00)	10 (71.43)	20 (54.05)
>30 μg/ml	4 (57.14)	3 (18.75)	2 (14.28)	9 (24.33)

Table 4.7 Trough concentrations in different gestational age groups

Trough serum level	Number of the patients (%)			
	Group I (n=7)	Group II (n=16)	Group III (n=14)	Total (n = 37)
<2 μg/ml	4 (57.14)	15 (93.75)	6 (42.86)	25 (67.57)
2-5 μg/ml	3 (42.86)	0	8 (57.14)	11 (29.73)
>5 μg/ml	0	1 (6.25)	0	1 (2.70)

Figure 4.1 Peak and trough concentration of amikacin



Mean ± SD of pharmacokinetic parameters of the patients are shown in Table 4.8. Average mean ± SD of K_e and $t_{1/2}$ are 0.094 ± 0.035 hr^{-1} and 8.90 ± 5.41 hr, respectively. Mean ± SD of K_e increases in patients with older GA while mean ± SD of $t_{1/2}$ decreases with GA. Significant difference has been shown between groups for K_e but not for $t_{1/2}$ ($p = 0.003$, $p = 0.057$). Moreover, we also have observed significant differences between groups for Cl but not for V_d ($p = 0.020$, $p = 0.641$, respectively). Average mean ± SD of Cl and V_d are 0.058 ± 0.022 l/kg/hr and 0.6959± 0.231 l/kg, respectively.

Table 4.8 Comparison of pharmacokinetic parameters of amikacin in three gestational groups

Parameters	Mean ± SD (Range)				P Value*
	Group I (n=7)	Group II (n=10)	Group III (n=14)	Total (n = 31)	
K_e (hr^{-1})	0.067±0.023	0.082±0.030	0.115±0.032	0.094±0.035	0.003
	(0.0369-0.0963)	(0.0208-0.1376)	(0.0789-0.1971)	(0.0208-0.1971)	
$t_{1/2}$ (hr)	11.41±3.95	10.65±8.10	6.39±1.51	8.90±5.41	0.057
	(7.20-18.79)	(5.04-33.03)	(3.52-8.79)	(3.52-33.03)	
V_d (l/kg)	0.684±0.277	0.703±0.247	0.616±0.205	0.659±0.231	0.641
	(0.4331-1.1213)	(0.4585-1.2678)	(0.3950-1.1485)	(0.3950-1.2678)	
Cl (l/hr/kg)	0.044±0.018	0.053±0.016	0.069 ±022	0.058±0.022	0.020
	(0.0248-0.0788)	(0.0264-0.0778)	(0.0406-0.1091)	(0.0248-0.1091)	

P Value * analyzed by using ANOVA, K_e = elimination rate constant, $t_{1/2}$ = elimination half life, V_d = volume of distribution per body weight, Cl = clearance per body weight

Post Hoc analysis has been done for K_e and Cl as shown in Table 4.9.

Table 4.9 Post Hoc analysis

Parameters	Group	P Value
K_e	I vs. II	0.577
	I vs. III	0.007
	II vs. III	0.046
Cl	I vs. II	0.630
	I vs. III	0.034
	II vs. III	0.170

K_e = elimination rate constant, Cl = clearance

Correlation between pharmacokinetic parameters and gestational age or postnatal age have been analyzed by linear regression using SPSS version 10.0.1 and the results have been shown in Table 4.10.

Table 4.10 Correlation between pharmacokinetic parameters and gestational age or postconceptional age

		Equations	R^2	r	P Value
K_e	=	0.0074GA-0.1456	0.2801	0.529	0.002
K_e	=	0.0015PCA-0.145	0.2801	0.529	0.002
$t_{1/2}$	=	-0.7852GA+34.378	0.1348	0.367	0.042
V_d	=	-0.0119GA+1.0452	0.0169	0.130	0.486
Cl	=	0.0039GA-0.0672	0.2064	0.454	0.010

K_e = elimination rate constant, $t_{1/2}$ = elimination half life, V_d = volume of distribution per body weight, Cl = clearance per body weight

There is a significant correlation between GA for K_e, Cl, and $t_{1/2}$ ($r = 0.529$, 0.454 and 0.367, respectively, $p < 0.05$). Correlation for K_e and Cl is proportional to GA while for $t_{1/2}$ shows inversely relationship compare to GA. There is no correlation between V_d and GA ($r = 0.130$, $p > 0.05$). Figure 4.2, 4.3, 4.4 and 4.5 present correlation between GA and K_e, Cl, $t_{1/2}$ and V_d. There is also significant correlation between K_e and PNA ($r = 0.529$, $p < 0.05$).

Figure 4.2 Linear relationship between elimination rate constant and gestational age

Figure 4.3 Linear relationship between amikacin clearance and gestational age

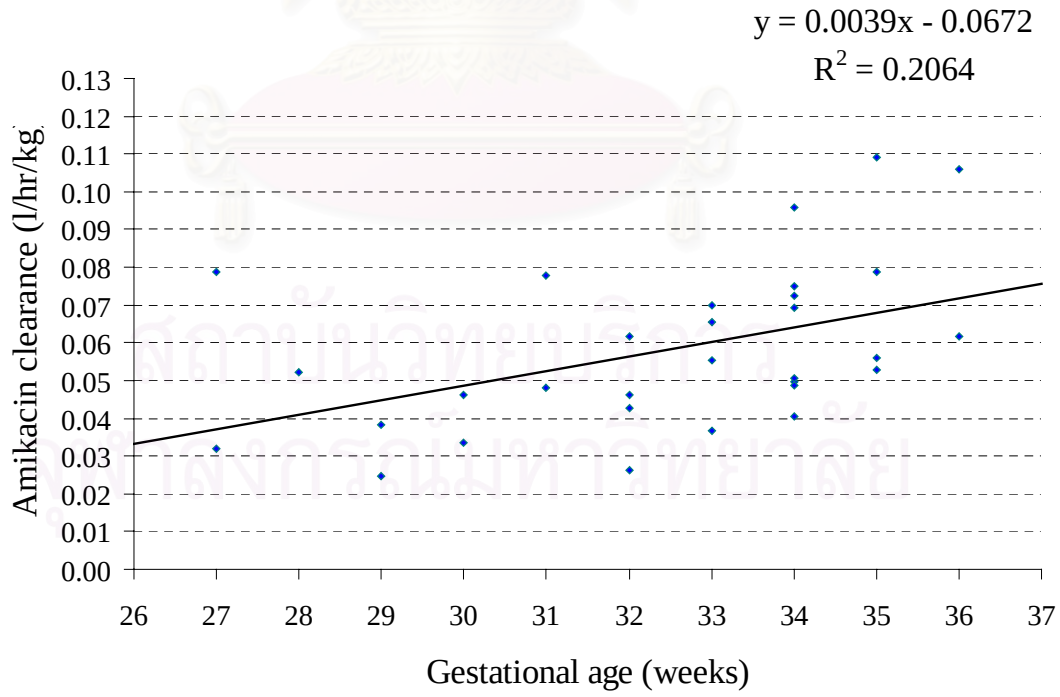


Figure 4.4 Linear relationship between volume of distribution and gestational age

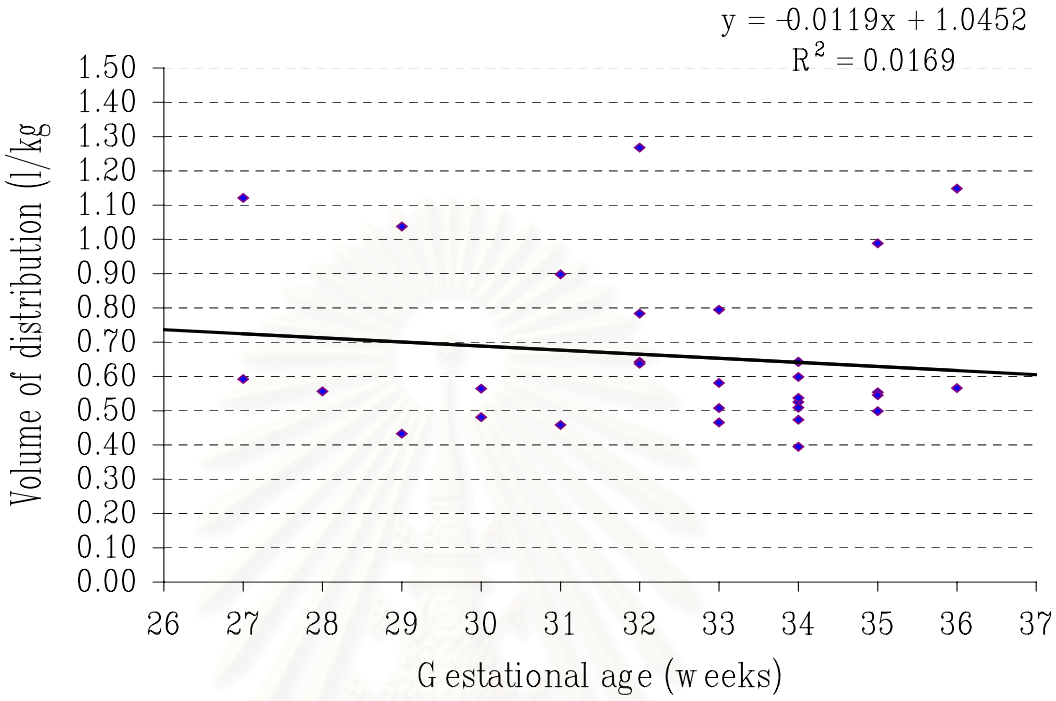
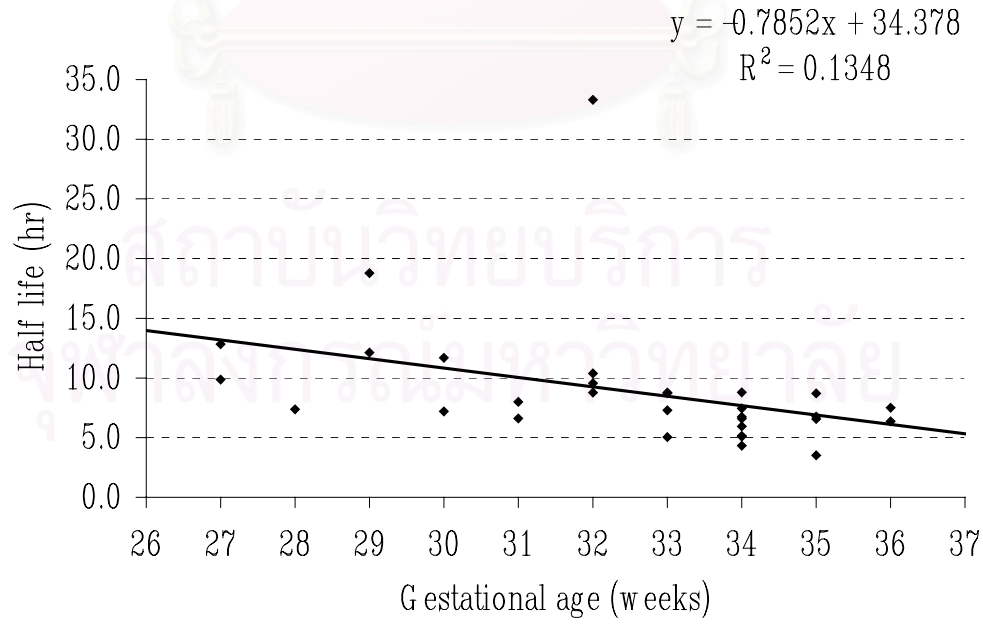


Figure 4.5 Linear relationship between half life and gestational age



Correlation between Apgar score at 1 and 5 minutes and both elimination rate constant and half life have been analyzed by ANOVA. Significant correlation has not been found between Apgar score and elimination rate constant for both at 1 and 5 minutes. Additionally, correlation between Apgar score at 1 and 5 minutes and half life are not significantly different. The results have been shown in Table 4.11 and Figure 4.6a and 4.6b.

Table 4.11 Correlation between elimination rate constant and elimination half life and Apgar score.

		Equations	R^2	r	P Value
K_e	=	$-3.08x10^{-3}$APGS1 +0.114	0.039	0.197	0.287
K_e	=	$-8.02x10^{-3}$APGS5 +0.163	0.092	0.303	0.098
$t_{1/2}$	=	0.560APGS1+4.876	0.044	0.210	0.257
$t_{1/2}$	=	0.688APGS5+2.795	0.025	0.159	0.393

APGS1 = Apgar score at 1 minute, APGS5 = Apgar score at 5 minutes.

Figure 4.6a Linear relationship between Apgar score and elimination rate constant

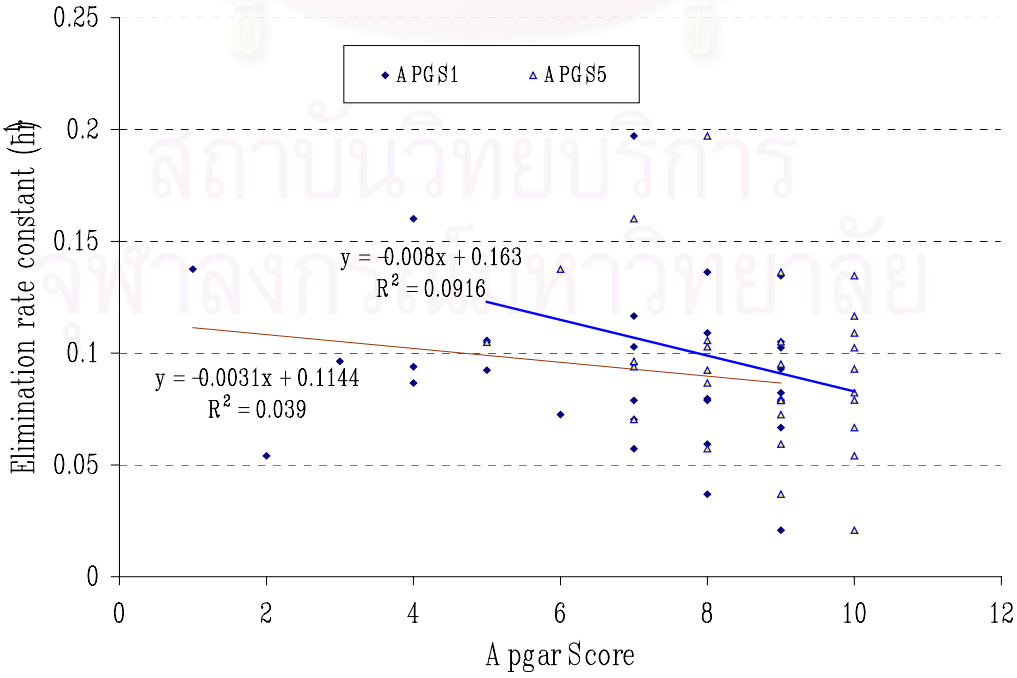
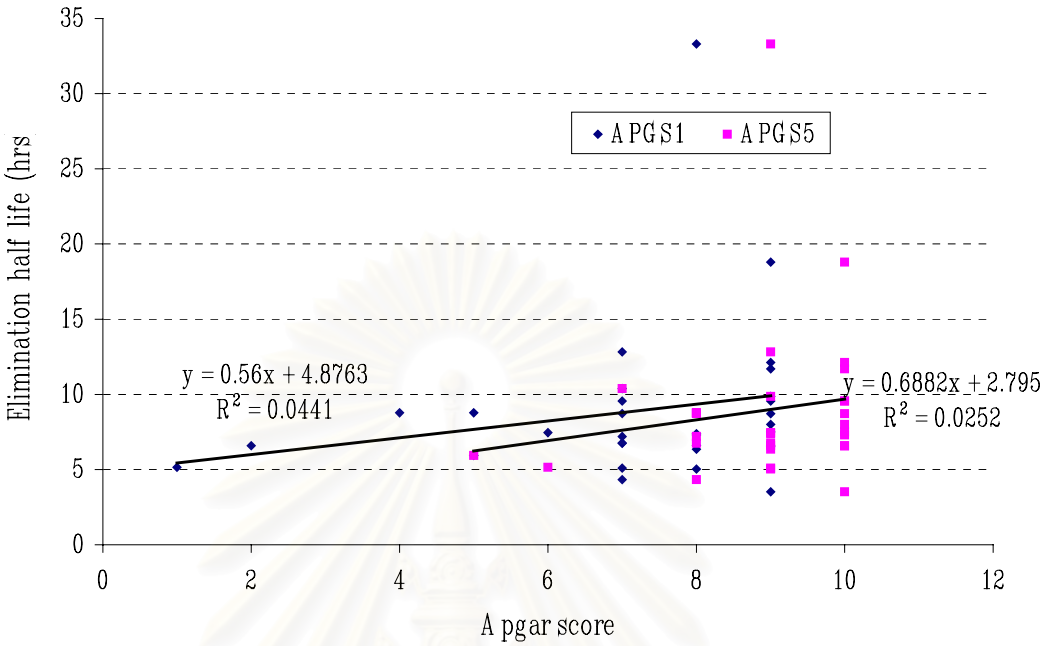


Figure 4.6b Linear relationship between Apgar score and elimination half life



III. Renal parameters

The index parameters of renal function such as serum creatinine and creatinine clearance on day 1 of life and day 2 of the discontinuation of therapy have been shown in Table 4.12. Almost all subjects have normal serum creatinine concentration (SCr) before and after amikacin treatment. There is no patient having serum creatinine rising equal to or greater than 0.5 mg/dl except five patients have slightly rising serum creatinine from baseline.

Mean $\pm$ SD of SCr and CrCl at day 1 and within day 2 of the amikacin therapy discontinuation have been presented in Table 4.13, Figure 4.7 and 4.8. There is no significant difference between groups in SCr and CrCl at day 1 ($p > 0.05$). In contrast, there are significant differences between groups in SCr and CrCl at day 2 after the discontinuation of therapy ($p < 0.05$).

Table 4.12 Serum creatinine and creatinine clearance in patients

No.	GA	Ht	Ct	SCr (mg/dl)		CrCl (ml/min/1.73 m^2)	
	(wks)	(cm)	(μg/ml)	Day 1	after discon.	Day 1	after discon.
1	32	40	7.17	1.37	0.95	9.64	13.89
2	32	41	1.06	1.23	0.96	11.00	14.09
3	33	37	0.79	1.04	1.05	11.74	11.63
4	34	42	2.57	0.93	0.75	14.90	18.48
5	29	36	3.63	1.09	1.01	10.90	11.76
6	29	41	2.9	1.00	0.97	13.53	13.95
7	33	43	<0.8	1.09	1.01	13.02	14.05
8	30	39	1.94	1.28	1.40	10.05	9.19
9	27	36	0.59	1.01	1.04	11.76	11.42
10	35	45	2.8	0.86	0.92	17.27	16.14
11	34	45	0.59	0.72	0.52	20.63	28.56
12	36	44.5	1.65	1.67	0.54	8.79	27.19
13	33	38	<0.8	1.10	0.84	11.40	14.93
14	28	37	0.35	0.72	0.59	16.96	20.69
15	31	39	0.30	1.02	0.78	12.62	16.50
16	31	41	<0.8	1.24	0.65	10.91	20.82
17	30	43	0.39	1.20	0.89	11.83	15.94
18	32	42	0.50	0.89	0.84	15.57	16.50
19	31	42	<0.8	1.14	0.83	12.16	16.70
20	35	41	0.27	1.23	0.90	11.00	15.03
21	36	46	2.10	0.76	0.70	19.97	21.69
22	34	41	1.57	1.44	0.57	9.40	23.74
23	35	41.5	2.72	1.11	0.58	12.34	23.61
24	27	34	2.43	0.87	0.83	12.90	13.52
25	35	48	2.65	0.87	0.87	18.21	18.21
26	32	40	<0.8	1.47	0.61	8.98	21.64
27	32	41	<0.8	1.26	0.71	10.74	19.06
28	34	47	3.47	1.14	0.89	13.61	17.43
29	34	48	1.24	0.98	0.86	16.16	18.42
30	31	40	0.24	0.83	0.71	15.90	18.59
31	33	47	0.41	1.00	0.49	15.51	31.65
32	33	43	0.30	1.24	0.86	11.44	16.50
33	32	44	0.82	0.86	1.11	16.88	13.08
34	34	44	1.54	1.09	0.45	13.32	32.27
35	34	47	1.29	0.71	0.38	21.85	40.82
36	33	44	0.05	0.62	0.34	23.42	42.71
37	34	41.5	3.61	0.78	0.48	17.56	28.53

GA = gestational age, Ht = height, C_t = trough concentration, SCr = serum creatinine, CrCl = creatinine clearance (calculate from Schwartz equation)

Table 4.13 Comparison of mean serum creatinine and creatinine clearance

Measurement	Mean ± SD (Range)				P Value*
	Group I (n = 7)	Group II (n = 16)	Group III (n =14)	Total (n = 37)	
SCr at day 1	1.02±0.19 (0.72-1.28)	1.09±0.22 (0.62-1.47)	1.02±0.28 (0.71-1.67)	1.05±0.24 (0.62-1.67)	0.718
SCr at day 2 of discontinuation	0.96±0.25 (0.59-1.40)	0.80±0.21 (0.34-1.11)	0.67±0.19 (0.38-0.92)	0.78±0.23 (0.34-1.40)	0.017
CrCl at day 1	12.56±2.26 (10.05-16.96)	13.18±3.58 (8.98-23.42)	15.36±4.14 (8.79-21.85)	13.89±3.71 (8.79-23.42)	0.161
CrCl at day 2 of discontinuation	13.78±3.72 (9.19-20.69)	18.90±7.90 (11.63-42.71)	23.58±7.27 (15.03-48.02)	19.70±7.77 (9.19-48.02)	0.016

SCr = serum creatinine, CrCl = creatinine clearance (calculate from Schwartz equation)
P Value * analyzed by using ANOVA

Figure 4.7 Serum creatinine in each groups

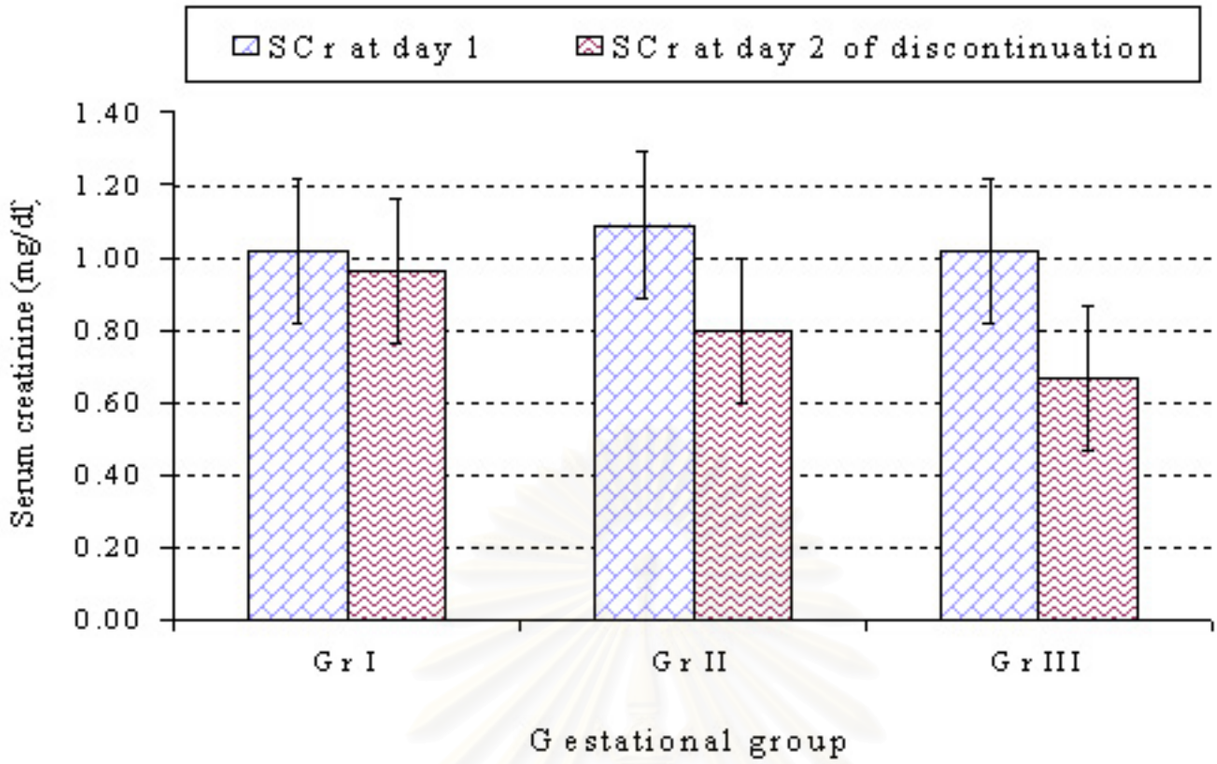
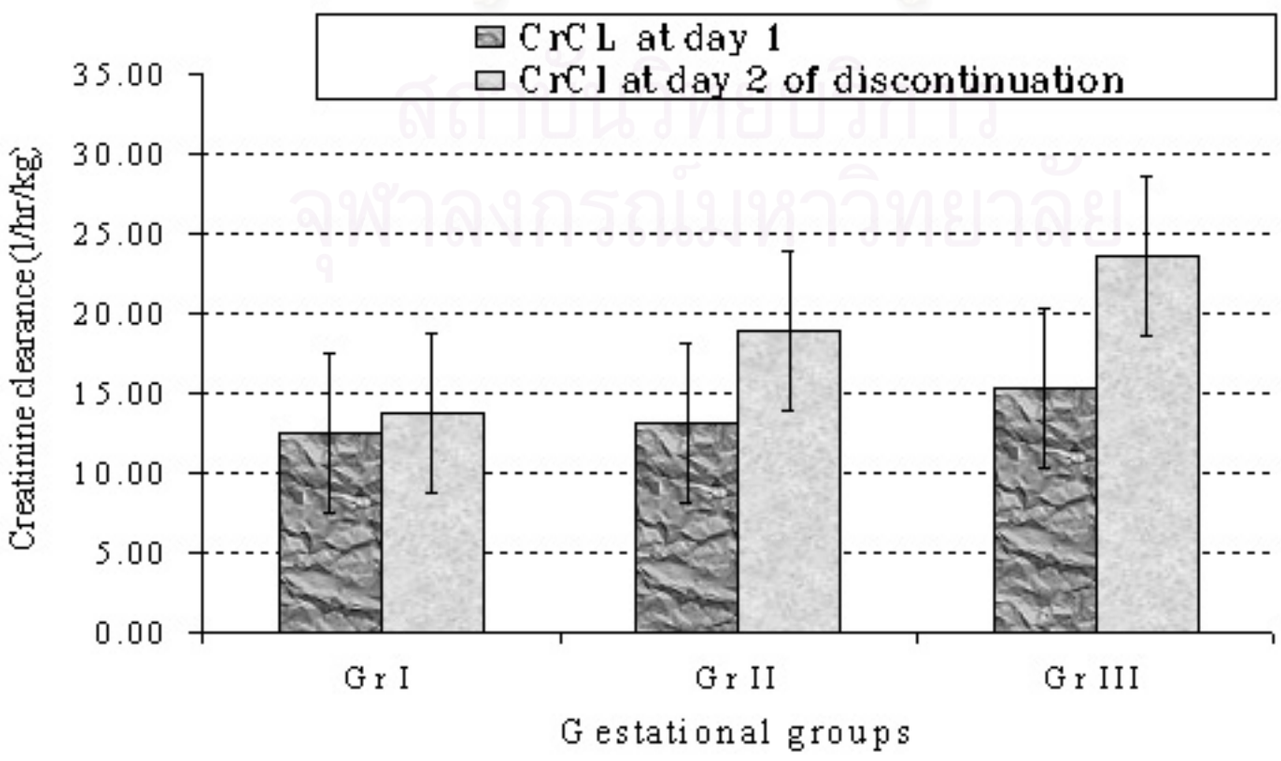


Figure 4.8 Creatinine clearance in each group

Correlations between GA and SCr or CrCl at day 1 have been



presented in Figure 4.9 and 4.10. There is no significant difference between

Correlations between GA and SCr or CrCl at day 1 have been presented in Figure 4.9 and 4.10. There is no significant difference between GA and SCr or CrCl at day 1 ($r = 0.058$, 0.252, respectively, $p > 0.05$) while there are significant differences between GA and SCr together with CrCl at day 2 of the discontinuation therapy ($r = 0.363$, 0.427, respectively, $p < 0.05$). Correlation between GA in weeks and SCr in mg/dl or CrCl in l/hr/1.73m^2 at day 2 of the discontinuation therapy have been presented in Figure 4.11 and 4.12. There is no correlation between C_t and SCr at day 2 of the discontinuation therapy ($r = 0.006$, $p = 0.673$). The result has been shown in Figure 4.13.

Figure 4.9 Linear relationship between serum creatinine at day 1 and gestational age

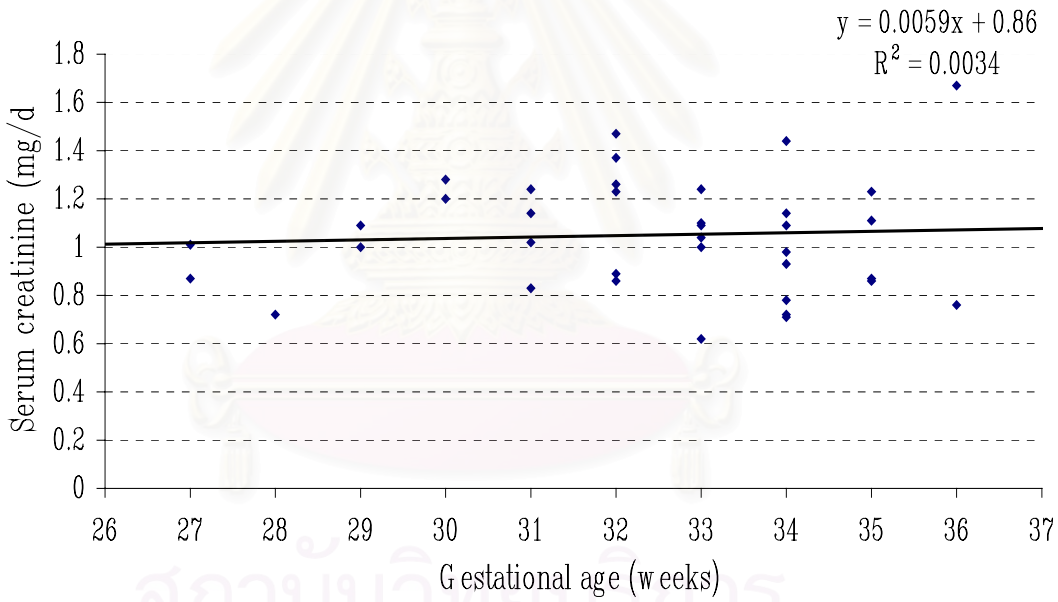


Figure 4.10 Linear relationship between creatinine clearance at day 1 and gestational age

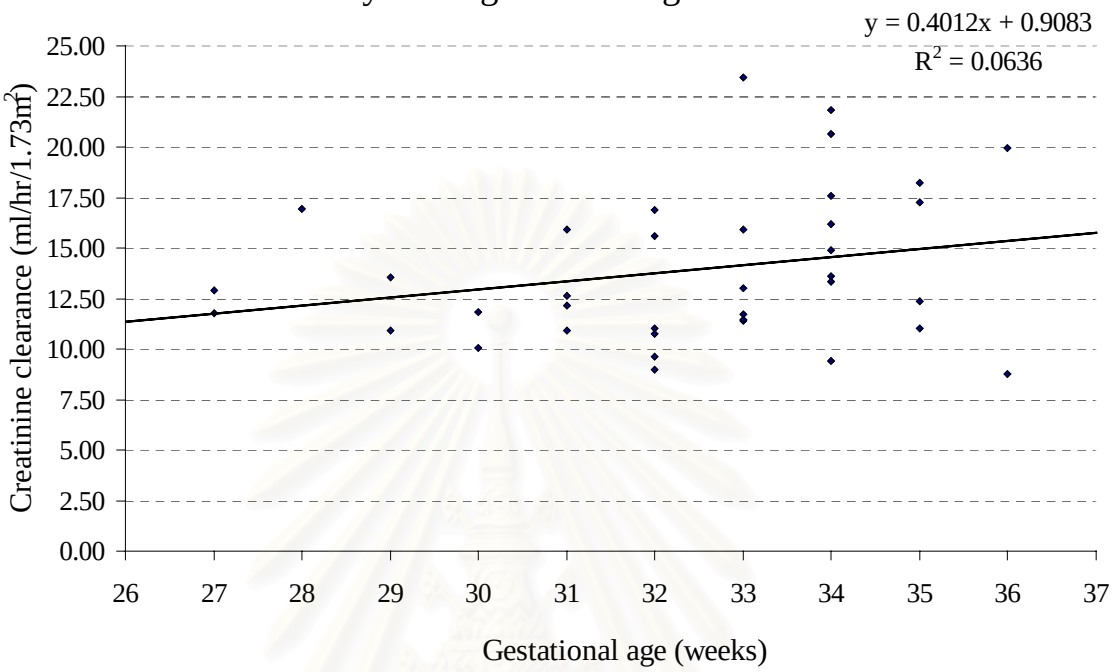


Figure 4.11 Linear relationship between serum creatinine at day 2 of discontinuation and gestational ages

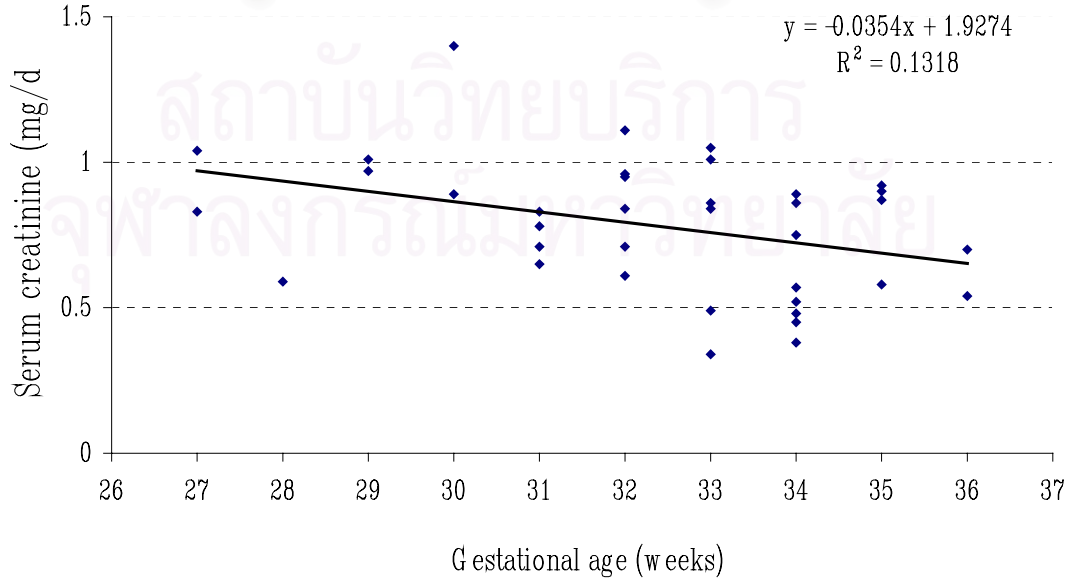


Figure 4.12 Linear relationship between creatinine clearance at day 2 after discontinuation

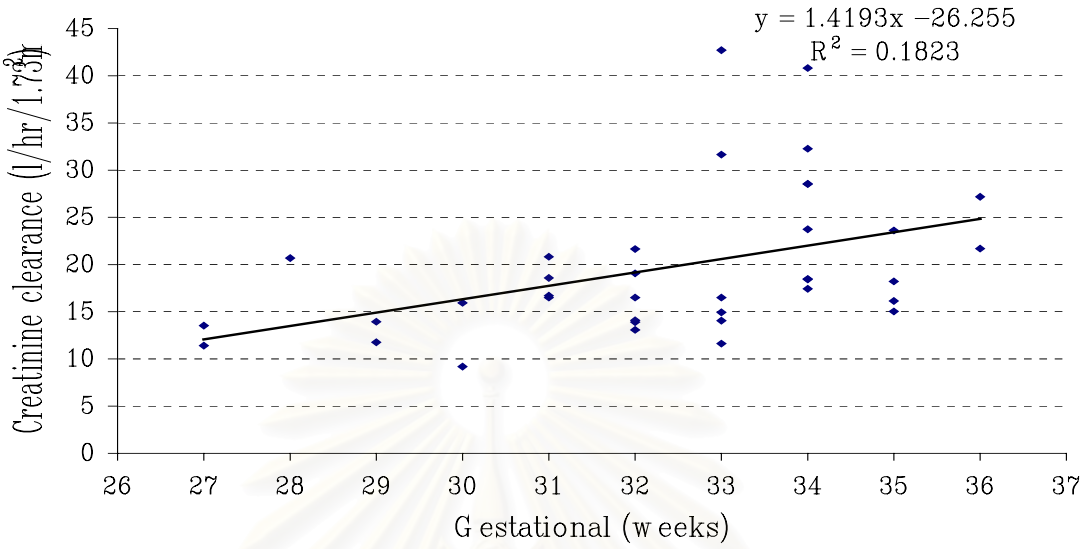
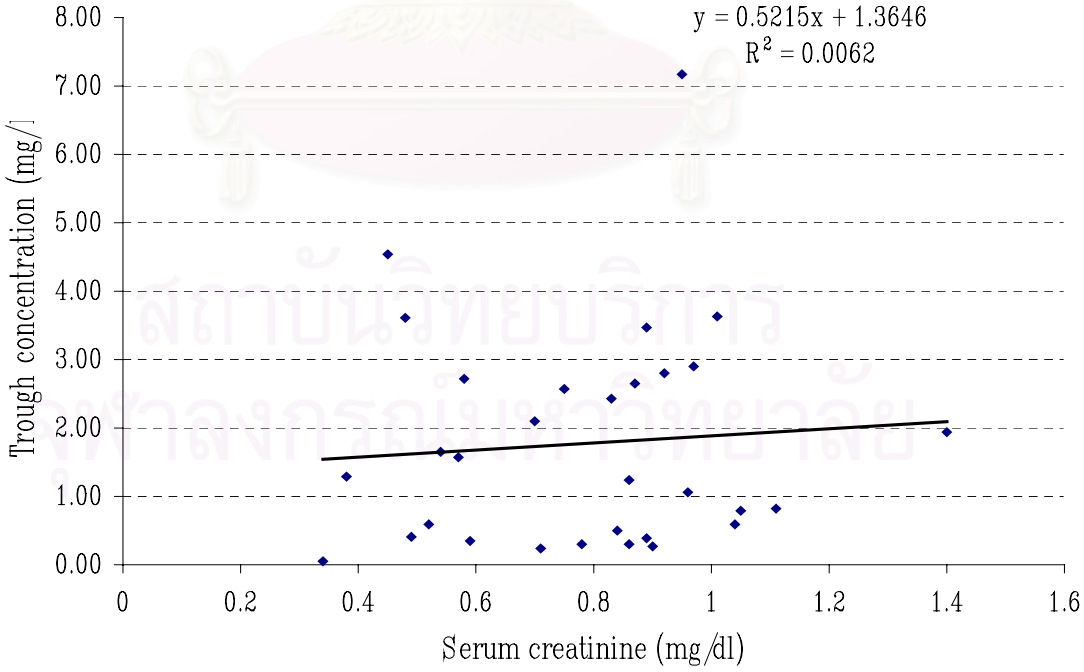


Figure 4.13 Linear relationship between trough concentration and serum creatinine at day 2 after discontinuation



Correlations between clearance of amikacin and serum creatinine together with creatinine clearance have been shown in Figure 4.14 and 4.15. There is no good correlation between Cl and SCr or CrCl (r = 0.113, p = 0.545 and r = 0.105, p= 0.574, respectively).

Figure 4.14 Linear relationship between serum creatinine and clearance of amikacin

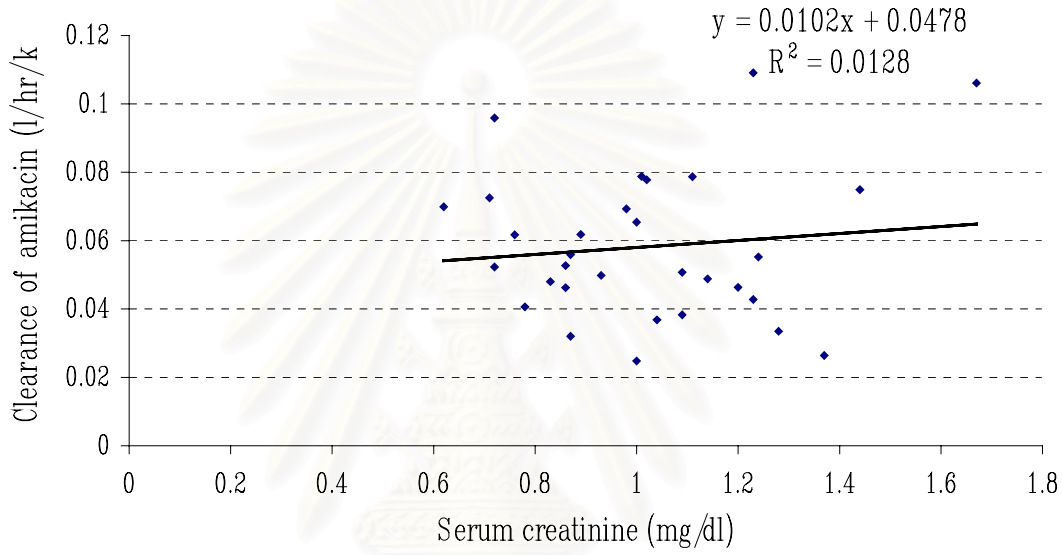
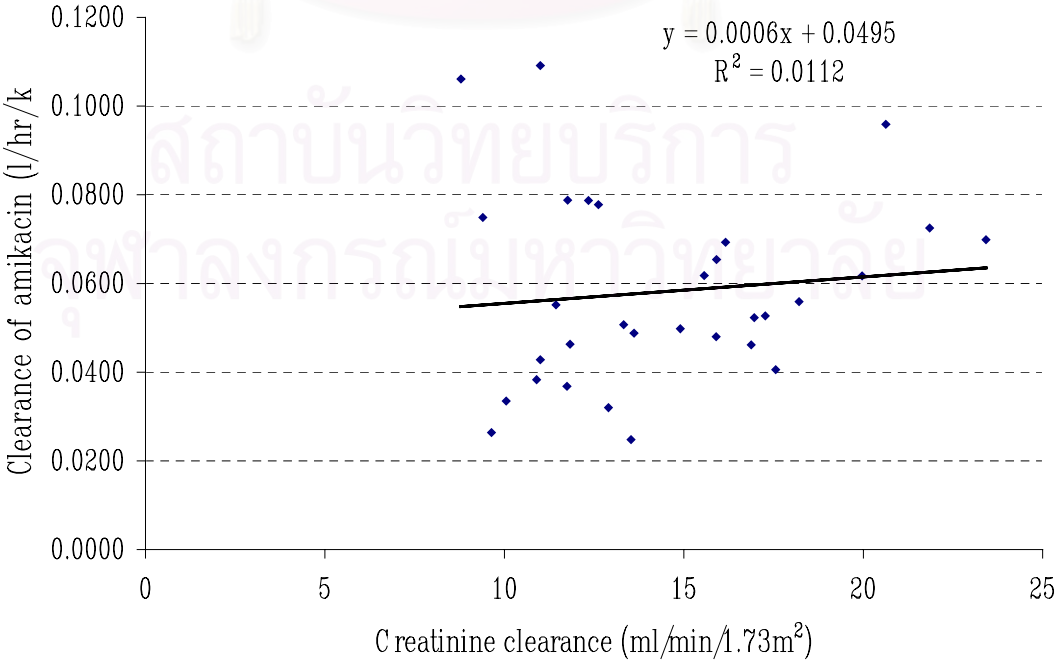


Figure 4.15 Linear relationship between creatinine clearance and clearance of amikacin



IV. Efficacy

Several clinical signs of patients have been monitored to indicate efficacy of amikacin therapy such as clinical symptoms, body temperature, white blood cell counts and hemoculture. Most patients have good clinical response to therapy. Patients with abnormal temperature at the initial of therapy showed favorable response after receiving the medication. Almost all patients present normal white blood cell count (9,000- 35,000 cell/cm^3) before amikacin therapy. Patient number twelfth and twenty-forth had white blood cell count higher than normal value at the begining of therapy. The high white blood cell count of these patients have been decreased to normal level after receiving the medication. Patient number two had low white blood count at the initial of therapy and it did not increase to normal level. No patient has positive hemoculture at the beginning of therapy.

Indication, dose and peak concentrations at steady state, duration and efficacy of amikacin therapy in three gestational groups of preterm neonates have been shown in Table 4.14. Patient number two which was in group II showed unfavorable efficacy. The subject was expired due to sepsis and intracranial hemorrhage. Her white blood cell count was low and not increase to normal level after four days of treatment. Peak concentration in this patient was 23.51 μg/ml. There is no significant relationship between peak concentration and efficacy when analyzed by logistic regression ($r = 0.089$, $p > 0.05$).

Table 4.14 Indication of treatment and efficacy

No.	Indication	Dose (mg)	Cp (mg/l)	Duration (days)	Efficacy
1	Clinical Sepsis	25	18.87	4	F
2	PROM, Congenital pneumonia	22	23.51	8	UF
3	Clinical Sepsis	19	31.09	7	F
4	Clinical Sepsis	28	27.41	5	F
5	Mild RDS	18	20.15	11	F
6	RDS, Congenital pneumonia	27	41.4	8	F
7	Clinical Sepsis	32	23.37	5	F
8	Clinical Sepsis	22	30.59	6	F
9	RDS, Clinical Sepsis	21	15.45	7	F
10	RDS, Congenital pneumonia	40	30.16	7	F
11	Hypoglycemia	45	21.60	8	F
12	PROM >24 hr	32	13.17	6	F
13	Hypoglycemia	22	16.11	6	F
14	RDS	18	27.57	7	F
15	PROM > 24hr, Clinical Sepsis	24	16.66	6	F
16	PROM > 24hr, Clinical Sepsis	25	23.85	8	F
17	Hypoglycemia	28	34.21	5	F
18	Clinical Sepsis	30	19.47	5	F
19	RDS, Congenital pneumonia	22	21.26	6	F
20	Clinical Sepsis	27	22.83	5	F
21	Clinical Sepsis	33	24.42	5	F
22	Clinical Sepsis	22	21.64	5	F
23	Clinical Sepsis, RDS	30	16.29	4	F
24	RDS	16	30.05	7	F
25	PROM > 24 hr	36	26.6	7	F
26	Clinical Sepsis, NEC	30	29.28	7	F
27	Clinical Sepsis, NEC	30	38.05	7	F
28	Clinical Sepsis, PROM > 24 hr	30	28.03	6	F
29	Clinical Sepsis, PROM > 24 hr	38	26.56	7	F
30	RDS	30	36.60	6	F
31	PROM > 18 hr ,Clinical sepsis	40	18.71	4	F
32	Clinical Sepsis	25	25.04	5	F
33	Suspected Neonatal Sepsis	30	23.84	5	F
34	Congenital pneumonia,RDS	30	26.80	7	F
35	PROM > 24 hr, Chorioamnionitis	38	26.81	4	F
36	Birth asphyxia	32	27.20	5	F
37	RDS	24	36.43	7	F

Cp = Peak concentration, F = Favorable, UF = Unfavorable, PROM = Prolonged rupture of membrane, RDS = Respiratory distress syndrome

V. Discussion

Pharmacokinetics of aminoglycosides are quite widely variate among populations. The pharmacokinetics strongly depend on age, weight, disease condition and renal function. In neonatal period, gestational and postnatal age are also the determinants. Kenyon et al have shown that administration of amikacin in preterm neonates using recommended dose for adults resulting in inadequate peak concentration, while trough concentration is high.[12] They suggested the modification of the trough concentration by extending the dosing interval, while the peak concentration would also be improved by increasing the dose. Regarding this recommendation, Langhendries et al have conducted sequential studies in neonates to assess this concept.[16,63] Based on the pharmacokinetic populations, a dosing chart for neonates, which are relatively higher, doses and longer interval in small gestational group have been proposed. Besides, the validity of their recommended dosing regimen has been assessed. From this study, they have concluded that the proposed dosing chart of amikacin administration allows even high-risk neonates to achieve adequate serum peak and trough concentrations in any gestational ages at birth and drug-induced nephrotoxicity and ototoxicity were also kept in minimal.

From these studies and the other investigations, dosage guidelines have been reviewed and published on Neofax every year. In 2001, dosage regimens of amikacin are divided into four gestational groups. Dose for small GA is higher than larger GA and dosing interval in small neonates also longer. Since some dosing interval is every 36 hours which is quite inconvenience dosing period for nurses, many hospitals have extended the dosage regimen to 48 hours interval. As most studies have conducted in neonates from foreign countries that might have different pharmacokinetic parameters from Thai neonates, this research has been conducted to assess the validity of the new dosage regimen and obtain pharmacokinetic parameters in Thai newborns. This study have been generated in patients of less than 48 hours of life hospitalized

in neonatal intensive care unit. The characteristics of subjects are not significant different between groups except GA, BW and Ht. Since GA is the character using in grouping the patients, so GA are significantly different between groups. BW and Ht are correlated with GA that makes them also significantly different between groups. As a result, GA and BW are taken into the account for dosage regimen. Apgar score is the value represent lack of oxygen and need of resuscitation at birth. Preterm neonates usually have respiratory problems resulting in low Apgar score. Severe asphyxia is defined as Apgar score less than or equal to 2 at 5 minutes.[64] This value were used to exclude patients from the study due to the safety of neonates. In addition, blood perfusion of vital organs such as renal and heart decreases in severe birth asphyxia, resulting in changing of pharmacokinetics. Apgar score is not significantly different between groups and Post Hoc analysis result as the same. Significant correlation between Apgar score and elimination rate constant or half life has not been found, therefore, Apgar score has no effect on pharmacokinetics of amikacin. The duration of therapy, notably, was longer in smaller GA than larger GA group. This might be because the clinical of patients in small GA did not improve as well as that in the larger GA group.

In this study, peak concentrations have been obtained from blood samples drawing, all trough concentrations are the extrapolated concentration from the calculation. Almost all patients have true peak concentration at the exactly time 30 minutes after the completion of infusion. Only two patients, number five and nine, have obtained blood sampling at an hour after completion of infusion due to the difficulty in blood sampling technique. Therefore, peak concentrations in these two patients are the extrapolated concentration. In the same way, the second blood sample at the eighteen or thirty-six hours have not been collected in three patients. However, the exact sampling time was documented as shown in Appendix III. Time of blood sampling for peak concentration must not earlier than 30 minutes after the

completion of infusion since it takes approximately 30 minutes for distribution phase.

Mean C_p and C_t are also not significantly different between groups. Although mean C_p in group I is the highest. Post Hoc analysis with Scheffe has shown that there was no significant difference among all groups. We have divided peak concentration in three different groups regarding to the desired therapeutic peak concentration noted in Neofax 2001. Most patients in group I have peak concentration over therapeutic range. There is an evidence of correlation between high peak concentration and therapeutic outcome, however, there is no clearly evidence of the disadvantage of the over therapeutic level.[39] Besides, high peak concentration does not increase renal accumulation in neonates, especially premature neonates, because of the immature proximal tubule cells and lower number of binding sites in neonates.[8] However, patients in this group should be closely monitored and further research in larger sample size need to be performed before making any conclusions. About 54 % of total subjects have desired C_p while about 21 % have C_p in subtherapeutic range and 24% have C_p in over therapeutic range. Percentage of patients with desired therapeutic concentration in this study is lower than the results reported by Labaune et al.[15] Nevertheless, Moore et al have shown that peak concentration was equal to or over than 20 μg/ml relate to therapeutic outcome.[39] Regarding to this finding, 78 % of total patients have peak concentration equal to or over than 20 μg/ml.

All patients obtained C_t levels lower than 2 μg/ml except in one patient with 7.17 μg/ml. Trough concentration over than 10 μg/ml is considered to cause nephrotoxic.[65] Therefore, C_t indicates no renal toxic in this study. However, most C_t is quite low, amikacin has PAE that serum levels may be allowed to fall below the MIC of pathogen without compromising antimicrobial effects. The MIC_{90} of amikacin for *E. Coli,* a major microorganism in neonatal infection, is 1 μg/ml.[27] Nevertheless, most patients in this study do not have real infection at birth and they also have co-administration of ampicillin during

the same time. As a result, microorganisms do not have enough time to adapt resistance to antibiotic.[66]

Pharmacokinetic characteristics of amikacin are explainable from both multi-compartment and one-compartment model depend on route of administration and time of blood collection. In this study, one-compartment model and first order kinetics are assumed because it does not need too many serum concentrations and the blood samples have been collected during the elimination phase in which the errors for calculation are minimized.

Laders et al and Pons et al have suggested that pharmacokinetics during the neonatal period were dependent on the postconceptional age.[67,68] This study was conducted in patients with postnatal age less than 48 hours so only gestational age is the determinant factor. As expected, pharmacokinetic parameter from this study varies between gestational groups. K_e and Cl are different between groups and proportional relate with GA. Significant difference between groups for K_e results from significant between group I versus group III and group II versus group III (p=0.007and 0.046, respectively). Significant difference between groups for Cl results from significant between group I versus group III ($p = 0.034$). K_e is not only related to GA but also significantly related to postconceptional age ($p < 0.05$). Therefore in the same gestational age with different postnatal age, K_e is greater in older infants than younger infants. As a consequence, an appropriate value of parameters must be choosen in each group of patients. Half-life is significant inversely correlated with GA though there is no significant difference between groups. Half life is a value reciprocal correlate with elimination rate constant. It should be significantly different between groups as same as elimination rate constant. Small sample size and extreme value in this study may be an explanation. One patient in group II who had a very long half life compare to other patients. In statistical method, this extreme value should not been included and, because of the exclusion, a significant different between groups ($p < 0.05$) will be found. This results from significant different between group I versus II and group I

versus III. Volume of distributions are not significant different between groups. We have not found significant correlation between volume of distribution and gestational age. The lack of the relationship between postconceptional age or gestational age might be due to the small sample size and reflects the fluctuation state of hydration in the early days of life. Volume of distribution is considered as a physiological indicator of extracellular fluid and can show wide variation between patients and in individual patients at different times.[21] Since volume of distribution was not significant different between groups, peak concentration in group I was quite higher than group II and III. Volume of distribution of preterm neonates was relatively larger than in adults. In this study V_d is about 38% of BW while it is about 25% of lean body weight in adults since there are larger extracellular fluid volume in neonates than in adults. The results in this study agree with the previously published data.[8,12,57,67] Data obtained from this study are more similar to the results demonstrated by Padavoni et al than by Kenyon et al.[12,57] Explanation for this finding, possibly, because of Kenyon's study is a single-dose design while this study and Padavoni's is the multiple-dose design. Process of pharmacokinetic parameters estimation is known to differ between a single dose versus multiple doses.[69]

Mean $\pm$ SD of SCr and CrCl at day 1 of life and day 2 of discontinuation are 1.05 $\pm$ 0.24, 0.78 $\pm$ 0.23 mg/dl and 13.89 $\pm$ 3.71, 19.70 $\pm$ 7.77 ml/min/1.73 m^2, respectively. SCr and CrCl are not significant different between the different gestational groups at day 1 while they are significant different at day 2 of the discontinuation ($p < 0.05$). This finding might be because of SCr at birth does not all come from neonates, it partially origins from mothers.[70,71] SCr and CrCl after the discontinuation are different between groups demonstrated that the maturity of renal function after birth depends on the gestational age at birth. This finding agrees with the results reported by Rudd et al and Counahan et al that glomerular filtration rate was proportional relate to GA.[70,71] Even though SCr is suggested to be a more reliable indicator for glomerular filtration rate than CrCl, in this study, CrCl was more associate

to GA than SCr (r = 0.427, 0.363, respectively). Clearance of amikacin shows no significant difference with both serum creatinine or creatinine clearance at day 1 of life. This may be because serum creatinine and creatinine clearance at the first day of life is not a good indicator for glomerular filtration and serum creatinine at birth partly be maternal origin.

Nephrotoxicity or ototoxicity is difficult to detect in neonate because of methodological problem. Ototoxicity has not been monitored in this study. In newborns, serum creatinine in the first day of life is vary and cannot be reliable as an indicator for renal function. Nephrotoxicity usually occurs after several days of treatment and is reversible. In this study, amikacin has been used as an empirical therapy in short term and serum creatinine was measured within two days after discontinuation, not during the therapy. Serum creatinine has not been found to be significantly rising in this study except in the five patients with serum creatinine slightly rising from baseline. These five patients have trough concentration range from 0.59 to 2.80 mg/dl. Rising of serum creatinine does not significant relate to trough concentration. Nephrotoxicity, defined as an increasing in serum creatinine equal to or greater than 0.5 mg/dl, has not been found in this study. This might partly due to the high serum creatinine at birth or there is really no renal toxic developed. Therefore, we cannot conclude that the dosage regimen in this study has no nephrotoxicity.

Efficacy of amikacin in this study was clinical efficacy, quite higher than previous data because there was only one patient in group II who had real infection. The rest of the treatment is for only suspected infection. Peak concentration level of amikacin in this infected neonate was 23.51 μg/ml. Therefore, intracranial hemorrhage may be a major cause of her death. Further research should be conducted in the real infection groups before drawing any conclusions.

CHAPTER V

CONCLUSION

Infection is the life threatening conditions that needed an early effective therapy especially in neonatal period. Because signs and symptoms of infection are not specific, empirical treatment with antibiotics is needed. The predominant infecting organisms are *E. coli*, group B streptococci and *Listeria monocytogenes*. The most popular regimen is a combination of gentamicin and ampicillin to cover both gram negative and gram positive, respectively. However, amikacin is an alternative against the resistant gram negative strains. Due to wide inter and intra - patient variability of pharmacokinetics of aminoglycosides, individualize dosing has been recommended. However, individual dosage cannot be done in every institution. Guideline of the dosage regimens, Neofax, has been proposed for convenient treatment. Many hospitals use this recommended dosage regimen, so does Phramongkutklao hospital with the modification of some dosing intervals. Therefore, the purpose of this study is to evaluate these modified dosage regimens and examines pharmacokinetics of amikacin in Thai premature neonates.

Thirty-seven premature neonates hospitalized at Neonatal Intensive Care Unit, Phramongkutklao Hospital have been enrolled in this research. Seven patients with gestational age not greater than 30 weeks are 5 males (71.42%) and 2 females (28.57%), 16 patients with gestational age of 31-33 weeks are 7 males (43.75%) and 9 females (56.25%) and 14 patients with gestational age of 34-36 weeks are 8 males (57.14%) and 6 females (42.86%). There is no significant difference in characteristics of patients between gestational groups in gender, Apgar score at 1 and 5 minute, postnatal age and duration of therapy ($p > 0.05$). The results obtained from this study could be conclude as follow:

1. Gestational age has effects on pharmacokinetics of amikacin while Apgar score shows no effect.

2. Mean peak concentrations of amikacin at steady state are in the range of the therapeutic levels (20-30 μg/ml) in every groups and has no significant difference between groups. Only 54% of all patients have drug concentration in therapeutic range. Twenty-two and twenty four percent of all patients have peak concentrations in subtherapeutic and over therapeutic range, respectively. Patients with peak concentrations in over therapeutic range should be monitored closely. Almost all patients except one neonate have trough concentrations less than 5 μg/ml.

3. Elimination rate constant and clearance of amikacin are significantly different between gestational groups ($p < 0.05$). Elimination half life has a trend to have significantly different between groups. The volume of distribution is not significant different between gestational groups ($p > 0.05$).

3. Good correlation has been found between elimination rate constant, clearance of amikacin and half life with gestational age ($p < 0.05$).

In conclusion, the results of this study show that the modification of dosage regimens result in peak serum concentrations equal to or greater than 20 μg/ml in most patients with trough concentration less than nephrotoxic level. Due to empirical treatment and small sample size, this study cannot demonstrate the relationship between peak serum concentrations and bacteriological efficacy of this regimen. Nevertheless, we have found that amikacin pharmacokinetics of Thai preterm neonates are not different from previous studies in the western countries.[5,12,57]

Suggestion for future research

From this study, we have found that there are wide variation of pharmacokinetic parameters in newborns especially preterm neonates, six of sixteen patients with gestational age between 31 and 33 weeks have too low serum concentrations at the thirty-sixth hours after infusion. Therefore, we cannot obtain pharmacokinetic data of these patients. This finding suggests that drug serum sample in patients receiving amikacin every 48 hours should be earlier collected. The optimal time of blood collecting should be at 24 hours after infusion.

Peak concentration of amikacin widely varies in neonates and dosing chart of the medication cannot replace therapeutic drug monitoring.

Since this is the first study conducted in Thai preterm neonates for empirical therapy, further research should be continued with large sample size in the infected patients.

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APPENDIX

APPENDIX I

No………

ADMISSION SUMMARY NOTE

Demographic data

Name…………………….…….…HN……….…/……..AN…………/.…Bed No……...
Admission date………………………………Discharge date……...……………….….
Birth date…………………Time……..……Gender……......................GA….………..wks
Type of labour ……………BW…………grams Ht.………cm Apgar Score……/…..
CC.……………………………………………………………………………………
Diagnosis..……………………………………………………………………………
Other disease…………………………………………………………………………

Drug profile

Date / Drug													

Vital signs

Date	Time	BP	BT	HR	RR

Date	Time	BP	BT	HR	RR

Laboratory Data

Date / Data										
WBC										
PMN										
BUN										
SCr										

Culture

Date / specimen										
H/C										
CSF										
U/C										
Sputum										
Stool										

……

……

……

Pharmacokinetics

Date	Peak/Trough	Time of infusion	Time of collection	Serum level

APPENDIX II

Manual of analytical operation

A. Analytical Method

Amikacin plasma levels were determined by immunoassay method using TDx Analyzer system, Abbott Laboratories based on fluorescence polarization technique. Coefficient of variation is less than 5 % at a concentration range of 5-30 ug/ml.

B. TDx Assays Manual

Manual of using TDx analyzer was regenerated from batch assay procedure in operation manual, Abbott Laboratories Inc.

1. Perform an assay calibration

The equipments consisted of calibration carousel, cuvettes, sample catridges, reagent pack, calibrators and controls. The calibration procedures are done as follow:

a. Preparing the Carousel

1. Select a calibration carousel.
2. Load 15 sample catridges and cuvettes. Starting from the first position and continue sequentially. Do not skip a position. Ensure that all disposables are clean and free of foreign matter before use.
3. Lock cuvettes into the carousel by turning the lock mechanism clockwise until it clicks.
4. Invert the calibrator pack gently five times. Pipette, in duplicate, 50 microlitres of calibrators A through F into sample wells 1 through 12. Avoid splashing, foaming, or bubbling.

   Example : Pipette calibrator A into positions 1 and 2, B into 3 and 4, C into 5 and 6, etc.

Recap each calibrator vials as it used, and return the pack to proper storage as described on the labeling.

5. Invert the control pack gently five times. Pipette 50 microlitres of H, M and L controls into sample wells 13 through 15. Avoid splashing, foaming or bubling. Position 16 through 20 are available for patients samples.
6. Inspect the samples for bubbles and remove any bubbles with applicator sticks (use a different applicator stick for each level of calibrator or control).

b. Preparing the reagent pack

1. Select the amikacin reagent pack.
2. Invert gently for five times.
3. Open the reagent pack and check to be sure the vials read S, T, P.
4. Remove the vial caps and place them upside-down in the lid spaces provided.
5. Inspect the surface of the liquid in the vials for bubbles and remove any bubbles with applicator sticks. (Use a different apllicator stick for each vial).

c. Run Calibration

1. Insert the reagent pack into the proper position in the analyzer.
2. Place the loaded calibration carousel into the instrument and close the access door.
3. Press RUN.
4. Verify that the correct assay name and the word [CALIBRATION] displays. The observed data and the calculated curve print at the end of the run. Do not press STOP before the printout is complete. Since the processing will be terminated and also prevent storage of the

calibration curve. When calibration is complete, the instrument displays DONE- REMOVE RPAK.

2. Performing an assay run

The equipments consisted of calibration carousel, cuvettes, sample catridges, reagent pack, calibrators and controls. The assay procedures were done as follow:

a. Preparing the carousel

1. Select a numbered assay carousel.
2. Load the carousel with one sample catridge and cuvette for each sample to be assayed. Ensure that all disposables are clean and free of foreign matter. Starting from the first position and continue sequentially. Do not skip a position. Ensure all cuvettes are right side up.
3. Lock cuvettes into the carousel by turning the lock mechanism clockwise until it clicks.
4. Pipette at least 50 µl of patient samples into each sample well. Avoid splashing, foaming, or bubbling.
5. Inspect the samples for bubbles and remove any bubbles with applicator sticks (use a different applicator stick for each sample catridge).

b. Preparing the Reagent Pack

1. Select the amikacin reagent pack.
2. Invert gently five times.
3. Open the reagent pack and check to be sure the vials read S, T, P.
4. Remove the vial caps and place them upside-down in the lid spaces provided.
5. Inspect the surface of the liquid in the vials for bubbles and remove any bubbles with applicator sticks. (Use a different apllicator stick for each vial).

c. Run Assay

1. Insert the reagent pack into the proper position in the analyzer.
2. Place the loaded assay carousel into the instrument.
3. Close the access door.
4. Press RUN.
5. Verify that the correct assay name is displayed .
6. Wait for the entire printout. The assay is complete when the display reads: D-REMOVE RPAK.
7. Remove the carousel, close the access door and discard the contents of the carousel.

APPENDIX III

RAW DATA

No.	Sex	GA (wks)	BW (gm)	Dose (mg/dose)	Interval (hrs)	T_1 (min)	C_1 (μg/ml)	T_2 (hrs)	C_2 (μg/ml)
1	M	32	1620	25	48	30	18.87	36	9.11
2	F	32	1420	22	48	30	23.51	36	2.28
3	F	33	1240	19	48	30	31.09	36	1.96
4	F	34	2110	28	24	30	27.41	18	4.59
5	M	29	1000	18	48	60	19.81	36	5.54
6	M	29	1520	27	48	30	41.40	36	5.60
7	F	33	2060	32	48	30	23.37	36	<0.8
8	M	30	1275	22	48	30	30.59	42	2.69
9	F	27	1170	21	48	60	14.90	36	1.32
10	M	35	2600	40	24	30	30.16	21.5	4.27
11	M	34	3030	45	24	30	21.6	18	1.42
12	F	36	2165	32	24	30	13.17	18	2.74
13	F	33	1400	22	48	30	16.11	36	<0.8
14	M	28	1080	18	48	30	27.57	36	1.03
15	F	31	1500	24	48	30	16.6	36	0.80
16	F	31	1600	25	48	30	23.85	36	<0.8
17	M	30	1560	28	48	30	34.21	36	1.18
18	F	32	1860	30	48	30	19.47	36	1.23
19	M	31	1410	22	48	30	21.26	36	<0.8
20	M	35	1770	27	24	30	22.83	18	0.8
21	M	36	2310	33	24	30	24.42	18	3.83
22	F	34	1500	22	24	30	21.64	18	2.98
23	M	35	2020	30	24	30	16.29	18	4.21
24	F	27	920	16	48	30	30.05	36	4.53
25	F	35	2450	36	24	30	26.60	18	4.66
26	M	32	1820	30	48	30	29.28	36	<0.8
27	M	32	1900	30	48	30	38.05	36	<0.8
28	M	34	2080	30	24	30	28.03	18.5	5.52
29	M	34	2550	38	24	30	26.56	18	2.62
30	F	31	1895	30	48	30	31.30	36	0.80
31	M	33	2510	40	48	30	18.71	36	1.05
32	F	33	1600	25	48	30	25.04	36	0.90
33	M	32	1880	30	48	30	23.84	36	1.89
34	M	34	2100	30	24	30	26.80	18	7.01
35	F	34	2500	38	24	30	26.81	18	2.70
36	M	33	2020	32	48	30	27.2	36	0.22
37	F	34	1645	24	48	30	36.43	36	6.35

No. = patient number, GA = gestational age, BW = birth weight, T_1 = time after completion of infusion, C_1 = serum concentration at time T_1, T_2 = time after start of infusion, C_2 = serum concentration at T_2

VITAE

Major Chanokporn Boonthariksiri was born on the 17th April 1967 at Hua Chiew Hospital, Bangkok. She graduated Bachelor degree in Pharmaceutical Sciences in 1990 from Faculty of Pharmaceutical Sciences, Chulalongkorn University. Her current position is a pharmacist at the Pharmacy Division, Phramongkutklao Hospital, Bangkok