Tobramycin and vancomycin use in newborns:

pharmacokinetic and pharmacodynamic aspects

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Tobramycin and vancomycin use in newborns:

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farmacokinetische en farmacodynamische aspecten

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Contents

General introduction and aims of the studies

Chapter

1

Partly based on: "the use of aminoglycosides in newborn infants".
M. de Hoog, J.W. Mouton, J.N. van den Anker,
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1.1. Use of aminoglycosides in neonates

1.1.1. General aspects of aminoglycosides

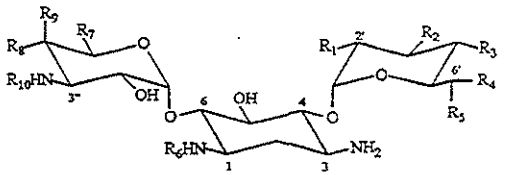
Introduction

Aminoglycosides, including tobramycin, have played a major role in antimicrobial therapy since their discovery in the 1940's, now more than 50 years ago[1]. Their bactericidal efficacy in gram-negative infections, synergism with ß-lactam antibiotics, limited bacterial resistance and low cost have given these agents a firm place in antimicrobial treatment. However, the successful use of streptomycin (1944), gentamicin (1963), tobramycin (1967), amikacin (1972) and netilmicin (1976) has been complicated by nephrotoxicity and ototoxicity in a significant number of treated patients.

This review summarizes the available data on aminoglycoside use in neonates. General aspects of aminoglycosides will be discussed, followed by a detailed description of specific aspects of aminoglycoside use in neonates. Pharmacokinetic parameters, drug resistance, toxicity and dosing schedules of the four commonly used aminoglycosides will be reviewed. Special attention will be paid to recent insights into increasing dose and prolonging dosing interval in preterm infants.

Structure and chemical properties

Figure 1: Basic structure of the main aminoglycosides (Adapted from: Mingeot-Leclerq et al.)[2]



Substituents on R-numbered spaces on basic aminocyclitol										
Aminoglycoside	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}
Tobramycin	NH_2	H	OH	H	NH_2	H	CH_2OH	OH	H	H
Gentamicin	OH	OH	OH	H	NH_2	H	H	CH_3	OH	CH_3
Netilmicin	-	-	-	-	-	CR	H	CH_3	OH	CH_3
Amikacin	OH	OH	OH	H	NH_2	COR'	CH_2OH	OH	H	H

R= CH_2CH_3, R'= $CHOH(CH_2)_2NH_2$

Figure 1 shows the basic structure of the main aminoglycosides. Aminoglycosides are comprised of two or more amino sugars attached via glycosidic bonds to an aminocyclitol nucleus and have a molecular weight of 445 to 600 Dalton. This structure consists of a six-membered ring with amino-group substituents, from which the term aminocyclitol is derived. To this central ring two or more amino-containing or non-amino-containing sugars are bound via glycosidic bonds, which led to the term aminoglycosides for this group of antibiotics. The central aminocyclitol for most aminoglycosides, including tobramycin, is 2-deoxystreptamine.

Aminoglycosides can be divided into chemical families with related structures (table 1). Tobramycin belongs to the kanamycin family, derived from *Streptomyces* spp[3]. The kanamycin family is linked to two cyclic sugars at positions 4 and 6 of 2-deoxystreptamine. Tobramycin is 3'-deoxykanamycin. The relation between structure of aminoglycosides and activity is incompletely understood.

Table I: Families of aminoglycoside antibiotics, divided in main groups and group members (adapted from Mandell)[4]

Family	Member
Streptomycin	Streptomycin
Kanamycin	Kanamycin A
	Kanamycin B
	Amikacin
	Tobramycin
	Dibekacin
Gentamicin	Gentamicin C1, C1a, C2
	Sisomycin
	Netilmicin
	Isepamicin
	Neomycin
	Paromomycin
Spectinomycin* (an aminocyclitol, no glycosidic bonds)	

Aminoglycosides are water soluble, cationic at normal pH, and distribute in plasma water. Protein binding is minimal. They have a stable structure over a wide range of temperature and pH[5, 6]. Aminoglycosides are inactivated in vitro by concomitant use of β-lactam antibiotics[7-9]. Tobramycin seems to be more easily inactivated than netilmicin, amikacin or isepamicin.

Method of action

Aminoglycosides act by altering the integrity of the bacterial cell membrane in growing bacteria by way of disturbing protein synthesis through binding to prokaryotic ribosomes[10, 11]. These cationic antibiotics bind rapidly and passively to the negatively charged parts of phospholipids and other proteins in the bacterial cell membrane[12]. They can not pass through the porin channels because of their relatively large size[13]. They enter the bacterial cell by way of a self promoted uptake process, described in detail below, wherein aminoglycosides displace Mg^{2+} and Ca^{2+} bond between adjoining lipopolysaccharides in the bacterial cell membrane[14, 15]. This leads to a rearrangement of lipopolysaccharides, which creates transient holes making the outer membrane more permeable to the antibiotic[16]. This ionic binding of aminoglycosides to the cell membrane is followed by a 2-staged energy dependent cellular uptake[10, 17, 18]. Phase I is slow, rate limiting, electron transport dependent and is termed energy dependent phase I (EDP-I). EDP-I precedes the loss of viability and inhibition of protein synthesis[12]. It is related to the concentration of aminoglycoside and can be inhibited by low pH, anaerobic conditions and hyperosmolarity[19, 20]. Aminoglycosides themselves close the "holes" made in the cell membrane through decreasing transmembrane electrical potential, causing aminoglycosides to be trapped inside the bacteria[21] resulting in far higher intracellular than extracellular concentrations. Phase II is characterized by an accelerated uptake of aminoglycoside in the cytosol and binding to the 30S subunit of ribosomes in a process utilizing energy from electron transport and ATP[18, 22, 23]. Specific binding may differ for different aminoglycosides[24-26]. After binding to ribosomes, aminoglycosides disturb the proofreading process controlling the accuracy of proteins under construction. These aberrant proteins, when inserted in the cell membrane, lead to an increase of permeability and increased uptake of aminoglycosides[27]. In man, inhibition of protein synthesis can also occur at supratherapeutic concentrations, probably through non-specific binding to eukaryotic ribosomes[28]. Although inhibition of protein synthesis plays a major part in bacterial cell death, it is not the sole explanation for the bactericidal effect of aminoglycosides. Other antibiotics that inhibit protein synthesis, like chloramphenicol, are only bacteriostatic. Binding of aminoglycosides to the bacterial cell membrane itself may play a role in rapid bacterial cell death[29].

Aminoglycoside antimicrobial activity

In vitro activity

Aminoglycosides have a concentration-dependent bactericidal spectrum encompassing aerobic and gram-negative bacteria like Enterobacteriacae, *Escherichia coli*, *pseudomonas* spp. and *Haemophilus* spp. Table 2 shows the in vitro spectrum of aminoglycosides compared with selected other antibiotics.

Table 2: In vitro spectrum of activity of aminoglycosides against selected microorganisms. (Adapted from Mandell)[4]

	Aminoglycoside			
Organism	**Gentamicin**	**Tobramycin**	**Amikacin**	**Netilmicin**
Gram-negative				
Escherichia coli	+	+	+	+
Proteus mirabilis	+	+	+	+
Klebsiella sp.	+	+	+	+
Enterobacter sp.	+	+	+	+
Morganella sp.	+	+	+	+
Citrobacter sp.	+	+	+	+
Serratia sp.	+	+	+	+
Salmonella sp.				
Providencia sp.	+	+	+	+
Aeromonas sp.	+	+	+	+
Acinetobacter sp.	0	±	0	
Pseudomonas aeruginosa	+	+	+	+
Burkholderia cepacia	0	0	0	0
Stenotrophomonas maltophilia	0	0	0	0
Neisseria gonorhoeae	0	0	0	0
Haemophilus influenzae	+	+	+	+
Yersinia pestis	+			
Francisella tularensis	+			
Gram-positive				
Streptococcus pneumoniae	0	0	0	0
Staphylococcus aureus	+	+	+	+
Staphylococcus aureus (MRSA)	0	0	0	0

MRSA = methicillin-resistant *S. aureus*

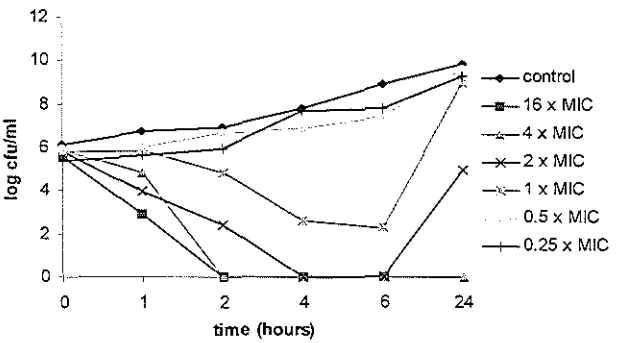
The susceptibility of most gram-negative bacteria to gentamicin, tobramycin, netilmicin and amikacin is relatively similar[30]. Although susceptibility to amikacin is three to fourfold less than the other aminoglycosides, this is compensated by its lower toxicity and therefore higher allowable dose. Gentamicin and tobramycin are comparable in activity, although tobramycin is slightly more active against *P. aeruginosa*. They are susceptible to the same modifying enzymes and resistance rates are therefore very similar. In contrast, amikacin is resistant to many of these enzymes and therefore often an alternative if strains are resistant to tobramycin or gentamicin. Netilmicin susceptibility is comparable to that of gentamicin and tobramycin, although netilmicin is resistant to some of the gentamicin inactivating enzymes and thus in some cases a good alternative.Antimicrobial activity of aminoglycosides has four distinct and clinically important aspects:

I. Concentration-dependent killing
II. Postantibiotic effect
III. Adaptive resistance
IV. Synergism with other antibiotics

Concentration-dependent killing

In vivo and in vitro studies have shown that aminoglycoside induced rate of bacterial killing as well as induction of resistance is peak concentration dependent[31-35]. This is illustrated by figure 2. Other in vitro investigations, mimicking in vivo fluctuations of drug concentrations, have shown a single bolus of aminoglycoside to be superior in rate and total amount of bacterial killing to the same dose in a multiple daily dosing regimen, in non-neutropenic animals[36, 37].

Figure 2: killing curves of tobramycin. Inocula of 5 × 10⁵ cfu/ml P. aeruginosa were incubated with tobramycin for 24 hours in a range from 0.25 –16 × MIC (Adapted from J. W. Mouton)[38]



Postantibiotic effect

Aminoglycosides are often reported to have a postantibiotic effect (PAE), meaning that there is suppression of bacterial growth for several hours after antibiotic serum concentrations have dropped below the MIC[39-41]. However, the PAE in these studies was not determined under conditions where decrease of concentrations was similar to that seen in patients. The PAE was most often determined by exposing bacteria to an antibiotic for limited a period of time, usually one hour, whereafter the drug is abruptly eliminated. Delay in resumption of growth is compared to a control curve. Recent in vitro pharmacokinetic models simulating the normal gradual decrease of serum concentrations in patients however, have failed to show evidence of a PAE beyond the MIC, especially with longer serum half-lifes[42, 43]. In vivo experiments indicate that there is a PAE, which even increases with increasing serum half-life[44, 45]. The difference between in vivo and in vitro models is partly explained by the fact that in vivo regrowth of bacteria is determined at sub-MIC concentrations[42]. Other host-related environmental factors may also play an important role. PAE should therefore be labeled as a postantibiotic sub-MIC effect. The clinical relevance is still unclear, and the emphasis on this effect in discussions on extending dose intervals of aminoglycosides is questionable.

Adaptive resistance

Antimicrobial activity of aminoglycosides is associated with a temporary adaptive resistance[46, 47]. It is a reversible form of resistance which develops within 1 to 2h of initial exposure to an aminoglycoside and disappears several hours after removal of the antibiotic. In this time period the bacterial population surviving the initial exposure are less susceptible to a renewed dose of aminoglycosides.

Synergy with other antibiotics

Synergy of aminoglycosides with other cell wall active antibiotics, like penicillins and cephalosporins has been established[4, 48]. This synergy is the basis of the clinical choice for combination therapy of aminoglycosides with penicillin or cephalosporins.

Issues of toxicity, which will be discussed later, concentration-dependent killing, postantibiotic effect (although doubtful), as well as adaptive resistance are the rationale for the change to extended interval dosing of aminoglycosides over the last decade[49-51].

Drug resistance and susceptibility

Resistance of aminoglycosides in the Netherlands is defined according to national guidelines on susceptibility and is divided in three groups as shown in table 3.

Table 3: Susceptibility criteria in mg/L according to the CRG[1] (Dutch committee on guidelines in susceptibility of microorganisms) compared with NCCLS guidelines

Antibiotic	CRG[1]			NCCLS[2]		
	S	I	R	S	I	R
	<=	> - <=	>	<=	>= - <=	>=
Amikacin	4	4-16	16	16	32	64
Gentamicin	1	1-4	4	4	8	16
Netilmicin	2	2-8	8	8	16	32
Tobramycin	1	1-4	4	4	8	16

[1] Commissie richtlijnen gevoeligheidsbepalingen, [2] National Committee for the Clinical Laboratory Standards, S= susceptible, I=intermediate resistance, R=resistant

Resistance to aminoglycosides can occur by three mechanisms, ribosomal resistance, decreased uptake and/or accumulation and enzymatic modification. Ribosomal resistance will not be discussed since it is only pertinent to streptomycin.

1. Decreased uptake and/or accumulation

A decrease in drug uptake is a clinical significant aspect of aminoglycoside resistance. The underlying mechanism, though probably related to membrane impermeabilization, is not really known[52]. It pertains to all aminoglycosides and is a stable characteristic resulting in a moderate level of resistance[2]. Another important phenomenon in aerobic gram-negative bacteria is called adaptive resistance, defined as a reduced antimicrobial killing in originally susceptible bacterial populations after initial incubation with an aminoglycoside[53]. It has clinical relevance especially for immunocompromised patients and in serious infections with gram-negative bacteria. Adaptive resistance is probably related to membrane protein changes and altered expression of regulatory genes of the anaerobic respiratory pathway[54]. It can be overcome by higher peak serum concentrations of aminoglycosides, which underscores the need for extended dose intervals[55].

2. Enzymatic modification

Aminoglycosides can be modified with subsequent loss of antimicrobial activity by enzymes produced by bacterial pathogens[4]. The three major classes of enzymes are *N*-acetyltransferases, *O*-nucleotidyltransferases and *O*-phosphotransferases. The genetic code for these enzymes is largely contained in plasmids, thereby rendering the resistance easily transferable. In addition, it is important to realize that all susceptible positions in aminoglycosides can be modified by several enzymes and that several inactivating genes can easily develop from a common ancestor leading to the conclusion that it will be unlikely that making aminoglycosides resistant to inactivation by a specific enzyme will be a worthwhile effort[2].

1.1.2. Specific aspects of aminoglycoside use in neonates

General aspects in neonates

Bacterial infections continue to be a major cause of morbidity and mortality in preterm infants admitted to neonatal intensive care units (NICU's)[56], though a decline in sepsis associated neonatal deaths has been reported[57]. The immunologically incompetent premature neonate is susceptible to invasive microbial infections through hospitalization, ventilation and invasive procedures, such as the introduction of central venous lines. Nosocomial infections in the neonatal period, including septicemia, meningitis, pneumonia or urinary tract infection occur in approximately 18% of very low birthweight (VLBW; <1500 g) infants[58]. Culture proven early onset sepsis, defined as within the first 3-4 days of life, occurs in approximately 2% of VLBW infants, but there are limitations to blood cultures in neonates and single blood cultures can be false negative[59-61].

Among major pathogens responsible for bacterial infections during the first month of life, Gram-negative bacteria like *Escherichia coli*, *Klebsiella* spp, *Enterobacter* spp and *Pseudomonas* spp, play an increasing role, possibly related to the increased prenatal administration of antibiotics and use of percutaneous central venous catheters in the NICU[58, 62-64]. Aminoglycosides are effective against most nosocomial acquired gram-negative infections in term and preterm infants and are synergistic with β-lactam antibiotics in treating group B streptococcal and coagulase-negative staphylococcal infections. They play an important role in the initial empiric treatment of neonatal septicemia[65]. After penicillins, aminoglycosides are the most commonly used drugs in the neonatal intensive care unit (NICU)[66]. A MEDLINE search shows that since 1966 more than 900 articles relating to

aminoglycoside use in neonates have been published. Despite this enormous amount of documentation, there is still much debate about the proper use of aminoglycosides in infants.

Pharmacokinetics

Aminoglycosides have a pharmacokinetic profile consisting of a rapid distribution phase ($t_{1/2\alpha}$), elimination phase ($t_{1/2\beta}$), and a second elimination phase ($t_{1/2\gamma}$). The gamma phase can only be determined after discontinuation of the drug. Distribution half-life is 5-10 minutes in adults, but has never been measured in newborns. The gamma phase in infants is long. Netilmicin was detectable in blood and urine 11 and 14 days after discontinuation, with a $t_{1/2\gamma}$ of 62.4h[67]. The tissue half-life in renal cortex is 4-5 days[68]. In most studies aminoglycosides are studied using a one-compartment open model with individual pharmacokinetic parameters estimated by way of the method described by Sawchuk and Zaske[69]. In general, the serum concentrations and pharmacokinetic data mentioned in most studies concern the elimination phase, which is adequately described by a one-compartment model. There are, however, some studies that have shown a two compartment model to be superior in predicting serum $t_{1/2\beta}$ and serum concentrations[70-72].

Aminoglycosides are eliminated from the body by way of glomerular filtration; it is therefore predictable that a relation between glomerular filtration rate (GFR) and serum concentrations exists. The link between GFR and aminoglycoside pharmacokinetics is often[67, 73-79], but not consistently[71, 79-81] described in neonates. Brion et al.[76] showed a linear relation between GFR and gentamicin $t_{1/2}$, but GFR was determined by serum creatinin. Some authors reported that serum creatinin measurements in newborns do not reliably predict GFR. They hypothesized that serum creatinin concentrations in the initial days after birth were a reflection of maternal serum creatinin. However, it was recently shown that the serum concentration is inversely related to gestational age[82]. If the serum creatinin concentration in the days after birth is indeed a reflection of maternal serum creatinin, the youngest infants should have had the lowest concentration because maternal serum creatinin is lowest at the beginning of the third trimester[83]. Therefore the elevated concentration of serum creatinin in these infants during the first postnatal days probably reflects the difficulty these children have to eliminate the excess creatinin transferred from their mother. Furthermore in adults it has been shown that aminoglycoside concentrations can change without concomitant change of serum creatinin[84].

Table 4: Results of pharmacokinetic studies of aminoglycosides in neonates

Reference	N	GA (weeks)	PNA (days)	Weight (grams)	CL (ml/min/kg)	Vd (L/kg^{-1})	$t_{1/2}$ (h)
Amikacin							
Padovani, 1993	32	32 ± 3.6		1740 ± 810	1.08 ± 0.51	0.655 ± 0.414	7.6 ± 4.4
Kenyon, 1990	28	30.5 ± 2.86		1380 ± 170	0.83 ± 0.28	0.57 ± 0.11	8.4
Kenyon, 1990	6	32-40	1-3	1500-3400	1.05 ± 0.30	0.70 ± 0.27	2
	5	36-40	5-8	2100-3600	1.08 ± 0.42	0.49 ± 0.11	5.6
	11	32-38	>8	1900-4600	1.78 ± 0.53	0.73 ± 0.13	5.1
Gentamicin							
Nakae, 1988	19		1	< 1500	0.75 ± 0.60	0.72 ± 0.45	13
	18		1	≥ 1500	0.97 ± 0.23	0.78 ± 0.39	13.8
	20		4	< 1500	0.50 ± 0.18	0.60 ± 0.26	10.9
	28		4	≥ 1500	0.72 ± 0.10	0.50 ± 0.18	8.1
Kildoo, 1984	15	< 33	< 7	< 1500	0.38 ± 0.15	0.53 ± 0.10	11.1
	15	< 33	8-30	< 1500	0.45 ± 0.17	0.50 ± 0.11	10.8
	6	< 33	> 31	< 1500	1.18 ± 0.45	0.50 ± 0.11	4.4
Koren, 1985	12		1.8	<1000	0.52 ± 0.08	0.35 ± 0.07	7.9
	36		1.8	≥1000	0.65 ± 0.13	0.38 ± 0.13	6.5
	20	≤ 30	1.8		0.58 ± 0.12		7.4
	28	>30	1.8		0.63 ± 0.13		6.5
Izquierdo, 1992	11	28-33	2-30		1.00	0.597	6.53
	31	35-38	2-30		1.22	0.538	4.95
	55	39-43	2-30		1.15	0.542	5.17
Williams, 1997	216	32.39 ± 2.83	?	1850 ± 670	0.75 ± 0.25	0.54 ± 0.13	8.98 ± 2.86
	106(PDA)	29.02 ± 2.92	?	1160 ± 530	0.67 ± 0.28	0.61 ± 0.15	12.24 ± 7.43
Watterberg, 1987	24 (PDA)		?	< 1500	0.93 ± 0.33	0.64 ± 0.20	8.49 ± 2.69
	16		?	< 1500	0.83 ± 0.4	0.41 ± 0.08	6.23 ± 1.92
Dahl, 1986	11	26-33	1-10				13
	6	34-40	1-10				6
Isemann, 1996	16	30.6 ± 0.86	< 12 h	1600 ± 154		0.57 ± 0.03	10.2 ± 0.89
	18	29.2 ± 0.81	< 12h	1294 ± 145		0.58 ± 0.02	12.0 ± 0.84

Abbreviations: n= number of patients in study, PDA= patent ductus arteriosus, ECMO= extracorporeal membrane oxygenation, GA= gestational age, PNA= postnatal age, PCA= postconceptional age, AS5= 5' apgar score, CL= total body clearance, Vd= volume of distribution; $t_{1/2}$= serum half-life

Table 4 (continued): Results of pharmacokinetic studies of aminoglycosides in neonates

Reference	N	GA (weeks)	PNA (days)	Weight (grams)	CL (ml/min/kg)	Vd (L/kg^{-1})	$t_{1/2}$ (h)
Gentamicin (continued)							
Southgate, 1989	10 ECMO	36-43	< 7		2.78 ± 1.55	0.51 ± 0.11	9.55 ± 4.38
Thomson, 1988	113	>34+AS5≥7	0-50	500-4500	0.88	0.47	
		≤34+AS5<7			0.73		
		≤34+AS5≥7			0.6		
Faura, 1991	165	37 ± 4.5	7.8±11.7	2432 ± 952		0.64 ± 0.22	8.2 ± 4.8
Pons, 1988	15	< 37	0-2		1.03 ± 0.37		
	27	≥ 37	0-2		1.40 ± 0.47		
	8	< 37	3-7		1.78 ± 0.63		
	16	≥ 37	3-7		1.78 ± 0.38		
	1	< 37	8-28		1.67		
	14	≥37	8-28		1.97 ± 0.43		
Netilmicin							
Granati, 1985	22	27-40	< 16	800-3400	1.07 ± 0.28	0.034 ± 0.11	9.6
Kuhn, 1986	12	28-33	< 28	770-2050	0.83 ± 0.27	0.63 ± 0.24	8.6
Siegel, 1979	16		<7	< 2000		0.609	4.7
	8		>=7	< 2000		0.599	4.1
	9		<7	> 2000		0.472	3.4
	23		<7	> 2000		0.617	4.4
	4		>=7	> 2000		0.510	3.8
Tobramycin							
Nahata, 1984	19	29-40	2-4	1000-3555	1.15 (0.70-1.83)	0.82 (0.54-1.76)	8.6(3.5-14.1)
			4-7	1000-3555	1.14 (0.62-1.56)	0.68 (0.40-1.06)	7.1(4.6-11.6)
	8		2-4	1000-1500	1.09 (0.74-1.15)	1.04 (0.64-1.36)	11.1(6.6-14.1)
			4-7	1000-1500	1.02 (0.62-1.55)	0.73 (0.46-1.06)	8.7 (5.7-11.6)
Nahata, 1984	9	28-30	2-6		1.04 ± 0.22	0.84 ± 0.31	9.3 ± 2.8
	11	30-34	2-6		1.13 ± 0.35	0.81 ± 0.20	8.9 ± 3.0
	6	34-40	2-6		1.28 ± 0.31	0.61 ± 0.14	5.6 ± 1.2
	7		2-6	1000-1250	1.05 ± 0.20	1.02 ± 0.27	11.3 ± 3.0
	6		2-6	1260-1500	1.12 ± 0.39	0.74 ± 0.16	8.2 ± 2.0
	7		2-6	1500-2000	1.10 ± 0.32	0.69 ±0.16	7.5 ± 1.6
	6		2-6	2100-3500	1.28 ± 0.31	0.61 ± 0.14	5.6 ± 1.2
Nahata, 1986	8	24-30	3-5	<1000	0.69 ± 0.10	0.59 ± 0.10	9.9 ± 1.5

Abbreviations: n= number of patients in study, PDA= patent ductus arteriosus, ECMO= extracorporeal membrane oxygenation, GA= gestational age, PNA= postnatal age, PCA= postconceptional age, AS5= 5' apgar score, CL= total body clearance, Vd= volume of distribution; $t_{1/2}$= serum half-life

Keyes showed that serum trough concentrations could not be reliably predicted in newborns with serum creatinin[80]. In conclusion these studies suggest that, though there is a relation, serum creatinin in the first week of life can not be accurately used to predict aminoglycoside clearance.

Effect of gestational age and birthweight on pharmacokinetics

The volume of distribution (Vd) of most drugs is larger in neonates, especially in prematures, primarily due to a higher percentage of extracellular water[85, 86]. As can be seen in table 4, this also holds true for aminoglycosides. There is a consistently higher Vd for prematures, especially in the VLBW-group/ gestational age (GA) group below 30 weeks. Most authors have found birthweight (BW)[71, 77, 87-89] to be the best predictor of Vd, some found Vd to be independent of GA[76, 90]. In practice, this means that prematures will end up having lower peak serum concentrations. The interpatient variability of Vd in these groups is greater and therefore serum concentrations are difficult to predict in the individual premature infant. Total body clearance (CL), associated with GA[71, 78, 88] and BW[71, 87-89], is lower and elimination half-life ($t_{1/2\beta}$) is longer in preterm infants, leading to higher serum trough concentrations in this group. This can be explained by the significant increase of GFR with GA and BW described in recent studies[91, 92]. CL and $t_{1/2}$ are also highly variable in the VLBW infants. Substantial effort has been put into developing formulas, mostly based on population pharmacokinetic studies, which potentially will lead to better prediction of serum concentrations in the individual patient[76, 87, 89]. Despite these efforts variability persists and therapeutic drug monitoring (TDM) remains common practice in all infants.

Effect of postnatal age on pharmacokinetics

Diminishing extracellular fluid in the neonatal period[93] leads to a corresponding decrease in Vd with increasing postnatal age (PNA), again especially in the VLBW group. In contrast, Faura et al.[94] found no difference in Vd between 48h and 144h PNA, most likely explained by the mean GA of 37 weeks in neonates included in this study. Recent data have shown a significant postnatal increase in GFR[91, 92]. A concomitant change in CL and $t_{1/2}$ has been shown for amikacin[95], gentamicin[76, 78, 79, 96, 97], netilmicin[67, 71] and tobramycin[98], but has been refuted by others[77, 94]. These data suggest that repeated TDM should be performed in the first week of life.

Effect of patent ductus arteriosus, indomethacin, extracorporeal membrane oxygenation (ECMO) and corticosteroids

Patent ductus arteriosus (PDA) as well as postnatal exposure to indomethacin alter the rapid postnatal increase in GFR[99], possibly through decreased renal blood flow, leading to an increase in Vd and a reduction of CL. Increase in Vd of gentamicin is found in infants with PDA[90, 100]. In this patient group fluid overload is common. A recent study in prematures[90] showed that a gentamicin Vd exceeding 0.7 L/kg has 92% specificity for presence of a hemodynamically significant PDA. However, important deficiencies in the design of this study were reported[101]. Closure of PDA leads to significant decrease in Vd of more than 30 %[100, 102]. Dosage adjustments, based on TDM, should be made in patients with PDA. The effect of indomethacin, used for closure of PDA, as well as surgical closure itself necessitates TDM. Information on gentamicin disposition in infants on ECMO is scarce. Two small studies[72, 103] showed Vd to be increased. Serum half-life in patients on ECMO was 9.55h and 9.24h respectively, and decreased to 3.87h in the same patients off ECMO in the study by Dodge et al[103]. Southgate et al. showed that serum creatinin is a significant predictor for $t_{1/2}$[72]. On the basis of these data, dosage adjustments should be made in this group of infants. They advise an initial dose of 4.3 mg/kg, with a maintenance dose of 3.7 mg/kg/18h[103]. Prenatal exposure to corticosteroids, which is seen increasingly in the VLBW group, leads to increased intra-uterine maturation of kidney function, possibly through direct vasodilatation mediated by glucocorticoid receptors[91]. Though this point has not been investigated yet, it might have a significant effect on pharmacokinetic parameters in this group of infants. These studies indicate that extra therapeutic drug monitoring (TDM) is warranted in patients who are either on ECMO, have an open ductus Botalli, or are exposed to indomethacin.

Drug resistance and susceptibility

Broad-spectrum antibiotics are generally used in NICU's as empiric treatment of (suspected) neonatal sepsis. Drug resistance in NICU's is an important factor related to this empirical use. Over the years aminoglycosides remain antibiotics of first choice in the initial empirical treatment of neonatal septicemia, both because of their broad spectrum as well as their activity[65].

Since most serious infections in the NICU are caused by Enterobacteriaceae and coagulase negative staphylococci (CONS), these are the organisms that are now

considered with respect to emergence of resistance in the clinical setting. In one study, a change from gentamicin to amikacin was initiated because of gentamicin resistant CONS. Although amikacin resistance quickly emerged in the CONS, the bacteria that caused the serious infections remained susceptible[104]. In another study, a change from gentamicin to amikacin because of the emergence of gentamicin resistant Enterobacteriaceae did not lead to an increase in amikacin resistant strains. In contrast, there was a decrease in gentamicin resistance[105]. In a third study on a NICU, the change from gentamicin to amikacin led to an outbreak of amikacin resistant *Serratia* spp, which remained susceptible to gentamicin[106]. However, this could have been due to the intrinsically higher activity of gentamicin against *Serratia* spp. In general, although the number of studies are limited, emergence of aminoglycoside resistant strains other than coagulase negative staphylococci is relatively slow[107], which is a definite advantage over the third generation cephalosporins. The frequent use of these latter drugs has been shown to rapidly lead to a significant increase in the emergence of multiple drug resistant strains[107-109].

Toxicity

The major specific side-effects of aminoglycosides are nephrotoxicity and ototoxicity. Neurotoxicity by way of blockade of neuromuscular synapses with prolonged muscle weakness after the use of muscle relaxants has not been described in infants. The delayed type hypersensitivity reaction of the skin is mostly seen after use of topical application of neomycin or framycetin and has not been described in neonates. Nephro- and ototoxicity will be described in further detail.

Nephrotoxicity

Aminoglycoside nephrotoxicity is induced by way of proximal tubular injury leading to cell necrosis. The mechanisms of toxicity have been mostly studied in animals. Less than 5% of filtered aminoglycosides binds to the brush-border membrane of proximal tubular cells and is actively reabsorbed, finally causing cell death. Absorption through the basolateral surface has also been described[68]. Intracellular mechanisms resulting in cell death are formation of hydroxyradicals, increase in phospholipidosis, inhibition of Na-K-ATPase, effects on microsomal protein synthesis, lysosomal and mitochondrial injury, increased tromboxane A_2 synthesis, and vascular factors like activated renin-angiotensin system[110]. Because of the site specific damage, aminoglycoside nephrotoxicity

does not initially induce azotemia and decreased GFR, but leads to polyuric renal failure, defective urinary concentration ability, cylinduria, glucosuria, phosphaturia, aminoaciduria, microproteinuria, enzymuria and a slow rise in serum creatinin. High doses (> 40 mg/kg) are needed to create cortical necrosis and overt renal dysfunction[111]. Tubular cells undergo a proliferative response after treatment leading to repair. This response is diminished in ill patients[112], which is a possible explanation as to why patients are more sensitive to aminoglycosides. The degree of nephrotoxicity is determined by the quantity of aminoglycosides stored in the proximal tubular cell and the intrinsic potency of the drug to damage subcellular structures[113]. Aminoglycosides show drug-specific saturable uptake into tubular cells in animals[114] and humans[115, 116]. Comparison between once daily dosing (ODD) and multiple daily dosing (MDD) or continuous dosing for amikacin, tobramycin, gentamicin and netilmicin in adults showed considerably less renal accumulation for ODD in gentamicin, netilmicin and amikacin. There was no difference between dosage regimens for tobramycin, though the study group was small (n=18). In several large meta-analytical studies toxicity seems to be related to high trough concentrations, indicating that these concentrations are not low long enough to prevent renal accumulation. Nephrotoxicity related to ODD opposed to MDD was found to be equal or less in these studies[49, 50, 84, 117, 118]. A recent prospective study in adults showed that both probability and time of occurrence of nephrotoxicity are negatively influenced by multiple daily dosing[119]. There is no clear distinction in level of nephrotoxicity between the four aminoglycosides in studies in adults[68].

The incidence of aminoglycoside nephrotoxicity in neonates is not well known, but seems to be considerably lower than in adults. Enduring glomerular filtration impairment has not been conclusively shown in prospective studies. GFR was not affected by tobramycin given for less than 6 days[120]. The normal postnatal decline of plasma creatinin was temporarily decreased in preterm and term infants treated with amikacin, gentamicin or netilmicin. This diminished decline persisted for at least two days after discontinuation of therapy. After 10 days, these differences could no longer be detected, except for term infants treated with gentamicin[121]. Other studies showed no difference in serum creatinin in treated patients versus controls[122, 123]. No difference in renal function was found between ODD and MDD for amikacin and gentamicin[123, 124]. No relation between serum concentrations and GFR disturbances has been demonstrated in infants.

Tubular dysfunction has been shown in many studies involving neonates[120-122, 125-129], and is more pronounced in term infants than in preterm newborns[120, 123, 128], possibly

explained by maturity dependent blood supply differences of the outer parts of the kidney and lower binding constants in the immature kidney[130]. Several studies showed the dysfunction to be reversible[121, 122, 125]. One study showed enzymuria during treatment to be more pronounced with gentamicin than with amikacin in the low birthweight infant[129]. In infants with a GA above 34 weeks no difference in proximal tubular damage was found between ODD and MDD of amikacin[123]. A reversible increased loss of sodium, calcium and magnesium has been shown to exist during and just after treatment with amikacin, netilmicin and gentamicin. Urinary electrolyte loss is higher at peak serum concentrations[121, 125]. In the ill term and especially preterm infant, who is already at risk for electrolyte disturbances, the increased loss during aminoglycoside therapy might be clinically relevant. In summary aminoglycoside related loss of renal function is rare. Reversible tubular dysfunction is seen often and already occurs early in therapy.

Ototoxicity

Aminoglycosides are potentially cochleo- and vestibulotoxic. They accumulate in the lymphatic fluid of the inner ear from which they are only slowly eliminated (24-36h)[131]. There is evidence for saturable uptake in animals[132]. Some authors suggest that ototoxic effect is only possible after conversion in serum of aminoglycosides to a cytotoxin[133, 134]. Certain gene mutations lead to a familial increased risk of aminoglycoside induced sensorineural hearing loss[135]. Sequentially outer hair cells, inner hair cells and spiral ganglional neurons are damaged. Aminoglycosides seem to give a polyamine-like enhancement of glutamate N-methyl-D-aspartate (NMDA) receptor activity, resulting in excessive entry of sodium and calcium which leads to excitotoxic cell death. Protective trophic factors like platelet derived growth factor are antagonized by aminoglycosides[136]. NMDA receptor blockade has been shown to protect against aminoglycoside induced ototoxicity[137]. Hearing loss is usually bilateral, symmetrical and permanent[131], and can also have a delayed onset of months[131]. Most authors suggest that ototoxicity is related to total dose and duration of therapy rather than to serum aminoglycoside concentrations, but the relation to aminoglycoside serum concentrations remains unclear. This form of toxicity usually occurs in patients who have received either long, or repeated, courses of aminoglycosides[138]. No difference in incidence between ODD and MDD could be demonstrated[118, 119]. In experimental studies amikacin appears to be more cochleotoxic than gentamicin and tobramycin. Netilmicin is probably the least ototoxic

aminoglycoside[139]. Although vestibulotoxicity is a disabling side effect in adults, it has not been shown in neonates.

There are many pitfalls in relating use of aminoglycosides to loss of hearing in infants. Hearing loss in neonates occurs in 0.1-0.3% of cases[140]. Numerous risk factors for neonatal hearing loss have been identified. Perinatal infections, meningitis, prematurity, hyperbilirubinemia, birthweight < 1500 grams, asphyxia, respiratory distress syndrome, mechanical ventilation, antibiotics, and diuretics have all been incriminated[141]. Even though some studies show a relation to administration of aminoglycosides, it remains difficult to separate the effect of aminoglycoside use from concomitant factors[140]. Furthermore initial investigations concerning hearing loss, before the introduction of brain stem evoked response audiometry (BERA), were hampered by the fact that conventional and behavioral audiometry were used, which are not reliable under the age of 12-24 months.

Table 5: Reported ototoxicity of aminoglycosides in neonates

Drug and reference	N	GA	PNA	Duration of therapy	Total dose (mg/kg)	Serum conc.	Ototoxicity
Amikacin							
Finitzo-Hieber, 1985	50	28-41	0, before discharge, follow up	5.3 ± 2.0	< 403	Yes	2% (N.S.)
Langhendries, 1993	10 ODD 12 MDD	≥34	0,3,9	8.8 ± 1.8 8.0 ± 2.4	N.A.	Yes	No
Gentamicin							
Kohelet, 1990	7	39.3	3	N.A.	N.A.	Yes	Yes, prolonged latencies in the treated group
Tsai, 1992	17	?	3,10	N.A.	N.A.	Yes	Yes, transient
Bernard, 1981	26(6 tobra)	37	5,10	8.7 ± 1.3	163 ± 81	Yes	Yes
Netilmicin							
Finitzo-Hieber, 1985	49	27-41	0, before discharge, follow up	5.2 ± 2.6	< 129	Yes	2% (N.S.)

N.A= not available, N.S.= not significant, GA= gestational age, PNA= postnatal age, conc. = concentrations (mg/L), value, n = number of subjects, ODD= once daily dosing, MDD= multiple daily dosing

Table 5 shows the results of studies concerning aminoglycoside related ototoxicity. Of the many studies, only those using BERA will be discussed below. Ototoxicity is an infrequent occurrence in these studies. Many studies did not find any aminoglycoside-related toxicity. Cox et al.[142] found that 14% of infants had abnormal latencies at 4 months in a group of 43 infants with multiple risk factors, but there were no controls. Interestingly, 3 infants had an initial normal BERA followed by an abnormal BERA at follow-up. This means that auditory evaluation during, or just after discontinuing aminoglycosides, will not capture all patients with induced hearing loss. In the other study with a high rate of ototoxicity, the 8 patients with abnormal BERA's and aminoglycoside treatment less than 10 days, also had another reason for hearing loss[143]. The best study so far, with a baseline value, follow-up and a control group, found 3 patients with hearing loss; 1 patient each treated with netilmicin or amikacin, and 1 control[144]. Several studies found a transient hearing loss[144, 145]. Some studies found a relation with duration[141, 143] and total dose[141, 146]. No clear relation was found to peak and trough concentrations. Concomitant treatment with furosemide and vancomycin was associated with hearing loss[123, 139, 147]. The results mentioned above lead us to conclude that aminoglycoside related hearing loss in infants is infrequent, possibly transient and might be late in appearing.

Aminoglycoside therapeutic drug monitoring and dosing

Dose and dosing interval are determined by the desired therapeutic range and pharmacokinetic as well as pharmacodynamic properties of a drug. It is difficult to define the desired therapeutic range for aminoglycosides. Peak concentrations of > 4-5 mg/L are generally accepted as necessary for antibacterial efficacy when dosing thrice daily[34, 84, 148, 149], but questions are being raised about the underlying basis of this assumption[138]. What is known, is that efficacy of aminoglycosides is related to the ratio of peak serum concentration to the minimal inhibitory concentration (MIC) of the infecting microorganism and the area under the time versus concentration curve (AUC). In vitro ratios of 10:1 prevent emergence of aminoglycoside-resistant pathogens[34, 35]. In several large meta-analysis studies toxicity seems to be related to high pre-dose concentrations[49, 50, 84, 117]. Commonly accepted trough concentration goals in adults are < 2 mg/L, but when dosing once a day most authors keep < 1 mg/L as a safe limit [51, 150, 151]. As described before, neonatal data on nephro- and ototoxicity as well as efficacy, in relation to aminoglycoside serum concentrations are not available, and

therefore have to be extrapolated from adult and experimental models. Based on the aminoglycoside susceptibility of gram-negative pathogens involved in neonatal septicemia, a reasonable target range for neonates would therefore be peak serum concentrations of 5-10 mg/L for gentamicin, netilmicin and tobramycin and 15-30 mg/L for amikacin. Trough concentration goals are < 2 mg/L when dosing thrice daily and < 0.5-1.0 mg/L for ODD in gentamicin, netilmicin and tobramycin and 1.5-3 mg/L for amikacin. As described before, investigations concerning the pharmacokinetics of aminoglycosides and other drugs in neonates have shown that elimination half-lives are longer in neonates, especially in preterms. This is primarily due to a higher percentage of extracellular water and thus a larger volume of distribution and reduced clearance. Most dosing schedules for preterm and term neonates take this into account. Currently recommended doses are 2-2.5 mg/kg for gentamicin, netilmicin and tobramycin and 7.5-10 mg/kg for amikacin, with dosing intervals, dependent on gestational age (GA) and postnatal age (PNA), between 8 and 24h. A critical look at the available data shows that required serum concentrations as mentioned above are not reached with most of these dosing regimens, stressing the need for other dosing strategies.

In larger studies, inadequate serum peak concentrations and elevated serum trough concentrations were found, especially in VLBW-infants[80, 152-155]. It has to be noted that many studies used steady state peak serum concentrations after the fourth dose, which are often higher than initial peak serum concentrations. From the viewpoint of efficacy it is important to obtain an adequate peak serum concentration after the first dose, which led several authors to advise a loading dose of aminoglycosides[102, 150, 154, 156]. It seems clear, that given the desired goals and the pharmacokinetic properties, aminoglycoside dosing in newborns should be revised towards larger doses at extended intervals. Several studies to that effect have been published recently[96, 124].

Due to the large interindividual variability of elimination half-life, especially in preterms, dosing interval has to be individualized. Currently this is performed by taking blood samples in steady state, around the third or fourth dose. Antibiotic courses in neonates are often relatively short. Antibiotics are initially started in a large percentage of patients in the first 24h on clinical grounds. They are discontinued if cultures remain negative after 2-3 days. Furthermore it is essential, when giving aminoglycoside antibiotics, to obtain efficacious peak levels after the first dose. These arguments point to a need for early TDM in neonates. A simple method for early TDM might be obtaining two serum samples 1h and 6h after the first dose. Individual dosing interval is then determined by

the calculated $t_{1/2}$, the target serum trough concentration being 0.5 mg/L. This method will have to be prospectively validated.

In conclusion, on the basis of these recent data, dosing of aminoglycosides in newborns should be revised to higher doses per kg with longer dosing intervals, as has been propagated in adults over the last few years. The rarity of aminoglycoside related toxicity in studies in infants, the paucity of case reports on this subject, the lack of evidence for relation to serum concentration, and the data in adults, justify starting infants on higher initial doses with longer intervals and early TDM for clinical trials.

1.2 Use of vancomycin in neonates

1.2.1. General aspects of vancomycin

Introduction

Vancomycin is an antibiotic first isolated from an Indonesian jungle soil sample in 1956 and was the first of the new class of glycopeptide antibiotics. Its initial clinical use in 1958 was facilitated by the emergence of penicillinase-producing staphylococci. It was largely supplanted by methicillin sodium, introduced in 1960, due to the frequent occurrence of side effects associated with vancomycin use, including generalized skin eruptions, phlebitis, fever and more importantly deafness and renal failure[157, 158]. Several factors have contributed to the "rediscovery" of vancomycin in the 70's. First, important changes in the preparation of vancomycin have led to a reduction of impurities present in the product and a concomitant decrease in incidence of side-effects[159-161]. Second, the emergence of methicillin-resistant staphylococci has necessitated a change in antibiotic policy for these infections.
Despite the emergence of vancomycin resistant enterococci, vancomycin still is the most widely used glycopeptide antibiotic, and is a cornerstone in antibiotic treatment of gram-positive infections in adults as well as neonates.

Structure and chemical properties

Vancomycin and teicoplanin comprise the commonly used glycopeptide antibiotics and are unrelated to other antibiotics. They are complex soluble glycopeptides, consisting of a seven-membered peptide chain, in the form of three large rings. Five of the seven amino acid residues are common to all glycopeptides[162-164]. A disaccharide, composed of glucose and vancosamine, is also present but is not part of the cyclic structure. The molecular weight of vancomycin is 1,448 Da[165].
Vancomycin is hydrophobic, but less so than teicoplanin[163]. It has a moderate protein binding (10-55%) and exerts its activity over a wide pH range of 6.5-8 [166, 167]. Vancomycin can be inactivated by heparin in high concentrations[168].

Method of action

The bactericidal activity of vancomycin is based on the inhibition of bacterial cell wall synthesis. It complexes, by way of hydrogen binding, to the D-alanyl-D-alanine portion of peptides found only in bacterial cell walls. The binding of this large molecule to the peptide side chain shields the substrate from the enzyme peptidoglycan synthetase[169]. This interferes with cross-linking of cell wall peptidoglycans and therefore bacterial cell wall rigidity can not be achieved[163, 165, 170]. The mechanism of action implies that vancomycin can only exert its effect on growing bacteria. Other, less important modes of action are alteration of the permeability of cytoplasmic membranes and selective inhibition of RNA synthesis[171, 172].

Vancomycin antimicrobial activity

Vancomycin is bactericidal for a host of aerobic and anaerobic gram-positive bacteria. Strains of *Staphylococcus epidermidis* and *Staphylococcus aureus* are susceptible to vancomycin, although emergence of vancomycin intermediate resistant strains are a growing concern, which will be discussed later[173].
Normal minimum inhibitory concentrations (MIC's) are in the range of 1-3 mcg/L [170].
Vancomycin is bacteriostatic for enterococci[167].
Important aspects of antimicrobial activity of vancomycin for clinical practice are:

I. Lack of concentration-dependent killing
II. Postantibiotic effect
III. Synergism with other antibiotics

Lack of concentration-dependent killing

Several recent studies have shown that the extent of bacterial killing is not related to peak serum concentrations but to the time the antibiotic concentration is maintained above the MIC[174-176]. This may however be dependent on time of exposition. An in-vivo study showed that in the first 12h the MIC was the most important factor, while for the total first 24h the AUC was more important[177]. There are conflicting conclusions in the translation of in-vitro and in-vivo results to vancomycin dosing. Most authors advocate 12h intervals in adults. Some give continuous infusion and others dose once daily[178-80]. Treatment failures due to once daily dosing have been described[181, 182]. Continuous infusion of vancomycin was proven to be as effective as intermittent dosing[179].

Postantibiotic effect

Vancomycin shows an in-vitro postantibiotic effect (PAE) against *S. aureus*, *S. epidermidis* and enterococcal species, lasting 1-6h[175, 183-185]. As with aminoglycosides, PAE should be studied under conditions simulating time versus concentration curves seen in clinical practice The duration of PAE effect seems to be far longer when bacteria remain exposed to vancomycin concentrations of 0.1-0.3 mg/L, indicating a sub-MIC effect[175]. In vivo experiments relating PAE to vancomycin concentrations are scarce. A definite conclusion on the clinical importance of the PAE of vancomycin can not be drawn.

Synergy with other antibiotics

Combination of vancomycin with an aminoglycoside or rifampicin is synergistic for *S. aureus* (both methicillin-sensitive and methicillin-resistant) and *S. epidermidis* and enterococcal infections[186, 187]. In enterococcal infections synergy can be achieved in most cases by adding an aminoglycoside as well[188].

Drug resistance

Clinically important resistance to vancomycin is seen in enterococci, *S. aureus* and *S. epidermidis*. An unsettling increase of vancomycin-resistant enterococci has been noted in the United States, related to selection pressure by indiscriminate use of vancomycin[189, 190]. Resistance in enterococci has been linked to at least four genes and types of resistance, Van A, Van B, Van C and Van D. Van A and Van B resistance can be transferred by way of plasmid conjugation to other enterococci[178, 191]. Van A leads to vancomycin and teicoplanin resistance, Van B resistance retains susceptibility to teicoplanin[167]. Van C phenotype shows low level vancomycin resistance but remains susceptible to teicoplanin[178, 192, 193]. There are two alarming features to these resistance genes. First, a transfer of Van A resistant enterococci from poultry and domestic animals to humans has been noted, possibly as a result of avoparcin use as a growth promoter in animals[194, 195]. Second, transfer of vancomycin resistance from enterococci to *S. aureus* has been demonstrated in the laboratory and an emergence of this phenomenon in the clinical situation is feared[196]. There are an increasing number of reports on intermediate resistance in *S. aureus* and *S. epidermidis*[173]. MIC's as high as 16 mg/L with minimal bactericidal concentrations (MBC's) of 64 mg/L have been reported for *S. epidermidis*, with a concomitant resistance to teicoplanin[197]. After the first report in Japan, a number

of papers have addressed the emergence of vancomycin intermediate resistant strains of *S. aureus* (VIRSA)[173, 198, 199]. Resistance seems to be related to thickened and aggregated cell walls, though the precise mechanism is not yet known[200]. There is cross-resistance to teicoplanin. Infection with VIRSA is associated with treatment failure of vancomycin[201].
An important mechanical factor in clinical resistance of *S. epidermidis* infections to vancomycin is the production of a biofilm by the bacteria, thereby shielding it from the antibiotic with a consequent reduction of antibiotic efficacy[202, 203]. Furthermore vancomycin is a large molecule, which inhibits diffusion into localized infection sites like endocarditis.

1.2.2. Specific aspects of vancomycin use in neonates

General aspects in neonates

The immunologically incompetent premature neonate is especially susceptible to invasive gram-positive infections through invasive procedures such as central venous lines. Late-onset neonatal septicemia, defined as occurring after the first 4 days of life, is seen in up to 31% of very low birthweight (VLBW) infants[204]. *Staphylococcus aureus* and coagulase-negative staphylococci (CONS) account for up to 55% of late onset nosocomial infections in newborn infants[204-207]. A substantial increase in the number of CONS infections in neonatal units has been reported[208, 209]. Especially VLBW infants have shown an increase in this type of bloodstream infection, associated with length of stay, neonatal risk scores, increased use of central venous lines and administration of parenteral nutrition[210-212]. Late-onset neonatal septicemia has significant impact on outcome and length of hospital stay. Length of stay is prolonged by nosocomial bacteremia by 14-25 days[207, 210, 213]. Mortality in this group is at least twofold higher than in neonates without late-onset sepsis and sepsis accounts for up to 45% of deaths occurring after two weeks of admission[207].

Vancomycin is widely used as empiric antibiotic for treatment of line-related infections in neonates. This glycopeptide antibiotic has been used in pediatric patients, including neonates, since the late 1950's[214]. As in adults, it has come into disuse in the 60's because of side effects. The resurge of interest in the 80's was instigated by the establishment of CONS as a clinical significant pathogen for neonatal septicemia and the emergence of methicillin resistant *S. aureus* and *S. epidermidis* in neonatal intensive care units (NICU's) [215-218]. With the increase of CONS as a cause of late-onset neonatal sepsis, the continuous use of low dose vancomycin or teicoplanin added to parenteral nutrition has been advocated [219-224]. Although a reduction in number of gram-positive infections in preterm infants has been shown, no decrease in mortality or length of stay has been proven[225]. Given the concerns about development of resistance by overuse of vancomycin, routine prophylaxis with low-dose vancomycin should not be given[225, 226]. Frequent administration of vancomycin for intravenous catheter-related infections in neonates will remain necessary however, and knowledge about pharmacokinetic- and dynamic aspects of vancomycin use in neonates is needed to rationalize treatment.

Pharmacokinetics

Vancomycin has a pharmacokinetic profile consisting of a distribution phase, which is longer than in aminoglycosides ($t_{1/2\alpha}$) and an elimination phase ($t_{1/2\beta}$). One, two or three compartment models have been described in adults. It has been suggested that a triexponential model best describes vancomycin disposition[227]. Vancomycin pharmacokinetics in neonates has been described using a model independent[228], one[180, 208, 229-243] or two[233, 238, 244-247] compartment model. A one compartment model seems to be a valid tool in predicting serum concentrations, as long as these are drawn in the post-distribution phase[238]. Since most studies take peak serum concentrations 1h after a 1h infusion this is very likely the case. Earlier serum sampling might lead to an underestimation of the apparent volume of distribution at steady state (V_{ss}).
Pharmacokinetic parameters of vancomycin in neonates are different from those in adults. These differences are largely determined by the change in amount of body water and maturation of renal function, which takes place in term and preterm newborn infants. These changes also result in higher inter-individual differences in neonates than in adults. Results of pharmacokinetic studies of vancomycin in neonates and infants are shown in table 6.

Distribution

Vancomycin is only used intravenously in neonates. Distribution half-life ($t_{1/2\alpha}$) is approximately 0.5-1 hour in adults[227]. In neonates and infants it ranges from 0.05-0.49h, but has only been determined explicitly in one study[245]. Others have suggested that $t_{1/2\alpha}$ might be longer, even up to four hours [238, 246, 247]. Seay et al. calculated $t_{1/2\ \alpha}$ from population parameter values and found values between 2.8 and 3.7h depending on gestational age and dopamine as co-medication [238]. Volume of distribution in steady state (V_{ss}) in neonates ranges from 0.38 to 1.06 L/kg, with the highest V_{ss} described in patients on extracorporeal membrane oxygenation (ECMO). This is in the same range as described in adults[227]. As mentioned by Rodvold[248], volume of distribution studied after a single dose or calculated with the elimination $t_{1/2\beta}$ was often larger than V_{ss}.
Since meningitis often accompanies sepsis in neonates, penetration of vancomycin in cerebrospinal fluid (CSF) is of possible concern. Vancomycin dosing leads to CSF concentrations of 7-21% of the serum concentration in adults [178].

Table 6: Results of pharmacokinetic studies of vancomycin in neonates

N	Subgroup/ Remark	GA	PNA (days)	PCA (week)	BW (grams)	Vd (L/kg)	CL (ml/kg/min)	$T_{1/2\beta}$ (h)	Ref
7	Dose 10 mg/kg	32	3.3		1230	0.736[h]	15[a]	9.8	245
7	Dose 15 mg/kg	34	4.7		1570	0.706[h]	27[a]	5.9	
7	Dose 15 mg/kg	40	2.6		3070	0.690[h]	30[a]	6.7	
3	Weight ≤ 1 kg		29[b]	30[b]	830[b]	0.970±0.426[b]	1.099±0.293	9.92±2.59	242
6	Weight > 1 kg		40[b]	32.7[b]	1378[b]	0.647±0.362[b]	1.030±0.223	5.35±0.77	
11	No means available	27-40	27	29-41	850-4380			3.5-9.6	247
14	PCA < 41	26-40	8-66	32-41		0.481±0.165	1.34±0.46	4.87	236
6	PCA > 43	31	90-210	54.2		0.377±0.036	1.67±0.61	3.04	
6	PDA[c]+indomethacin		7	29.0		0.71±0.36	0.38±0.15	24.6±12.4	249
5	No PDA[c] controls		15	32.0		0.48±0.17	0.90±0.57	7.0±1.8	
20		26.5		36.4	1300	0.693±0.149			229
15	First dose	28.4	21	31.4	1069	0.53±0.13	1.22±0.7[d]	6.0±2.0	228
12	Steady state					0.52±0.1	1.16±0.6[d]	6.6±2.1	
13		29.8[b]	30[b]	38[b]	1375[b]	0.47±0.15[b]	1.44±0.89[b]	5.1±3.0[b]	230
15		29.0	29	33.2	1297	0.48±0.09	1.07±0.34	5.6±1.6	244
11			10[b]	30.9[b]	1262[b]	0.51±0.03[b,h]	0.74±0.20[b]	8.5±2.8[b]	232
4	Exposed to indomethacin	26	18	28.5	810	0.57±0.06	0.6±0.17	11.9±3.7	241
19	No indomethacin	29.3	34	34.2	1780	0.52±0.08	1.2±0.53	5.6±1.6	241
192	NONMEM[e] 2 compartment	29.6	15		1480	0.764±54.1%[e]			238
	NONMEM[e] 1 compartment	27.6	16		1305	0.496±19.3%[e]			
29		31.2	18	33.4	1860	0.551±0.205	1.01±0.37		237
16	PCA 27-30	26.6	18	29.4	972	0.55 ± 0.02	1.00±0.07	6.63±0.35	208
15	PCA 31-36	29.4	23	32.9	1379	0.56 ± 0.02	1.17±0.08	5.59±0.36	
13	PCA ≥37	35.9	24	39.2	2616	0.57 ± 0.02	1.33±0.08	4.90±0.39.	
15	Day 2		90		6400	0.81±0.6	1.5±0.5	5.3±3.2	231
15	Day 8		90		6400	0.44±0.19	1.2±0.4	3.4±1.2	
12	ECMO	39	2[f]		3300	1.06±0.45	0.78±0.19	16.9±9.5	250
11		30.8	18	33.4	1186	0.48 ± 0.13	0.63±0.18		246
15	ECMO	38.8	13		3100	0.45±0.18	0.65±0.28	8.29±2.23	240
15	No ECMO	39.7	8		3400	0.39±0.12	0.79±0.41	6.53±2.05	
24	Standard dose	29.2	30	33.5	1500		1.19±0.55		180
29	Adjusted dose	30.5	24	33.9	1800	0.61±0.39	0.99±0.41		
72	Development algorithm	29.4	26	32.9		0.65±0.34	1.26±0.55	6.9±4.5	235
17	Testing algorithm	28.4	39	32.0		0.67±0.28	1.40±0.67	6.5±3.3	
59	NONMEM analysis	29	19	32	1520	0.669±18%[e]			233

[a]ml/min/1.7 m^2, [b]calculated from individual values for all patients mentioned in the article, [c]Population mean ± interindividual variability, [d]ml/min, [e]Patent ductus arteriosus, [f]age when put on ECMO, [g]Population analysis, [h]Apparent volume of distribution of beta phase, GA = gestational age (weeks), PNA = postnatal age, BW = birthweight, N = number of patients

In children Spears reported CSF concentrations of <0.8 mg/L in seven samples 1-12h after the vancomycin dose[214]. In infants the first report by Schaad et al. found CSF concentrations of 7-21% of the serum concentration in three patients[245]. Later reports have mentioned CSF concentrations ranging from 0.2 to 17.3 mg/L, with vancomycin CSF penetration ranging from 7.1 to 68%[251-254]. No clear relation of CSF concentrations to serum concentrations was found. As in adults, there is a significant correlation between CSF concentration and markers for meningeal inflammation [167, 253]. Data on this subject are scarce, however, and vancomycin can not be relied upon to adequately treat gram-positive meningitis when given as the sole antibiotic.

Excretion

Vancomycin is eliminated from the body by way of glomerular filtration. After 24h 80-90% of an administered dose can be recovered from urine in adults[178]. A small amount is eliminated by non-renal mechanisms of unknown origin[255]. In neonates 44% of vancomycin was recovered unchanged after 8h[228]. Total body clearance (CL) in adults (0.71-1.31 ml/kg/min) is often higher than that reported in neonates and infants, although ranges are similar[256-259].

In neonates CL ranges from 0.63 to 1.5 ml/kg/min, depending on gestational age (GA) and/or postconceptional age (PCA) (table 6). Lower clearances were seen in special subpopulations, which will be described later. Vancomycin $t_{1/2\beta}$ in adults ranges from 4-8h in patients with normal renal function. Mean $t_{1/2\beta}$ in neonates of varying gestational and postconceptional ages ranges from 3.5-10h, with even longer half-lives in neonates exposed to indomethacin or ECMO treatment, which will be discussed in more detail below.

Given the route of elimination, an association between glomerular filtration rate (GFR) and excretion is logical. In adults vancomycin clearance was directly related to renal function, with a vancomycin clearance of approximately 100 ml/min in patients with normal renal function[257, 260-263]. In neonates this relation has been established as well. Serum creatinin and creatinin clearance have been correlated to clearance of vancomycin in several studies (table 7)[180, 229, 232-234, 237, 239, 244, 247]. In term and preterm infants vancomycin clearance indexed for weight shows a negative correlation with serum creatinin [229, 232, 234, 244, 248]. Pawlotsky et al. found vancomycin clearance (ml/kg/min) to be inversely related to serum creatinin, though the significance was lost in the multivariate analysis [180].

Kildoo and co-workers found a difference in vancomycin clearance between patients with serum creatinin <53 μMol/L or 62-106 μMol./L; respectively 1.38±0.24 vs 0.79±0.10 ml/kg/min [244]. Schaible also found a negative correlation. In a study of 11 infants vancomycin clearance (L/h) was best described by a combination of PCA and the reciprocal of serum creatinin ($1/Cr_S$)[247]. A recent study found a negative, but nonlinear relation in a population analysis of 59 neonates. Serum creatinin was the sole covariate included in the final analysis, and dose recommendations were purely based on this factor[233]. Rodvold et al. as well as Silva et al. showed a positive relation between creatinin clearance calculated according to Schwartz and vancomycin CL (ml/kg/min) [239, 248, 264]. In the latter study the authors concluded that creatinin clearance was not an important covariate in explaining vancomycin clearance.

Vancomycin $t_{1/2\beta}$ displays a positive correlation with serum creatinin[229, 232]. The largest study to date, performed in 192 neonates, did not include creatinin or creatinin clearance as a covariate in the model[238]. Clearance found in this study (0.29-0.98 ml/kg/min) was lower than reported in most studies, possibly due to the larger number of VLBW infants, sampling strategy or use of the NONMEM statistical approach.

In the case of terminal renal failure, vancomycin clearance by way of hemodialysis and/or peritoneal dialysis is slow with doses of 15 mg/kg leading to trough serum concentrations of >4-5 mg/L after 7 days in adults [265]. A significant increase of vancomycin clearance can be achieved with continuous veno-venous hemodiafiltration [266].

Taken together, the published evidence favors a clear relation between renal function in terms of serum creatinin or creatinin clearance and excretion of vancomycin.

Effect of gestational age, postnatal age and postconceptional age on pharmacokinetic parameters of vancomycin

Gestational age, postnatal age and postconceptional age can all be expected to alter pharmacokinetics of vancomycin. As mentioned in the chapter about aminoglycosides, the volume of distribution (Vd) of most drugs is larger in neonates, especially in prematures, primarily due to a higher percentage of extracellular water[85, 86]. Creatinin clearance (ml/min) shows a positive correlation with gestational age[82, 267]. On the basis of gestational age, premature neonates are expected to have a longer $t_{1/2\beta}$. The postnatal increase in GFR seen in neonates as well as the reduction of extracellular fluid[91-93] means that $t_{1/2\beta}$ for vancomycin should decrease with increasing postnatal age. There is also a positive relation between postconceptional age and kidney function [267].

Table 7: Correlations between demographic variables and pharmacokinetic parameters

Correlation	Coef.	p-value	Ref	Correlation	Coef.	p-value	Ref
Excretion (not standardized for weight)				*Excretion (standardized for weight)*			
CL (L/h) ⇔ GA	0.71	<0.0009	241	CL (ml/kg/min) ⇔ PCA	0.724	<0.005	234
CL(L/h) ⇔ GA	0.59	<0.01	237	CL (ml/min) ⇔ PCA	0.46[1]	0.0002	180
CL(L/h) ⇔ GA	0.48	<0.05	239	CL (ml/min) ⇔ PCA	0.74[1]	0.0001	180
CL(ml/min) ⇔ GA	0.54	<0.05	244	CL (ml/min) ⇔ PCA + BW	0.52[1]	0.0005	180
CL(L/h) ⇔ PNA	0.54	<0.01	237	CL(L/kg/h) ⇔ PCA	0.62	<0.0005	241
CL(ml/min) ⇔ BW	0.82	<0.05	244	CL(L/kg/h) ⇔ PNA	0.50	<0.008	241
CL(ml/min) ⇔ BW	0.38[1]	0.0013	180	CL(L/kg/h) ⇔ PNA	0.46	<0.05	239
CL(ml/min) ⇔ BW	0.67[1]	0.0001	180	CL(ml/kg/h) ⇔ BW	0.867	<0.00001	230
CL(ml/min) ⇔ BW[a]	0.83	<0.001	228	CL(ml/kg/h) ⇔ PCA	0.863	<0.00002	230
CL(ml/min) ⇔ BW[b]	0.89	<0.001	228	CL(ml/kg/h) ⇔ PCA	0.649	<0.001	236
CL(L/h) ⇔ BW	0.85	<0.0001	241	CL(ml/kg/h) ⇔ PNA	0.873	<0.00001	230
CL(L/h) ⇔ BW	0.90	<0.01	237	CL(ml/kg/min) ⇔ BW	0.78	<0.001	229
CL(L/h) ⇔ BW	0.68	<0.05	239	CL(ml/kg/min) ⇔ BW	0.62	<0.01	237
k(1/h) ⇔ BW	0.464	<0.04	236	CL(ml/kg/min) ⇔ PCA	0.8	<0.001	229
PCA ⇔ BW	0.89		233	CL(ml/kg/min) ⇔ PCA	0.41	<0.05	244
$T_{1/2}$ ⇔ BW	-0.88	<0.0005	229	CL(ml/kg/min) ⇔ PCA	0.48	<0.005	208
CL(ml/min) ⇔ BSA[a]	0.84	<0.001	228	CL(ml/kg/min) ⇔ PCA	0.27[1]	0.0094	180
CL(ml/min) ⇔ BSA[b]	0.89	<0.001	228	CL(ml/kg/min) ⇔ PCA	0.22[1]	0.01	180
CL(ml/min) ⇔ PCA + BW	0.77[1]	0.0001	180	CL(ml/kg/min) ⇔ PCA	0.62	<0.01	237
CL(ml/min) ⇔ PCA	0.88	<0.05	244	CL(ml/kg/min) ⇔ PNA	0.70	<0.01	237
CL(ml/min) ⇔ PCA	0.81	<0.00001	208				
CL(ml/min) ⇔ PCA		0.02	180				
CL(ml/min) ⇔ PCA		0.002	180				
CL(ml/min) ⇔ PCA[a]	0.56	<0.05	228				
CL(ml/min) ⇔ PCA[b]	0.62	<0.05	228				
CL(L/h) ⇔ PCA	0.92	<0.0001	241				
CL(L/h) ⇔ PCA	0.86	<0.01	237				
CL(L/h) ⇔ PCA	0.57	<0.05	239				
CL(L/h) ⇔ PCA	0.91	<0.0001	247				
$T_{1/2}$ ⇔ PCA	-0.91	<0.0001	229				
$T_{1/2}$ ⇔ PCA	-0.627	<0.01	234				

CL= vancomycin clearance, PCA= postconceptional age, BW=body weight, Cr_s= serum creatinin, [1]=r^2
GA= gestational age, V_{ss} = volume of distribution in steady state, PNA = postnatal age, CL_{cr} = creatinin clearance, K_{el} = elimination rate constant, BSA= body surface area, [a]first dose, [b]steady state, coef. = correlation coefficient

Table 7 (continued): Correlations between demographic variables and pharmacokinetic parameters

Correlation	Coef.	p-value	Ref	Correlation	Coef.	p-value	Ref
Distribution (L)				*Renal function*			
V_{ss}(L) ⇔ BSA	0.93	<0.0001	241	V_{ss}(L) ⇔ CL_{cr}(ml/kg/min)	0.70	<0.01	237
V_{ss}(L) ⇔ BSA[a]	0.80	<0.001	228	CL(L/h)⇔CL_{cr}(ml/kg/min)	0.86	<0.01	237
V_{ss}(L) ⇔ BSA[b]	0.89	<0.001	228	CL(L/h) ⇔ CL_{cr}(ml/kg/min)	0.27	<0.05	239
V_{ss}(L) ⇔ BW	0.94	<0.0001	241	CL(ml/kg/min)⇔ CL_{cr}(ml/kg/min)	0.59	<0.01	237
V_{ss}(L) ⇔ BW	0.86	<0.05	244	CL(L/kg/h) ⇔ CL_{cr}(ml/kg/min)	0.31	<0.05	239
V_{ss}(L) ⇔ BW	0.86	<0.01	237	V_{ss}(L/kg) ⇔ Cr_s	0.47	<0.01	237
V_{ss}(L) ⇔ BW	0.93	<0.05	239	V_{ss}(L) ⇔ Cr_s	-0.40	<0.05	237
V_{ss}(L) ⇔ BW[a]	0.77	<0.001	228	CL(L/h) ⇔ Cr_s	-0.65	<0.01	237
V_{ss}(L) ⇔ BW[b]	0.89	<0.001	228	CL(ml/kg/min) ⇔ Cr_s	-0.74	<0.005	229
V_{ss}(L) ⇔ GA	0.84	<0.05	244	CL(ml/kg/min) ⇔ Cr_s	-0.81	0.0027	232
V_{ss}(L) ⇔ GA	0.61	<0.01	237	CL(ml/kg/min) ⇔ Cr_s	-0.82	<0.05	244
V_{ss}(L) ⇔ GA	0.58	<0.05	239	CL(ml/kg/min) ⇔ Cr_s	-0.64	<0.01	237
V_{ss}(L) ⇔ PCA	0.89	<0.0001	241	CL(ml/min) ⇔ Cr_s	-0.35[1]	0.0165	180
V_{ss}(L) ⇔ PCA	0.67	<0.05	244	CL(ml/min) ⇔ Cr_s	-0.49[1]	0.0001	180
V_{ss}(L) ⇔ PCA	0.79	<0.00001	208	$T_{1/2}$ ⇔ Cr_s	0.91	<0.0001	229
V_{ss}(L) ⇔ PCA	0.80	<0.01	237	$T_{1/2}$ ⇔ Cr_s	0.84	0.0012	232
V_{ss}(L) ⇔ PCA	0.76	<0.05	239	$T_{1/2}$ ⇔ Cr_s	0.725	<0.01	234
V_{ss}(L) ⇔ PCA[a]	0.53	<0.05	228	Cr_s ⇔ PCA	-0.62	<0.01	229
V_{ss}(L) ⇔ PCA[b]	0.62	<0.05	228	CL(L/h) ⇔ BW÷1/Cr_s	0.96	<0.01	247
V_{ss}(L) ⇔ PNA	0.41	<0.05	237				
Distribution (L/kg)							
V_{ss}(L/kg) ⇔ GA	0.29	<0.05	239				

CL= vancomycin clearance, PCA= postconceptional age, BW=body weight, Cr_s= serum creatinin, [1]=r^2
GA= gestational age, V_{ss} = volume of distribution in steady state, PNA = postnatal age, CL_{cr} = creatinin clearance, K_{el} = elimination rate constant, BSA= body surface area, [a]first dose, [b]steady state, coef. = correlation coefficient

All three factors have been related to vancomycin pharmacokinetics in neonates (table 7). A significant relation between unstandardized vancomycin CL (ml/min or L/h) and gestational age has been noted, but in all of these studies significance disappears when clearance is normalized for body weight[237, 239, 244]. The same studies have described V_{ss} in relation to gestational age and again significance disappears in all but one study when V_{ss} is described in L/kg [239]. This implies that if weight is incorporated, GA is not an important determinant of vancomycin V_{ss} or CL. Several authors demonstrated the relation between PNA and standardized clearance (ml/kg/min) or V_{ss} (L) [230, 237, 239, 241]. No correlation was found by many others [208, 228, 232, 236, 244, 247]. V_{ss} (L) but not standardized V_{ss} (L/kg) has been related to PNA [237, 244]. Postconceptional age has been well described in relation to pharmacokinetic parameters for vancomycin. Unstandardized CL (ml/min) [180, 208, 228, 237, 239, 241, 244, 247] and standardized CL (ml/kg/min) [180, 208, 229, 230, 234, 236, 237, 241, 244] has been related to PCA. A concomitant change in $t_{1/2\beta}$ has been noted as well [229, 234]. As with PNA, only unstandardized V_{ss} (L) has a significant correlation with PCA[208, 228, 237, 241, 244]. The diminished influence of GA and PNA can be explained by several factors. First the combined effects of GA and PNA are integrated in PCA. Although a stronger increase in renal function in term infants has been described earlier, the frequent prenatal exposure of neonates to corticosteroids seen over the last 5-10 years might mitigate the difference between term and preterm neonates, and therefore limit the effect of GA on clearance [91]. Furthermore postnatal increase of GFR seems to be higher than intra-uterine increase [99]. At the same PCA, this might imply that the effect of slower maturation of kidney function in prematures is cancelled out by the difference in intra- and extrauterine development of GFR. A third and maybe more important factor is that vancomycin is seldom given in the first week of life. Since a large increase of kidney function in neonates takes place in this period, the dynamics of these changes and their influence on vancomycin pharmacokinetics are not seen in the studies mentioned here.

In most studies where both the influence of PCA as well as PNA were studied significance of PCA outweighed that of PNA [237, 239, 241]. These data suggest that clearance in relation to postconceptional age is the main determinant in the pharmacokinetic profile of vancomycin in neonates.

Effect of patent ductus arteriosus, indomethacin and extracorporeal membrane oxygenation

Prenatal as well as postnatal exposure to indomethacin has been shown to negatively affect increase of kidney function in neonates [91, 268, 269]. Open ductus Botalli can increase V_{ss} and decrease CL in neonates[99, 270]. Several studies have addressed the effect of indomethacin treatment of open ductus Botalli on vancomycin pharmacokinetics in newborns [239, 241, 249]. Asbury described 4 patients exposed to indomethacin and compared them to 19 with no exposure (table 6) [241]. Clearance was half that of the no indomethacin group, with a concomitant change in $t_{1/2\beta}$. The authors concluded however, that only in one patient the decrease of renal function could be attributed to indomethacin. Spivey et al. outlined a study in 11 neonates of whom 6 were exposed to indomethacin for closure of open ductus Botalli (table 6) [249]. Volume of distribution was higher and clearance substantially lower in the indomethacin group resulting in a $t_{1/2\beta}$ of more than 24h. No specifics about peak sampling were given and the non-indomethacin group had substantially higher GA and PNA. Therefore a clear relation with indomethacin treatment could not be ascertained. Silva et al. outlined a study in 44 patients; 26 received concomitant treatment with indomethacin and/or mechanical ventilation [239]. In these 26 patients clearance was lower than in the other 18 (0.07 vs 0.086 L/kg/h). Although a definitive conclusion can not be made on the grounds of these data, they suggest that indomethacin treatment of open ductus Botalli leads to an increase of V_{ss} and a decrease of CL, warranting extra therapeutic drug monitoring in these patients.

Hoie and others were the first to describe vancomycin pharmacokinetics in 6 patients on ECMO[271]. Values for V_{ss} (0.68±0.12), CL (1.10±0.32 ml/kg/min) and elimination half-life (7.71±2.61) were not different from values in the literature for patients without ECMO. Amaker and Bhatia studied 12 term neonates with a PNA of 0-6 days on ECMO. These patients had a CL of 0.78±0.19 ml/kg/min, a V_{ss} of 1.06±0.45 L/kg and a $t_{1/2\beta}$ of 16.9±9.5h. Clearance was lower than that seen in other groups of patients with a PCA>37 weeks and V_{ss} is higher [250]. Creatinin in these patient was relatively high though with values ranging from 53 to 168 µmol/L. Buck et al. did a case-control study in 30 patients, of which 15 were on ECMO[240]. Patients were matched with historical controls for underlying disease and several other clinical factors. Renal function expressed in terms of serum creatinin was worse in the ECMO group (0.8±0.1 vs 0.6±0.2 µmol/L). Patients on ECMO had a mean GA of 38.8 weeks and a mean PNA of 12.7 days. ECMO patients had a slight, but significant higher half-life (8.29 vs 6.53h) and lower elimination rate

constant (K_el; 0.09 vs 0.12 h^{-1}). Although these studies were relatively small and results were somewhat obscured by differences in renal function a longer half-life in vancomycin treated neonates on ECMO is likely.

Microbiological and clinical susceptibility and efficacy

Vancomycin is still widely used as the first-choice antibiotic for treatment of CONS infections in neonates. This choice is mostly based on the in-vitro bactericidal activity of this antibiotic against gram-positive infections. A second reason is that emergence of vancomycin resistant pathogens in the NICU is slow in contrast to cephalosporin induced resistance[108]. Data on clinical efficacy in adults are scarce. There is no correlation between serum vancomycin concentrations and clinical cure. Regimes associated with peak and trough concentrations ranging from 18-47 μg/mL and 2-13 μg/ml, respectively, showed acceptable rates of effectiveness, but failures in these treatment groups had the same serum concentrations[272]. Serum bactericidal titers (SBT) of more than 1:8 are related to treatment success and high minimal bactericidal titers to MIC ratio's to treatment failure, but exact information pertaining to efficacy does not exist[273, 274]. Fixed doses of 1g every 12 hours or 7.5 mg/kg every 6 hours have been documented to be effective against staphylococcal and streptococcal infections[265]. Information in neonates is also difficult to find. The first study describing vancomycin use in 23 children, described 6 infants with staphylococcal infections, of whom 4 were cured with doses of 40-180 mg/kg/day (i.v. or i.m.)[214]. An early study by Schaad et al. evaluated the susceptibility of 20 strains of S. aureus and 6 strains of *S. epidermidis* from a group of 55 neonates, infants and children. Except for one tolerant strain of *S. aureus* an SBT of 1:8 or greater was observed with vancomycin concentrations of ≥ 12 mg/L.

A further study by the same authors detailed antibiotic treatment of 33 patients of whom 10 were neonates and 11 were infants[254]. Indications for treatment were septicemia, shunt infections, pneumonia, abscesses, fasciitis and cellulitis. In 29 out of 33 patients *S. aureus* or *S. epidermidis* was cultured. Eleven of 33 patients received co-treatment with another antibiotic. Serum concentrations were 18.4-57.1 mg/L (peak) and 3.1-18.8 mg/L (trough). The relation between these concentrations and antistaphylococcal activity were evaluated for 21 patients. In one patient with recurrent septicemia by a tolerant strain of *S. aureus* no bactericidal activity was seen, all other samples had bactericidal titers of 1:8-1:32 against the isolated pathogen. In all 19 patients who were re-cultured, the causative pathogen was eradicated. All but one of 33 patients improved clinically. This patient, a 12-year old girl,

had possible brucellosis. No details on which of these patients were neonates or infants were given. In a study of 17 neonates and infants (14 patients with *S. aureus* and 3 with *S. epidermidis* infections), Naqvi et al. reported clinical success in 16/17 patients[236]. All patients had been pretreated with aminoglycosides. The one failure was a patient with recurrent endocarditis due to a vancomycin-tolerant *S. aureus*. Mean peak and trough serum concentrations in these patients ranged from 30.4- 57.8 mg/L and 9.5-15.1 mg/L, respectively.

Lisby-Sutch and others described 11 patients in whom bactericidal titers were determined for infecting organisms[230]. Serum inhibitory titers were ≥1:8 in 10/11 peak serum samples, with peaks ranging from approximately 10-45 mg/L. In the study by Reed et al., in which infants were treated for S. *epidermidis* sepsis, 14 out of 15 patients showed clinical recovery, with initial peaks ranging from 18.8-73.3 mg/L and trough from 5.1-38 mg/L, respectively. No details about susceptibility were given and all patients were pretreated with an aminoglycoside and a β-lactam antibiotic. Pawlotsky et al. showed that continuous infusion of vancomycin was effective in 13 documented invasive infections with concentrations ranging from 3-37.6 mg/L[180]. There are no definitive data relating serum concentrations to effect. These studies, with relative few numbers of patients, show that a wide range of vancomycin peak and trough concentrations are effective against gram-positive infections in neonates and infants. But, on a critical note, these results do not validate the therapeutic range of peak concentrations of 20-40 mg/L and trough concentrations of 5-10 mg/L often mentioned in the literature.

Choice of antibiotic and drug resistance

Enterococci are a related genus of gram-positive catalase-negative cocci [275]. They are a pathogen known to cause outbreaks of disease in NICU's [276-278]. Vancomycin resistant enterococci (VRE) infections in neonates can be accompanied with an increase of mortality[279]. Vancomycin use is a consistent risk factor for colonization and infection with vancomycin-resistant enterococci[280]. Emergence of VRE has become a major infection control problem, especially in the United States. In the Netherlands colonization with VRE of hospitalized patients seems to be relatively low [281]. Vancomycin is mostly used in the setting of late onset (occurring after 3 days of age) sepsis. The choice is made on the basis that CONS are the most common pathogens in this period [282, 283]. CONS is not however associated with fulminant late-onset septicemia in neonates. The mortality in 277 neonates with late-onset CONS septicemia was 1% in contrast to late-onset gram negative

septicemia, which had an associated mortality of up to 56% [282]. This has implications for choices of empiric antibiotic treatment. Several authors have shown prophylactic use of low doses of vancomycin or teicoplanin to prevent late-onset septicemia in VLWB infants[219-222, 224, 284, 285]. An increase of VRE was not reported, but the potential risk of development of vancomycin resistance in NICU's through injudicious use of vancomycin has been pointed out[221, 222, 282, 286]. Given the overall concern about vancomycin resistance, vancomycin prophylaxis does not seem to be warranted at this time. Alternative antibiotics for empiric treatment are effective in the initial treatment of late-onset sepsis neonates. Cephalosporins and β-lactam antibiotics have been effectively used as empiric treatment for gram-positive infections, with a switch made to vancomycin when methicillin resistance was determined[282, 287, 288]. The postponed use of vancomycin was not associated to treatment failure or increased mortality in these patients.
A highly selective use of vancomycin seems to be justified by these studies.

Toxicity

Toxicity related to vancomycin use has been the subject of numerous reports. Complications include Red man syndrome, neutropenia, thrombocytopenia, eosinophilia, trombophlebitis, chills, fever, rash, nephrotoxicity and ototoxicity. Three cases of cardiac arrest (two fatal) associated with rapid infusion of vancomycin have been described[214, 289, 290]. The most frequent problem encountered was the Red man syndrome, a histamine mediated rash of the face, neck, upper trunk, back and arms. This phenomenon, associated with pruritus, tingling flushing, tachycardia and shock, is related to the rate of infusion [170]. It has been described in neonates and children, related to an infusion rate of < 1h by Schaad et al., but also in 7 out of 20 patients with infusion rates of 1h by Odio[245, 252]. The incidence of most of these side effects has decreased enormously with the removal of impurities from early preparations in the 60's. Nephro-and ototoxicity will be described in more detail.

Nephrotoxicity

Vancomycin can cause reversible nephrotoxicity in man and has been studied extensively. Animal models have failed to demonstrate significant nephrotoxicity when vancomycin was given alone[291, 292]. Vancomycin can enhance aminoglycoside induced renal toxicity in animals and possibly in humans[161, 293]. The incidence mentioned in adults varies. Some

studies did not detect any toxicity with vancomycin monotherapy [294]. In other studies in adults nephrotoxicity ranges from 5% in patients receiving vancomycin alone to 18% in patients without control for other variables possibly influencing toxicity [272, 293, 295]. It has been related to trough concentrations >10 mg/L, but in most studies it remains unclear whether elevated serum trough concentrations are the cause or consequence of renal failure[295, 296]. Nephrotoxicity has been studied in several groups of neonates, though seldom explicitly. Many studies could not detect any nephrotoxicity [232, 236, 245, 247, 254, 297]. These studies total 61 patients treated from 4 to 28 days, with serum concentrations ranging from 1.9 to 92.5 mg/L. The earlier studies by Schaad and co-workers showed no difference between baseline and post-treatment serum urea and/or creatinin in 20 neonates infants and children [245, 254]. Jarrett et al. did not find clinical or biochemical signs of renal failure in 11 patients in whom a baseline creatinin was determined [232]. In another study 3 out of 12 VLWB infants had a rise of serum creatinin of more than 0.3 mg/dl [234]. In 2 out of 3 patients serum creatinin normalized within days of stopping treatment, the third patient died but obduction did not reveal renal abnormalities consistent with drug related nephrotoxicity. Gous et al. could not demonstrate a rise in mean serum creatinin in 15 infants between day 0,2 and 8 of treatment [231]. Three of these patients had an increase of more than 50% of serum creatinin, but all three were also exposed to aminoglycosides, as was the patient described in a case-report [298]. A case report showed that a vancomycin induced rise of serum creatinin in 2 children normalized after adjusting vancomycin concentrations to the therapeutic range. Naqvi and associates did not find evidence of renal toxicity in 17 neonates and infants treated for 10-42 days; all patients were also exposed to aminoglycosides[236]. Twenty VLBW infants displayed no change in serum creatinin or tubular function during vancomycin treatment (4-13 days)[297].

In a study using continuous infusion of vancomycin only one neonate out of 53 showed a reversible increase of serum creatinin [180]. The effect of simultaneous use of vancomycin and an aminoglycoside was prospectively evaluated in 61 infants[299]. No evidence of renal toxicity in terms of serum creatinin or urinalysis abnormalities was seen. Finally, Bhatt-Mehta et al. looked at the effect of peak serum concentrations on renal function in neonates with a mean PCA of 32.4 weeks[300]. Patients were divided in two groups, 61 patients with peak serum concentrations $\leq$ 40 mg/L and 8 patients with peaks > 40 mg/L. Nephrotoxicity was defined as a doubling of serum creatinin and was not seen in the group with high peak serum concentrations. In the other 61 patients this was noted 6 times, but a doubling of serum creatinin to values of >53 μmol/L was only seen

in 3 patients. Interestingly, peak concentrations > 40 mg/L were only seen in neonates with a baseline serum creatinin of >53 μmol/L (0.6 mg/dl). This association was also found for trough concentrations > 10 mg/L.
The overall conclusion from this information is that vancomycin induced nephrotoxicity in neonates is rare, reversible, and there is no clear relation to serum concentrations.

Ototoxicity

Information on vancomycin ototoxicity is scarce. Vancomycin is said to be potentially vestibulo- and cochleotoxic[301]. Tinnitus seems to precede hearing loss. As with aminoglycosides, hearing loss is more pronounced in the high frequency range (8-16 kHz) [293]. There are animal studies relating ototoxicity to vancomycin in combination with an aminoglycoside, but little evidence for ototoxicity of vancomycin alone[302, 303]. The first report of vancomycin related ototoxicity in humans was in 1958[166]. Reported incidence of ototoxicity in adults is fewer than 2% [272]. Reports on ototoxicity are fraught with methodological problems. Most studies were retrospective and included patients who had been exposed to other ototoxic medication, mainly aminoglycosides[301].
A relation between vancomycin related ototoxicity and serum concentrations could not be demonstrated from available literature[293]. A confounding factor, as in nephrotoxicity is, that the time of serum sampling in relation to dose is not always mentioned, which clouds interpretation of these serum concentrations. Data on vancomycin ototoxicity in neonates are almost non-existent. Neonates born to mothers who received vancomycin in the second or third trimester of pregnancy did not show hearing loss[304]. Brainstem evoked response audiometry and behavioral audiometry did not demonstrate any ototoxicity in 12 neonates and children[254]. One case report described a repeated accidental overdose in a 47 day old premature[305]. Although serum concentrations were in excess of 100 mg/L for 4 days, no hearing loss was found with brainstem evoked response audiometry during follow-up. Hearing loss in humans exposed to vancomycin is sporadic and no clear relation to serum concentrations or patterns of underlying illness can be detected. Although the absence of case reports of vancomycin induced hearing loss in neonates suggests that this is very uncommon, data in neonates are insufficient to form any conclusion on the relationship between vancomycin and ototoxicity.

Vancomycin dosing and dose interval

As in other drugs, vancomycin dose and dosing interval are determined by its desired therapeutic range and pharmacokinetic properties. Historically, vancomycin dosing has been titrated to obtain peak serum concentrations between 20-40 mg/L and serum trough concentrations of 5-10 mg/L. There is little scientific evidence for both ranges. The upper limit of 40 is based on the fact that the earliest study, describing ototoxicity with peak concentrations > 80 mg/L suggested that peaks should not exceed 50 mg/L[166]. As described before, there is no clear relation between oto- or nephrotoxicity and serum concentrations. Also, there is no microbiological or clinical evidence for increased effectiveness of vancomycin at advised peak concentrations. The lower limit of the range for trough concentrations seems to be reasonable. Susceptibility of most micro-organisms for which vancomycin is used is <1-2 mg/L. With a maximal protein binding of 50%, this means that vancomycin trough concentrations will have to exceed 4 mg/L to stay above the MIC[306]. Although there are some reports relating nephrotoxicity to trough serum concentrations > 10 mg/L, there is insufficient evidence to rigidly adhere to this goal[295]. Nevertheless, these desired ranges of concentrations have been the goal of dosing regimens advised in neonates and infants. The first report on vancomycin dosing in children used doses of 25-180 mg/kg/day, with the highest dose used in an infant[214]. Following this first report many dosing regimens, related to PNA, PCA, bodyweight or serum creatinin, have been defined (table 8). The first dosing advice, based on pharmacokinetic studies in 21 infants, related dose to PNA[245]. The advise of 10 mg/kg q 6h for infants older than 30 days was evaluated by Gous et al[231]. Despite the large interindividual differences most serum concentrations were within the desired range. Three out of 15 steady state trough concentrations were lower than 5 mg/L, though only one was below 4 mg/L. This study also demonstrated important changes in pharmacokinetic parameters between day 2 and 8 of treatment in the same patient, possibly related to a normalization of physiological changes occurring with septicemia. Alpert et al. studied vancomycin dosing in 44 infants and children and used doses of 10-15 mg/kg with an interval of 6-12h[243]. Trough serum concentrations were relatively high, especially when using a 6h interval. The guidelines based on this paper, though not mentioned in the article itself, were evaluated in the same institution in a group of 11 infants[247]. Results of this study suggested reducing vancomycin doses in the first two months of life, though no specific advice is given by the authors.

Table 8: Recommended dosing regimens in neonates and infants

PNA (days)	PCA (weeks)	BW (grams)	Serum creatinin (μmol/L)	Target peak/ trough	Dose (mg/kg)	Interval (h)	Reference
<7				25-40/<10	15	12	Schaad, 1980[245]
8-30					15	8	
> 30					10	6	
> 30 and CNS infection					15	6	
≥14	29-35	<1000		25-40/2-12	25 LD, 15*	12	Gross, 1985[242]
≥14	29-35	>1000		25-40/2-12	12.5 LD, 10*	12	Gross, 1985[242]
	<41			25-30/<10	15 LD, 10*	8	Naqvi, 1986[236]
	>43			25-30/<10	15 LD, 10*	6	Naqvi, 1986[236]
	< 27	<800		30/6	27	36	James, 1987[229]
	27-30	800-1200			24	24	James, 1987[229]
	31-36	1200-2000			18-27	12-18	James, 1987[229]
	≥37	>2000			22.5	12	James, 1987[229]
	≤36			25-35/5-10	10	12	Reed, 1987[228]
	30-34	<1200		25-35/5-10	10	12	Lisby-Sutch,1988[71]
	30-34	>1200		25-35/5-10	10	8	
	35-42	>1200		25-35/5-10	10	8	
	>42	>1200		25-35/5-10	10	6	
	25-32	< 1000		25-40/<10	15	24	Leonard, 1989[234]
>14	>30		≤0.6		10	8	Kildoo, 1990[244]
>14	>30		0.7-1.2		10	12	
≤7	<30			20-40/<10	15	24	Gabriel, 1991[308]
>7	<30		≤1.2		10	12	
≤ 14	30-36				10	12	
>14	30-36		≤0.6		10	8	
>14	30-36		0.7-1.2		10	12	
≤7	>36				10	12	
>7	>36		≤0.6		10	8	
>7	>36		0.7-1.2		10	12	
	<27	<800		25-35/5-10	18	36	McDougal,1995[208]
	27-30	800-1200			18	24	
	31-36	1200-2000			18	18	
	>36	>2000			15	12	

PNA= postnatal age, PCA= postconceptional age, BW = bodyweight, LD= loading dose,* maintenance dose,** maintenance dose per day, [1]Serum creatinin in mg/dl, [2]no indomethacin and/or mechanical ventilation, [3]indomethacin and/or mechanical ventilation

Table 8 (continued): Recommended dosing regimens in neonates and infants

PNA (days)	PCA (weeks)	BW (grams)	Serum creatinin (μmol/L)	Target peak/ trough	Dose (mg/kg)	Interval (h)	Reference
	≤32[2]				12.5	12	Silva, 1998[239]
	≤32[3]				10	12	
	>32[2]				10	8	
	>32[3]				7.5	8	
7-30		<1000			10	18	Schaible, 1986[247]
		1000-2000			10	12	Alpert, 1983[243]
		>2000			10	8	
31-60					10	6	
>60					10	6	
	25-26				7 LD, 10**	Continuous	Pawlotsky, 1998[180]
	27-28				7 LD, 12**	Continuous	
	29-30				7 LD, 15**	continuous	
	31-32				7 LD, 18**	continuous	
	33-34				7 LD, 20**	continuous	
	35-36				7 LD, 23**	Continuous	
	37-38				7 LD, 26**	Continuous	
	39-40				7 LD, 29**	Continuous	
	41-42				7 LD, 31**	Continuous	
	43-44				7 LD, 34**	Continuous	
	>45				7 LD, 40**	Continuous	
			20-29		20	8	Grimsley, 1999[233]
			30-39		20	12	
			40-49		15	12	
			50-59		12	12	
			60-79		15	18	
			80-100[1]		15	24	
			>100		15	Depending on trough	

PNA= postnatal age, PCA= postconceptional age, BW = bodyweight, LD= loading dose,* maintenance dose,** maintenance dose per day, [1]Serum creatinin in mg/dl, [2]no indomethacin and/or mechanical ventilation, [3]indomethacin and/or mechanical ventilation

Serum creatinin was higher than 53 μmol/L in 7 patients, which might have skewed results towards this conclusion.

Gross et al. suggested a dosing regimen based on neonates weighing either more or less than 1000 grams[242]. This advice was based on data in 9 prematures, of whom only 3 were < 1000 grams. One of these three had a high serum creatinin of 80 μg/ml, so results for this group are doubtful. A loading dose of 15 mg/kg followed by 10 mg/kg with an PCA-related interval based on average V_{ss} and K_{el} was advised by Naqvi and co-workers following a pharmacokinetic study in 20 neonates[236]. The group of patients with a PCA greater than 43 weeks comprised only 6 patients. James et al. proposed a very detailed PCA-defined dosing regimen, after finding an excellent correlation between $t_{1/2\beta}$ and PCA in 20 preterm infants[229]. When looking closely at the graphs, it is obvious that there were only two patients each in the PCA groups of < 27 and > 37 weeks, which undermines the validity for these PCA groups. In a subsequent study by the same authors they showed that using their dose recommendation in preterms improved the chance of achieving serum concentrations within the therapeutic range over the dosing regimen as proposed by Schaad et al[245, 307].

In a third study by the same group, the authors studied 12 infants weighing less than 1000g and revised the original advise of James et al. to use vancomycin once daily in this group[234]. Vancomycin was given once daily to 10 of these infants and doses varied between 9.4 and 27.3 mg/kg. Peak concentrations were adequate (32.6±9.3 mg/L), but trough concentrations were low (5.7±4.5 mg/L). Both the original as well as the revised regimen by James, was tested in a later study in 44 neonates[208]. Individual V_d and K_{el} per patient were used to simulate the original and revised regimen by James. These results were compared to the dosing regimen as advised by the authors and used in this study. On the basis of these simulations the authors concluded that their dose recommendations were more precise in achieving adequate serum concentrations in premature neonates, although they did not present data in patients with a PCA < 27 weeks. Kildoo and others investigated 15 preterm neonates using an institutional PNA and BW vancomycin dosing algorithm[244]. The substantial differences in clearance between patients due to renal function led to a proposed regimen based on serum creatinin higher or lower than 0.6 mg/dl. They also estimated that using the dosing regimen of James would have led to peak serum concentrations exceeding 40 mg/L in 11 out of 15 cases. Reed also advised an extended interval for neonates with a PCA ≤ 36 weeks[228]. Doses in this study were 9.8-17.8 mg/kg, with an interval of 8 hours in all but one of the 16 patients.

High steady state trough concentrations were seen, ranging from 8.1-38 g/L, leading to the advice of a 12h interval. A confounding factor in the interpretation of this study is the relatively high serum creatinin prior to therapy of up to 115 μmol/L. Lisby-Sutch and Nahata developed a PCA and weight based regimen on grounds of a study in 13 vancomycin treated infants[230]. There was a good correlation between daily vancomycin requirements and PCA, when 4 patients with hepatic or renal disease were excluded. With this in mind, the subdivision into 4 groups in the dosage guideline has a very small basis. Asbury et al. studied pharmacokinetics of vancomycin in 19 neonates without and 4 with indomethacin exposure [241]. The authors postulated that dose and dose interval in patients without indomethacin would lie in the range of 29.6 mg/kg/d with an interval of 6-18h. A limitation of this study was that some of the peak serum concentrations as well as all of the steady state concentrations were calculated and not measured. The authors themselves do not advise to calculate vancomycin dosing according to their equations, until this has been prospectively validated (table 9). Another study individualized dose and dosing interval on the basis of linear pharmacokinetic analysis on initial serum concentrations obtained after the initial dose of 15 mg/kg[232]. Steady state peak and trough concentrations were within the desired therapeutic range. Six out of eleven trough concentrations were lower than 5 mg/L however which suggests an overestimation of $t_{1/2\beta}$.

Seay et al. studied 192 infants with a population pharmacokinetic model and found a relation between clearance and exposure to dopamine and/or gestational age ≤32 weeks. Predictive performance of their dosing algorithm was prospectively validated in 30 patients. Though the study results suggest using longer dosing intervals, no advice was made by the authors.

Forty-four infants were evaluated resulting in a dose recommendation depending on PCA and exposure to mechanical ventilation and/or indomethacin[239]. Although the relation to indomethacin seems logical, effect of mechanical ventilation is harder to imagine. The authors described a relation between creatinin clearance and vancomycin clearance, but not with mechanical ventilation or indomethacin treatment alone. The clinical usefulness of the statistical relation to co-treatment found in this study is doubtful. Pawlotsky et al studied continuous administration of vancomycin after a loading dose in 29 prematures [180]. PCA related dosing was based on an evaluation of 24 other patients. This continuous dosing regimen led to steady state concentrations of 10-30 mg/L in 88%. As can be seen in table 8 the division in subgroups is very detailed and is, given the large inter-individual variation in pharmacokinetic behavior between patients of the same PCA,

probably not warranted. Grimsley and Thomson performed a population pharmacokinetic analysis in 59 infants and based their dosing regimen on serum creatinin alone[233]. This regimen was subsequently tested in 25 neonates and found to give more adequate trough serum concentrations. The number of patients with high serum creatinin in the prospective group was not mentioned.

In conclusion, several dosing regimens in neonates have been proposed and tested. Studies based on PCA and/or serum creatinin have shown to achieve serum concentrations within the therapeutic range[208, 231, 234, 238, 307]. Though an extension of dose interval to more than 8 hours has been suggested, especially in VLBW infants, trough serum concentrations < 5 mg/L found in several studies should lead to caution in that aspect[208, 230-234, 307].

Table 9. Formula's representing dosing schedules

Model	Formula	Author
1 compartment model	CL (L/h)=0.0626xBWx0.455^{Z1}x0.656^{Z2} V_d (L)=0.496 x BW	Seay, 1994[236]
1 compartment model	CL(L/h)=0.007+6.875 x 10^{-5} x CW (g) V_d(L) = 0.034 + 4.991 x 10^{-4} x CW(g) V_d=0.562±15% L/kg (PCA≤32 weeks) V_d= 0.498±16% L/kg (PCA>32 weeks) CL= 0.07±41% L/kg/h (indomethacin and/or mechanical ventilation) CL= 0.086±35% L/kg/h (no indomethacin or mechanical ventilation)	Silva, 1998[238]
1 compartment model	CL(L/h)=0.0281x PCA(weeks)-0.818 V_d(L)= 0.557x BW(kg)-0.051 Vancomycin dosage (mg/kg/d)=1.7835xPCA(weeks)-31.3655[1] Vancomycin dosage (mg/kg/d)=1.5357xPCA(weeks)-26.4576[2]	Asbury, 1993[241]
2 compartiments model	CL(L/h)=0.0224xPCA-0.639 CL(L/h)=0.06xBW(kg)+0.095x(1/S_{cr})-0.141 V_d(L)=0.563xBW(kg)+0.052	Schaible, 1986[247]

CL= clearance, BW= bodyweight, V_d = volume of distribution, PCA= postconceptional age, Z1=1 if exposed to dopamine, else 0, Z2=1 if GA≤32 weeks, else 0, [1]Targets= peak 25-35, trough 5-10 mg/L. [2]Target = steady state concentration of 15 mg/L.

Therapeutic drug monitoring

Therapeutic drug monitoring of vancomycin is mostly performed at steady state, with serum concentrations taken just before and 1h after completion of the intravenous infusion. Target concentrations are peaks between 20-40 mg/L and troughs of 5-10 mg/L. Peak serum concentrations depend on the timing of sampling and since there is a wide variety in sampling time in relation to dose, this should be taken into account when setting goals in therapy[309, 310]. Strangely enough, despite these differences in timing most authors adhere to the same peak level goals.

In general, routine therapeutic drug monitoring is only rational when the drug has the following characteristics[265]. First a good correlation must exist between serum concentration and effect or toxicity. Second when this correlation exists, there must be large interindividual differences in pharmacokinetic behavior between patients. Third the clinical effect or toxicity of the drug must be hard to determine or have a delayed presentation, otherwise TDM will not influence treatment. Fourth a readily available assay with an adequate assay error must exist. Fifth use of TDM should appropriately predict subsequent serum concentrations in the same patient.

In the case of vancomycin use in neonates the first condition is not met. As discussed before, neither efficacy nor toxicity show a clear relation to serum concentrations, and this is especially true for peak values. The second condition is true; there is a large inter-individual variation between neonates and infants with different PCA's with a concomitant effect on obtained peak and trough serum concentrations. There is however no clear relation of peak concentration to toxicity and effect, so the clinical importance of this inter-individual variation is doubtful. Furthermore it has been shown in neonates and adults that peak serum concentrations >40 mg/L are seldom seen with trough concentrations below 10-15 mg/L [311-313]. Given these considerations routine monitoring of peak serum concentrations is questionable. A case can be made for monitoring trough concentrations, although this is also debatable. Based on in-vitro studies, vancomycin trough concentrations should exceed 4-5 mg/L[306]. Acceptable cure rates with trough concentrations ranging from 2 to 18.8 have been described in neonates [230, 236, 254]. Except for endocarditis, there are no clinical studies in neonates, children or adults which have substantiated the clinical need for higher serum concentrations[167]. Several dosing regimens, which have been discussed before, especially those with dose intervals exceeding 8h, have shown that trough serum concentrations can be lower than 5 mg/L,

indicating a need for trough level monitoring [208, 230-234, 307]. Trough level monitoring should thus be aimed at ascertaining that serum concentrations remain > 5 mg/L.
The third and fourth point of requirements for effective TDM are met by vancomycin. Pertaining to point five, several studies have investigated the predictive performance of TDM with vancomycin in the neonatal setting[232, 235, 237, 238, 246, 311, 312, 314]. Controlling dose and/or dose interval with TDM can be performed using first order elimination kinetics, as proposed by Sawchuk and Zaske, or with a Bayesian method[69]. In adults Bayesian feedback is associated with a better predictive performance than the method of Sawchuk and Zaske[315]. Jarrett and associates obtained serum concentrations 2.7 and 12h after initiation of a 60-minute vancomycin infusion, and determined V_d and $t_{1/2\beta}$ using first order elimination kinetics [232]. Maintenance dose and dosing interval were calculated and results showed that 9 out of 11 infants had peak concentrations within the therapeutic range in steady state. All troughs were < 10 mg/L. They also concluded that using only the 2 and 12h serum concentration worked as well as using all three.
Two studies, using 132 routinely collected paired serum concentrations, demonstrated that, as long as there is no overt renal failure, serum trough concentrations < 10 mg/L are seldom accompanied by peak concentrations > 40 mg/L [311, 312]. One other study found no relation between pre-dose and post-dose concentrations in 100 paired samples, but only 3 patients had a high peak serum concentration associated with a low trough value[235]. In a group of 74 infants and children, including 30 neonates, therapy was optimized by using paired serum concentrations after the first dose[314]. TDM goals were peak and trough concentrations of 15-60 and > 4 mg/L, respectively. Standard vancomycin dosage guidelines, which were not specified, were used. Initial trough serum concentrations were low in 5 out of 30 neonates with. After optimization, only 1 neonate had an insufficient trough concentration. The authors conclude that monitoring of vancomycin concentrations is essential to prevent underdosing.
Burstein et al. studied pharmacokinetics in 11 neonates in a 2 compartment open model with serum concentrations taken 3 and 9h after initiation of a 1h infusion of vancomycin. They calculated an optimal sampling strategy with 2, 3 or 4 serum samples, using population based optimal sampling strategies. These strategies were tested on 100 simulated cases. All strategies underestimated actual distributional and total clearance (L/kg/h) as well as central compartment volume and V_d (L/kg). They concluded that no more than two samples (0.5h after a 1h infusion and a trough concentration) are needed for clinical purposes. A lot of assumptions were made in calculating individual

pharmacokinetic parameters with only 2 serum concentrations, which somewhat clouds the results obtained in this study. The best study has been performed by Rodvold and co-workers[237]. They developed a set of population based parameters (see table 9) based on data in 29 neonates. The precision of the dosing regimen based on these parameters was tested in 18 neonates, with 35 courses of vancomycin, in whom more than 1 set of peak and trough concentrations was available. Prediction of subsequent paired serum concentrations was performed using either the population parameters alone or with Bayesian feedback of the first paired serum concentrations. The Bayesian method performed slightly better when subsequent serum concentrations were taken within 30 days of the initial set. Bias and precision for this period were –1.62 and 4.72 mg/L (peak) vs 0.65 and 1.74 (trough). Population-based parameters were superior after 30 days, underscoring the potential change in individual pharmacokinetic parameters over time. The authors conclude that additional feedback concentrations are needed approximately every 14 days. These studies indicate that TDM with use of 2 serum concentrations can predict subsequent serum levels reasonably well. Peak concentrations exceeding 40 mg/L are unlikely with trough concentrations < 10-15 mg/L.

In conclusion, vancomycin has shown to be an effective and relatively safe antibiotic for treatment of gram-positive infections in the neonatal setting. Concerns about development of resistance warrants judicious use. Several dosing regimens were successful in achieving target serum concentrations. In the light of in-vitro and in-vivo studies of efficacy and toxicity it is unlikely that peak concentrations play a major role. TDM should therefore probably be aiming at producing trough concentrations > 5 mg/L, except for patients with certain specific illnesses like endocarditis. Neonates with renal failure should be monitored more closely.

Further studies will have to determine dosing regimens adhering to this new target range.

1.3. Aims of the studies

Chapter 1 sets out to review the literature pertaining to clinical, pharmacokinetic and pharmacodynamic aspects of aminoglycoside (including tobramycin) and vancomycin use in neonates. At the end it states the aims of the studies provided in this manuscript.

Chapter 2 will describe the use of pharmacokinetic modeling of tobramycin antibiotics in neonates. Two methods of pharmacokinetic modeling, non linear mixed effects modeling (NONMEM) and non parametric expectation maximization (NPEM), will be compared in the setting of routine therapeutic drug monitoring in a neonatal intensive care unit (NICU).

Chapter 3 addresses the questions relating to pharmacokinetics and therapeutic drug monitoring of tobramycin in neonates. In *chapter 3.1* a gestational age related tobramycin dosage regimen for neonates is developed and prospectively validated using routinely collected peak and trough serum concentrations. These data are analyzed in a population pharmacokinetic model with the following questions:

1. Is there a need for extended interval dosing of tobramycin in neonates ?
2. Is there a relation between gestational age and dose or dosing interval ?
3. Is there a need for a loading dose of tobramycin in neonates ?

This proposed dosing regimen is prospectively validated in a group of neonatal intensive care patients. In *chapter 3.2* the use of early therapeutic drug monitoring is investigated. The following questions are adressed:

1. Will the dosing regimen mentioned in chapter 3.1 lead to effective peak serum concentrations after the first dose ?
2. Is it possible to predict individual trough serum concentrations of tobramycin by two serum samples taken 1 and 6 hours after the first dose ?
3. What is the relation between individual dose intervals and gestational age of the patient ?

Chapter 4 sets out to describe the pharmacokinetics of vancomycin in neonates. In *chapter 4.1* a dosing regimen for vancomycin in neonates is developed. The following issues were studied:

1. Is there a need for a gestational age dependent dosing regimen in neonates ?
2. Is there a need for the routine measurement of peak serum concentrations of vancomycin in neonates ?

In **Chapter 5** we evaluate the relative risk of hearing loss in neonates exposed to tobramycin and/or vancomycin. In *chapter 5.1* we designed a study using otoacoustic emissions to determine high frequency hearing loss in three year old children who have been treated on the neonatal intensive care unit. The purpose of this study is to:

1. Determine whether high frequency hearing loss occurs in children who have been treated with tobramycin in the neonatal period.
2. Relate hearing loss to serum concentrations and duration of therapy of tobramycin.

In *chapter 5.2* we analyze results of routine neonatal hearing screening (A-ABR screening) performed in the NICU of the Sophia Children's Hospital. Screening results will be related to exposure to tobramycin and/or vancomycin.

Chapter 6 is the concluding chapter in which the results of the previous studies are discussed. Recommendations about dosing, dosing interval and individual therapeutic drug monitoring of tobramycin in neonates are made and suggestions for future research in this area are presented.

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Population modeling of tobramycin

Chapter

2

Population modeling of tobramycin in neonates:a comparison of NONMEM and NPEM2.
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Submitted

SUMMARY

Nonlinear mixed effects modeling (NONMEM) and nonparametric expectation maximization (NPEM2) have both been used in population modeling of tobramycin. We compared both methods for differences in population pharmacokinetic parameters in relation to error models used. Predictive performance was compared between models. A group of 470 neonates who had received tobramycin with a gestational age dependent dosing interval was analyzed according to a one-compartment model with NONMEM and NPEM2. Additional models were made where the assay error pattern in NPEM2 mimics NONMEM residual error and vice versa. Individual pharmacokinetic parameter estimates were compared. Predictive performance was evaluated in a separate group of 61 patients. Population estimates and variation coefficients (CV) for optimal models were: NONMEM K_{el} 0.071 h^{-1} (27%), V_d 0.59 L/kg (9%), NPEM2, K_{el} 0.079 h^{-1} (42%), V_d 0.65 L/kg (48%). Forcing NONMEM to use the NPEM2 error pattern as residual error or vice versa resulted in smaller differences in CV's of the estimates. NONMEM gave less bias ($p<0.05$) than NPEM2 and comparable precision with this approach. In conclusion NONMEM and NPEM2 are dissimilar in population estimates. Differences in ranges of pharmacokinetic parameter estimates between NONMEM and NPEM2 are largely determined by the method of incorporating error patterns in both programs.

INTRODUCTION

Therapeutic drug monitoring plays an important role in the optimization of aminoglycoside dosing regimes in neonates. Several nomograms and models incorporating gestational age, weight, postnatal age, co-medication and other possible descriptive factors have been tested to define the optimal a priori dosing regimen for this vulnerable population. However, due to the large inter-patient variability dose individualization based on serum concentration measurements early in therapy remains necessary. Bayesian feedback methods using population models have been shown to be clinically superior and cost-effective in this respect[1]. Nonlinear mixed effects model (NONMEM) and the nonparametric expectation maximization (NPEM2) algorithm have been used for population pharmacokinetic modeling of aminoglycosides in neonates[2-6]. Both methodologies give an estimate of the interindividual variability within a sample of subjects from the target population, given the data of past doses and responses (serum concentrations). Typically population pharmacokinetic models are defined as the mean or median pharmacokinetic parameter estimates with interpatient variability characterized by the standard deviation (SD) or coefficient of variation (CV). In addition, NPEM2 gives the full population probability distributions while NONMEM provides estimates (standard errors) of the precision of its parameter estimates, including those describing variability.

NONMEM assumes a unimodel normal or lognormal distribution of pharmacokinetic parameters in the population under study. NPEM2 makes no assumption about the distribution other than a limitation to the possible values. Both methods have theoretical advantages for clinical use. NPEM2 gives a graphic output of the probability distribution of a given combination of pharmacokinetic parameter estimates (joint density plot), and is able to discover and quantitatively describe unsuspected sub-populations that can give rise to multi-modal population distributions. With NONMEM clinically important covariates can be easily analyzed and incorporated into the model. Maire et al. were the first to describe preliminary data on relative differences between the modeling methods for amikacin in geriatric patients[7].Recently, NONMEM and NPEM2 modeling has been compared describing flucytosine pharmacokinetics[8].

To date, no study has addressed potential causes for differences found in population pharmacokinetic estimates generated by either method. The residual error models used in NONMEM and the assay error pattern plus additional environmental noise captured by gamma used in NPEM2 play an important role in determining population parameters,

and changes in these parameters are likely to influence the final model. Clinically the most relevant question is which method gives the most useful model for capturing interpatient variability and for predicting (and therefore control) serum concentrations in any next patient.

The aim of the present study was to compare results of population modeling with NONMEM and the nonparametric EM algorithm by re-analyzing tobramycin therapeutic drug monitoring data in a neonatal population as described recently [3]. The predictive performance of NONMEM and NPEM2 generated models was evaluated in a separate group of 61 neonates which was not part of the population modeling.

PATIENTS AND METHODS

Patients

The patient population for this study consisted of two groups of neonates admitted to the neonatal intensive care unit of the Sophia Children's Hospital, Rotterdam empirically treated with tobramycin for suspected neonatal sepsis in the first week of life.

Data of the first group of 470 patients were used to develop population pharmacokinetic models (model generation group). This group received the following tobramycin regimen in a 30-minute i.v. infusion: GA less than 28 weeks 3.5 mg/kg/24 hrs,28-36 weeks 2.5 mg/kg/18 hrs, more than 36 weeks, 2.5 mg/kg/12 hrs. Drug administration times, dosing regimens, blood sampling times and demographic data were collected and have been published previously[3].

Data of a second group of 61 patients (validation group) were used to validate the population models developed with data of the model generation group. The validation group received 4 mg/kg/dose of tobramycin with an interval of 48, 36 or 24 hours depending on their gestational age of < 32 weeks, 32-37 weeks and > 37 weeks, respectively. Data collected in both studies included gestational age, birthweight, 5 minute Apgar score and exposure to indomethacin and/or corticosteroids.

Tobramycin concentration monitoring

Serum concentrations were drawn just before and 30 minutes after completion of the fourth dose in the model generation group. In the validation group TDM was performed 3 and 8 hours after the first dose and just before the second dose. Concentrations of

tobramycin were measured by a Fluorescence Polarization Immuno Assay (FPIA) using a TDxFLx (Abbott Diagnostic Division, Amstelveen, NL). The assay error pattern for the tobramycin assay was: SD (mg/L)= 0.0599 + 0.0126 C+0.00438 C^2, where C is the measured serum concentration (mg/L).

Population pharmacokinetic modeling

Tobramycin data of the model generation group were analyzed according to a one-compartment open model, assuming the data were attributable to the fourth dose after birth, using NONMEM population pharmacokinetics software (NONMEM version V, NONMEM project group, University of California, San Francisco, CA) [9] and the NPEM2 algorithm (NPEM2, USC*PACK collection of PC programs, version 10.7, LAPK, Los Angeles CA)[10]. The model parameters were the elimination rate constant (K_{el}; h^{-1}) and volume of distribution (V_d; L/kg). For NONMEM and NPEM2 individual empirical Bayes' estimates were generated for K_{el} and V_d based on the population estimates.

Nonlinear Mixed Effects Model (NONMEM)

Data were analyzed using first order conditional estimation (FOCE). A constant coefficient of variation intra- and inter-individual error was assumed.
Two models were parameterized:

1. A model parameterized in terms of K_{el} and V_d in a standard NONMEM analysis. This standard model was defined as the optimal NONMEM model and was compared to the optimal NPEM2 model in the validation group.
2. A model parameterized in terms of K_{el} and V_d and a fixed residual error of 0.0599 plus a proportional error of 5%. In this way the input residual error in the NONMEM model is comparable to the assay error pattern in NPEM2 model 1 over the concentration range studied.

With each model individual empirical Bayes pharmacokinetic parameter estimates were generated by NONMEM.

Non-Parametric Expectation Maximization algorithm (NPEM2)

With NPEM2 the joint probability density functions (PDFs), population means (± standard deviation), medians (± dispersion factor) and individual parameter estimates for K_{el} and V_d were estimated using the assay error pattern for the tobramycin assay as an

explicit measure for intra-individual error. In the NPEM2 program this assay error pattern can also be multiplied by a factor (gamma) which can either be user defined or estimated by the program as a means to account for other environmental noise. A change of less than 0.001% in the likelihood function was taken as the convergence criterion for NPEM2. Calculations were based on 20.000 grid points.

Three different NPEM2models were parameterized:

1. A K_{el} and V_d model using the tobramycin assay error pattern and a fixed gamma of 1.0. This is the most widely used method in NPEM2 analysis. This model was compared to the optimal NONMEM model in the validation group.
2. A K_{el} and V_d model using the assay error pattern multiplied by a gamma of 2.82 as determined by the program. This way of modeling has recently been described, and may be a better way to model total error with NPEM2 [11].
3. A K_{el} and V_d model using an error pattern that mimics the residual error of 21% found in the first NONMEM analysis (SD (mg/L) = 0.21 C). By using this approach the NPEM2 error model becomes comparable to the residual error of the first NONMEM model.

For each model maximum a posteriori (MAP) Bayesian estimation was used to generate individual Bayesian posterior parameter estimates by using the 'population of one' utility in the NPEM2 program.

Predictive performance evaluation: comparison of NONMEM and NPEM2 models in the validation group

The relationship between the observed tobramycin concentrations and concentrations predicted by the population models was evaluated with data of the validation group. For this purpose the mean population pharmacokinetic parameters and standard deviations of both optimal models were defined in the MW\PHARM program (MW\PHARM, version 3.15A, MediWare; Groningen, The Netherlands)[12]. Three and eight hour serum tobramycin concentrations were used as Bayesian feedback to the models. Next, predicted trough concentrations estimated by the Marquardt algorithm were compared with the observed trough concentrations for each patient.

Statistical analysis

Statistical analysis was performed using SPSS (SPSS for Windows V9.0, SPSS, Inc., Chicago, IL).

Individual pharmacokinetic parameter estimates for both NONMEM and NPEM2 models were compared. The predictive performance of NONMEM and NPEM2 models was evaluated by comparing predicted serum concentrations with observed serum concentrations according to the method of Sheiner and Beal [13]. Bias was calculated as the mean prediction error (ME; mean difference between measured and predicted concentration), and is a measure of the systematic error. Precision was calculated as the mean squared prediction error (mean of the sum of squared differences between actual and predicted serum concentrations (MSE), and represents the accuracy of the systematic error. The root mean squared prediction error is the squared root of MSE and converts the measure of precision back to concentration units. Relative predictive performance was determined by comparing differences and confidence intervals of differences of MSE and ME for models.

Individual parameter estimates for comparable NONMEM and NPEM2 models were analyzed using descriptive statistics. Bayesian parameter estimates for NONMEM and NPEM2 were tested for significant differences with the Wilcoxon signed rank test. A significance level of $p < 0.05$ was accepted throughout.

RESULTS

Demographic parameters of both study groups are summarized in table 1.

Table 1: Demographics of both study groups

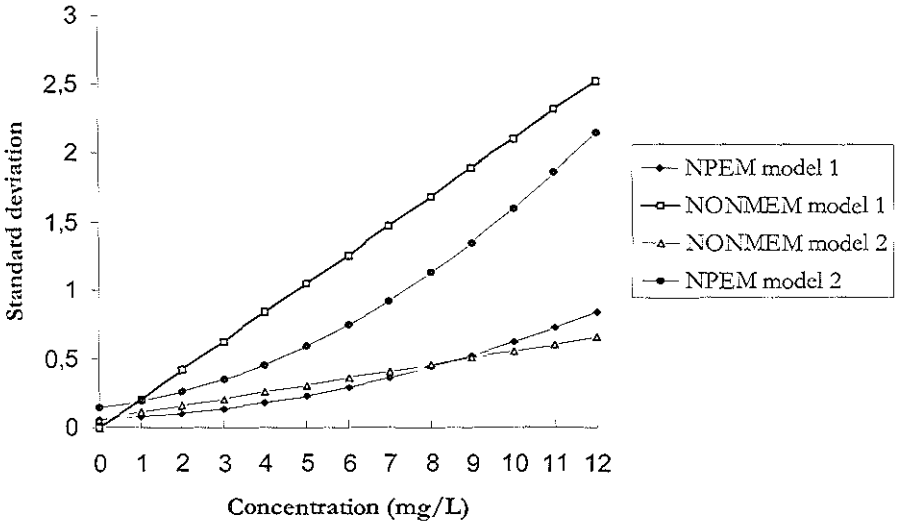
	Group 1 (n=470)	Group 2 (n=61)	
Variable	**Median (range)**	**Median (range)**	**Difference**
Gestational age (weeks)	31.6 (23.7-42.9)	33.4 (25.7-41.4)	N.S.
Birthweight (g)	1530 (485-5245)	2029 (765-4500)	N.S.
Male/female	267/203	34/27	N.S.

N.S., not significant

Data of 470 neonates in group I were used for the generation of population models. Population pharmacokinetic models were evaluated for predictive performance in the validation group consisting of 61 neonates. There were no significant differences in gestational age, weight or gender between groups.

Figure 1 shows the error patterns used in NONMEM and NPEM2 expressed as the relation between the serum concentrations and the standard deviation of the concentration over the working range.

Figure 1: Relation between serum concentration and error pattern used in NONMEM and NPEM2



The line portraying the residual error estimated by NONMEM (model 1) of 21% approximates the assay error pattern times the gamma of 2.82 found by NPEM2 (model 2). If residual error in NONMEM (NONMEM model 2) is fixed like the assay error pattern in NPEM2 (model 1), as described in the methods section, the lines representing assay error for both models are approximately the same over the concentration range in this patient group.

Table II: population pharmacokinetic parameters for NONMEM and NPEM2 models

	K_{el} (h^{-1}) (CV%)	V_d (L/kg) (CV%)
NONMEM model 1	0.0713 (27 %)	0.593 (9 %)
NONMEM model 2	0.0695 (41 %)	0.568 (32 %)
NPEM2 model 1	0.0789 (42 %)	0.646 (31 %)
NPEM2 model 2	0.0743 (42 %	0.816 (36 %)
NPEM2 model 3	0.0783 (35 %)	0.647 (30 %)

K_{el}, elimination rate constant; V_d, distribution volume, CV%, coefficient of variation in %

Population pharmacokinetic parameter estimates for NPEM2 and NONMEM models are listed in table II. As can be seen population pharmacokinetic parameter estimates from the NPEM2 analyses are higher in all models. For the two optimal models (NONMEM model 1 and NPEM2 model 1), NPEM2 estimates are 11% higher for K_{el} and 9% higher for V_d. These differences remain largely the same when residual error in NONMEM is modeled according to the assay error pattern in NPEM2 (NONMEM model 2 and NPEM2 model 1) or vice versa (NONMEM model 1 and NPEM2 model 3). The coefficient of variation is larger for NPEM2 in the optimal models, 15% for K_{el} and 22% for V_d. This difference decreases somewhat, to 8% and 21% respectively, when the NPEM2 error pattern is modeled as NONMEM residual error. When NONMEM residual error is modeled to resemble the assay error pattern of NPEM2 however, the difference in coefficient of variation is reduced to almost nothing (1%).

Table III: descriptive statistics of individual parameter estimates with NONMEM and NPEM2 models

	K_{el} (h^{-1}) Mean (SD)	K_{el} (h^{-1}) Median (range)	V_d (L/kg) Mean (SD)	V_d (L/kg) Median (range)
NONMEM model 1	0.073 (0.017)	0.071 (0.10)	0.59 (0.02)	0.59 (0.15)
NONMEM model 2	0.079 (0.030)	0.072 (0.19)	0.62 (0.23)	0.57 (1.71)
NPEM2 model 1	0.076 (0.037)	0.069 (0.24)	0.82 (0.44)	0.73 (2.98)
NPEM2 model 2	0.075 (0.030)	0.067 (0.19)	0.80 (0.28)	0.76 (1.70)
NPEM2 model 3	0.078 (0.025)	0.069 (0.15)	0.63 (0.17)	0.60 (0.98)

K_{el}, elimination rate constant; V_d, distribution volume

Table III and fig. 2-4 show descriptive statistics and histograms of individual Bayesian parameter estimates. The difference in means for K_{el} and V_d between all comparable NONMEM and NPEM2 models is statistically significant ($p<0.001$) except for K_{el} in NONMEM model 1 and NPEM2 model 1.

As can be expected, NPEM2 and NONMEM medians for Bayesian parameter estimates of K_{el} and V_d follow the differences found in population estimates of both models. Medians for K_{el} are higher for the NONMEM models than for comparable NPEM2 models, whereas V_d is consistently lower for NONMEM than NPEM2. The changes in parameter estimates and ranges are illustrated in figures 2-4. The distribution for K_{el} and Vd (optimal models) is normal (Kologorov-Smirnov test) for NONMEM and neither normal nor lognormal for NPEM2. Standard deviation for both K_{el} and V_d are higher for all NPEM2 models with a concomitant difference in distribution range. When NONMEM residual error mimics NPEM2 assay error pattern, the differences in parameter ranges almost disappears (fig. 3, table III). These differences are reduced, but still large when NPEM2 error pattern mimics NONMEM residual error (fig. 4, table III).

Table IV shows the predictive performance of both optimal NONMEM and NPEM2 models. Bias for NONMEM and NPEM2 are –0.25 and –0.33 mg/L, respectively. The bias for the NONMEM model is significantly better than that for NPEM2 ($p<0.05$). Precision for the models is 0.44 mg/L for NONMEM and 0.47 mg/L for NPEM2.

Table IV: Predictive performance of optimal NONMEM and NPEM2 models

	Bias[a]		**Precision**[a]		
Models used	Mean error (mg/L)	Ratio[c]	MSE[b]	Root MSE[b] (mg/L)	Ratio[c] (mg/L)
NONMEM model 1	-0.25	0.08 (0.01-0.014)*	0.20	0.44	-0.03 (-0.08-0.02)
NPEM2 model 1	-0.33		0.23	0.47	

[a] Data points are point estimates, with 95% confidence intervals in parentheses

[b] MSE, mean squared error

[c] Relative to NPEM2 model 1. *, $p<0.05$ (Wilcoxon)

Fig 2: Histograms for individual estimates of K_{el} and V_d : optimal NONMEM and NPEM2 models

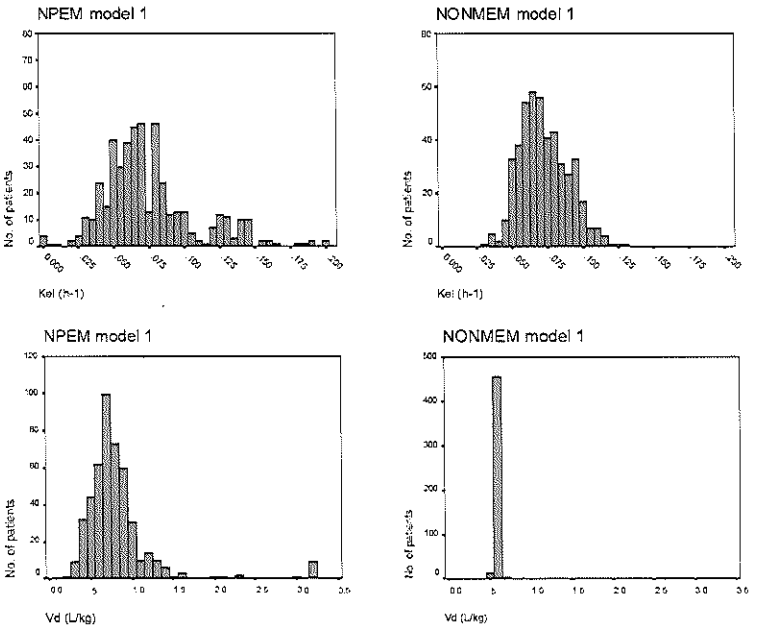


Fig 3: Histograms for individual estimates of K_{el} and V_d: NONMEM residual error mimicking NPEM2 assay error pattern

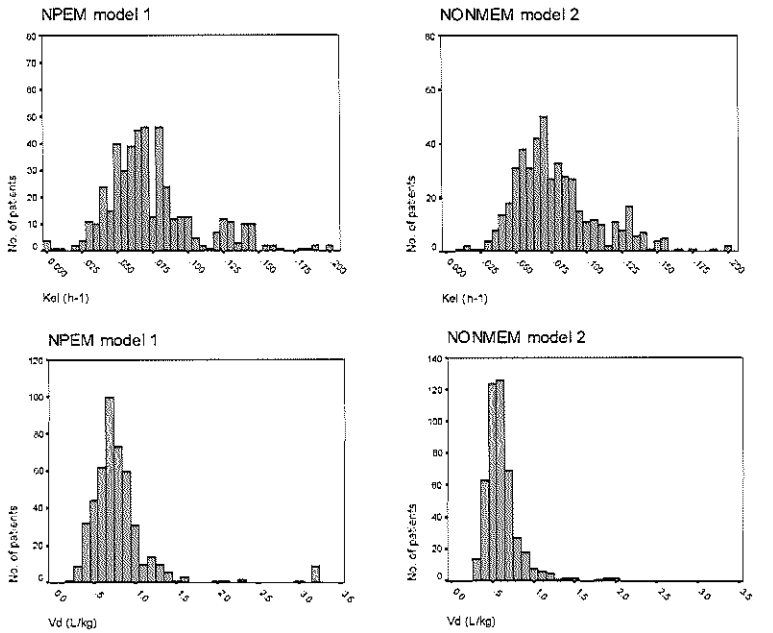
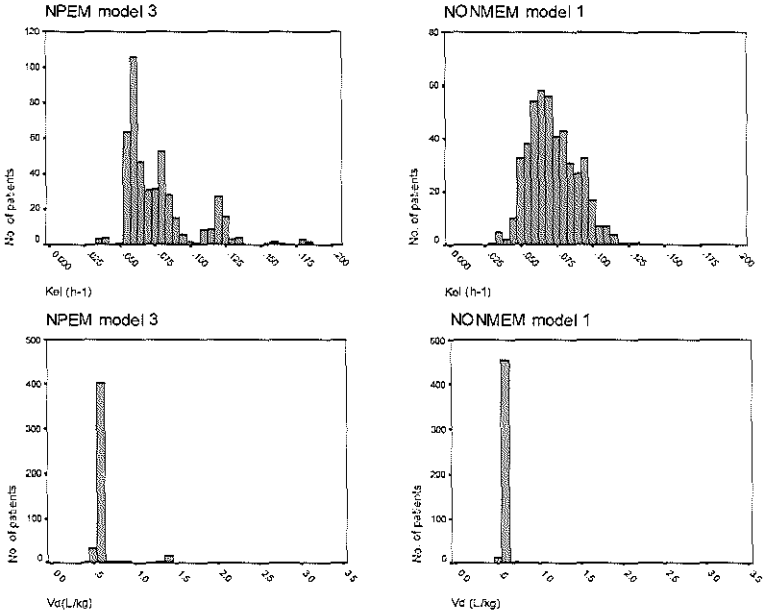


Fig 4: Histograms for individual estimates of K_{el} and V_d. NPEM2 assay error pattern mimicking NONMEM residual error



DISCUSSION

Several population pharmacokinetic methods have been used in order to determine the optimal model for individualizing aminoglycoside therapy in adults[14-16] and neonates of varying gestational ages[2-5, 17, 18]. The most widely used methods for population modeling are nonlinear mixed effects modeling (NONMEM) and the non-parametric expectation maximization (NPEM2) algorithm. Both methods can handle sparse data sets with varying amounts of information per subject[19, 20].

NONMEM describes the data using a mixture of fixed (e.g. time of measurement, dose) and random (variability within or between subjects) effects. Besides population pharmacokinetic parameters the residual error is determined by the analysis. This residual error is the difference between predicted and observed concentrations and accounts for unexplainable variability like dosing error, assay error, model misspecification and errors in recorded timing of measurements.

NPEM2 describes the data using the assay error pattern of the drug as the predominant source of error. This assay error can be multiplied by gamma, as described in the methods section. Differences are to be expected between population pharmacokinetic estimates generated by these programs. To date, there are only a few studies comparing these methods in the same patient group[7, 8]. In the study by Vermes et al. various NONMEM and NPEM2 models for flucytosine were compared using the population estimates as priors for MAP Bayesian forecasting[8]. Predictive performance was evaluated using bias and precision. In their cohort of patients the NONMEM model had better predictive ability, although at closer inspection, there were no statistical differences in bias or precision between optimal NONMEM and NPEM2 models. In the present study, using a comparable approach, we found the optimal NONMEM model to have a significantly smaller bias. There was no significant difference in precision. The clinical relevance of this small, but significant difference in bias is unclear however.

The intriguing question is why the population estimates, variation coefficients and individual Bayesian estimates differ between NONMEM and NPEM2 . NPEM2 calculates a higher V_d and a slightly lower K_{el} than NONMEM. The higher population V_d is partly explained by more extreme outliers for individual estimates of V_d in NPEM2; the difference in median of individual estimates for V_d is 0.14 L/kg versus 0.23 L/kg for mean values. The difference in standard deviation for both models is even larger. This can be explained by two reasons. First, NONMEM assumes a normal or log-normal distribution and it will therefore tend to put less weight on extreme outliers. The second important factor is that the assay error pattern used by NPEM2 is approximately three times lower than the residual error in NONMEM. Because of this small bandwidth of error, NPEM2 assumes serum concentrations lying outside of the expected range to be due to extreme pharmacokinetic parameters in the patient. These parameter estimates are incorporated in the expected distribution of parameters. NONMEM on the other hand does not find a wide distribution of pharmacokinetic parameters. This is mainly because extreme serum concentrations are considered to be a part of the residual (unexplained) error and are not ascribed to interindividual pharmacokinetic differences. This is illustrated by the histograms for individual parameter estimates. The initial differences in range decrease greatly when the assay error pattern in NPEM2 and residual error in NONMEM are fixed at the same level.

These results have implications for clinical use of these methods. As seen in this study, the use of gamma in NPEM2 may largely determine the difference between the final NONMEM and NPEM2 population estimates.

This study as well as the study by Vermes suggest NONMEM to have a better predictive performance, when using population pharmacokinetic estimates as the basis for Bayesian feedback[8]. This however does not mean that this will hold true for every setting. Use of parametric methods like NONMEM still imply that all the information in the population is reduced to a mean or median with a standard deviation or dispersion factor. This limits the ability to predict serum concentrations in new patients. With NPEM2 an interesting new feature is under development; multiple modeling[11, 21]. With this method proposed by Schumitzky and Jelliffe it is possible to retain all the information of the population and, using NPEM2, design an optimized regimen in which the weighted squared error in obtaining desired serum concentrations is minimized. It would be interesting to compare predictive performance of this method to NONMEM. Furthermore, if the distribution of parameters in the actual population has discrete subpopulations, NONMEM will not be able to detect these without further analysis, whereas NPEM2 will. In this particular setting predictive performance of NPEM2 may be better. NONMEM on the other hand is able to integrate covariates in the analysis, which is a valuable feature for clinical application. We propose that both approaches have complementary strengths. NPEM2 is useful for obtaining the full joint density of pharmacokinetic parameter estimates and discrimination of clinically relevant subgroups. NONMEM is the robust method for obtaining estimates of residual error, parameter point estimates and population pharmacokinetic covariate models.

In conclusion, this study shows that tobramycin population modeling in neonates with a parametric and nonparametric approach results in different individual parameter distributions. Differences in PK estimates can be largely explained by differences in method of incorporating error patterns in NONMEM and NPEM2.

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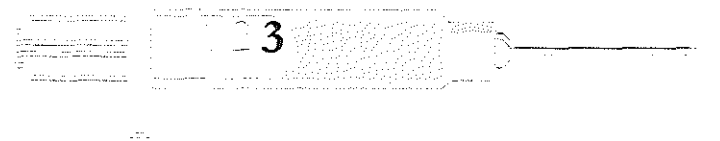
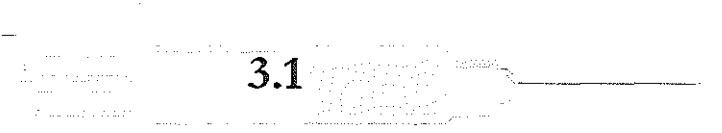
Tobramycin dosing and therapeutic drug monitoring in neonates

Chapter



Tobramycin population pharmacokinetics in neonates

Chapter



M. de Hoog, J.W. Mouton, R.C. Schoemaker, J. N. van den Anker

Clinical Pharmacology and Therapeutics, 1997; 62: 392-399

SUMMARY

Objective: Establish a tobramycin dosing schedule for neonates of various gestational ages.

Methods: This was a retrospective study with prospective validation. A retrospective study in 470 neonates, with suspected septicemia in the first week of life, was performed. All patients received tobramycin according to the following scheme: infants with a gestational age (GA) of less than 28 weeks: 3.5 mg/kg/24 hrs, 28-36 weeks: 2.5 mg/kg/18 hrs, more than 36 weeks: 2.5 mg/kg/12 hrs. Trough and peak tobramycin serum levels were determined before and 30 minutes after the fourth dose. Tobramycin data were analysed according to a one-compartment open model using NONMEM population pharmacokinetic software. Individual empirical Bayes' estimates were generated based on the population estimates, and used to calculate predicted peak and trough levels for different dose and dosing intervals. To establish an optimal dosing regimen, target trough levels were set at below 2 mg/L and target peak levels above 5-10 mg/L. The dosing regimen was prospectively evaluated in 23 patients.

Results: Of the 470 patients 19.1 % of measured peak and 32.8% of measured trough tobramycin serum levels were outside the desired therapeutic range. 48.8% of infants with a GA of less than 28 weeks had an aberrant trough level. Using population estimates the following dosing regimen was recommended:

- GA < 32 weeks: 4 mg/kg/48 hrs
- GA 32-36 weeks: 4 mg/kg/36 hrs
- GA ≥ 37 weeks: 4 mg/kg/24 hrs

With this dosing schedule predicted peak levels were higher than 5 mg/L in 95.1% of cases. Predicted trough levels were higher than 2 mg/L in 1.9% and higher than 1 mg/L in 7.6%. Prospectively measured peak levels were higher than 5 mg/L in all but one. Measured trough levels were higher than 2 mg/L in three patients and marginally higher than 1 mg/L in four patients.

Conclusions: With the use of this proposed schedule, taking into account differences in GAs, predicted peak levels will be therapeutic whereas predicted trough levels will minimize toxicity.

INTRODUCTION

During recent years there has been much debate about the optimal dosing interval and required serum concentrations of aminoglycosides in adults, in order to maximize efficacy and minimize toxicity[1-5]. Efficacy of aminoglycosides is related to the ratio of peak serum concentration to the minimal inhibitory concentration (MIC) of the infecting micro-organism and the area under the time versus concentration curve (AUC)[1, 5], whereas toxicity of these drugs seems to be related to high trough levels[1, 5]. Based on these pharmacodynamic characteristics and the results of clinical trials it was recently advocated in three meta-analytic studies[2, 4, 5] to administer aminoglycosides in adults once daily.

Aminoglycosides also play an important role in the initial empiric treatment of neonatal septicemia[6]. Various regimens for dose, dosing interval and monitoring have been suggested and implemented over the last two decades[7-14]. A significant relation between GA and the need for prolonged dosing intervals was established, and the more recent dosing regimens propose once daily dosing of aminoglycosides in very low birth weight infants[11-15]. We, however, had the impression, that even with once daily administration of aminoglycosides in these infants high trough levels were frequently encountered. We therefore performed this study to investigate the results of the dosing schedule we currently use, in order to find a more appropriate dosing schedule to administer aminoglycosides to newborns of different gestational ages during the first week of life. Using population pharmacokinetics on our own data over the last few years we established a dosing regimen that combines optimal efficacy with minimal toxicity. To validate this regimen, we prospectively tested it in our patient group.

PATIENS AND METHODS

Patients

This retrospective study with prospective validation comprised all neonates, in the first week of life, who were treated with tobramycin as part of their empiric treatment for suspected neonatal sepsis in the neonatal intensive care unit of the Sophia Children's Hospital between August 1992 and December 1994. Only infants whose paired peak and trough serum tobramycin levels were available were included. In the period between February and April 1997 additional patients were studied for validation.

Parameters

All parameters were abstracted from the patient files. GA and birthweights were recorded. GA was determined on the basis of the mother's menstrual history, confirmed by early ultrasound examinations if available, and by physical examination with the use of the criteria of Dubowitz et al[16].

Administration and dosage regimen of tobramycin

Tobramycin was given in combination with amoxicillin 50-100 mg/kg/day as empiric treatment for suspected neonatal sepsis. Patients with documented invasive bacterial infection received at least 10 days of intravenous therapy. Patients with sterile cultures and without a focus of infection received a total of 72 h of therapy. Administration of tobramycin was done in a 30 minute i.v. infusion with the following dosing regimen: GA less than 28 weeks 3.5 mg/kg/24 hrs, 28-36 weeks 2.5 mg/kg/18 hrs, more than 36 weeks 2.5 mg/kg/12 hrs. All doses and times of administration were recorded routinely. Trough and peak blood samples were taken before and 30 minutes after the fourth dose. Dosage adjustments were made according to the outcome, with the intention to keep trough levels below 2 mg/l and peaks between 4 and 10 mg/l.

Analytical Techniques

Concentrations of tobramycin were measured by a Fluorescence Polarization Assay using a TDxFLx (Abbott Diagnostic Division, Amstelveen, NL).

Data Analysis + Dosage recommendations and simulations

Tobramycin data were analyzed according to a one-compartment open model, assuming the data were attributable to the fourth dose after birth, using NONMEM population pharmacokinetics software (NONMEM version IV, NONMEM project group, University of California, San Francisco, CA). Based on the population estimates, individual empirical Bayes' estimates were generated. Scatterplots against weight and age indicated that both clearance and volume of distribution were related to age and weight. After estimation of clearance per kilogram birthweight, only a correlation between Vd and age or weight remained (age and weight are naturally highly correlated in this group). The empirical Bayes' estimates were used to calculate predicted peak and trough levels at

steady state for different dose and dose interval combinations, and scatterplots against gestational age were constructed.

Target serum tobramycin levels were set. The target trough level was below 2 mg/L (generally accepted as trough when dosing more than once daily) and preferably below 1 mg/L[4, 17]. Target peak levels were set at a minimum of 5 mg/L daily[18-21] and preferably ten times the MIC of the infecting micro-organism because of the possibility of emergence of aminoglycoside-resistant pathogens at lower ratios[21, 22]. The MIC of the most important gram-negative pathogen, *Escherichia coli*, is 1 mg/L in the Dutch population[23], so target peak levels were 5-10 mg/L.

Prospective study:

The predictive performance of the dosing regimen was evaluated prospectively in patients receiving tobramycin according to the dosing recommendation mentioned in the results:

- Gestational age ≤ 32 weeks: 4 mg/kg every 48 hours
- Gestational age > 32 but <37 weeks: 4 mg/kg every 36 hours
- Gestational age ≥ 37 weeks: 4 mg/kg every 24 hours

Tobramycin peak and trough serum levels were determined 30 minutes after the first dose and just before the second dose, and analyzed as described in the retrospective study.

RESULTS

Retrospective study:

Table I. Measured tobramycin concentrations in retrospective study

	Gestational age group (weeks)									
Tobramycin (mg/L)	GA<28		28≤GA<32		32≤GA<37		GA ≥ 37		**TOTAL**	
trough ≤ 2	42	(51.3)	103	(61.7)	104	(81.2)	67	(72.1)	**316**	**(67.2)**
trough > 2	40	(48.8)	64	(38.3)	24	(18.8)	26	(28.0)	**154**	**(32.8)**
peak < 5	4	(4.9)	37	(22.2)	32	(25.0)	17	(18.3)	**90**	**(19.1)**
5≤ peak ≤10	75	(91.5)	128	(76.6)	96	(75.0)	74	(79.6)	**373**	**(79.4)**
peak > 10	3	(3.7)	2	(1.2)	0	(0)	2	(2.2)	**7**	**(1.5)**
TOTAL	**82**	**(17.4)**	**167**	**(35.5)**	**128**	**(27.2)**	**93**	**(19.8)**	**470**	**(100.0)**

Numbers are number of patients, numbers in parentheses are percentages of total in group. GA=gestational age

Four hundred and seventy neonates were enrolled in the study. Their gestational ages (GA) and birthweigths (BW) ranged from 23 to 42 weeks (median 31.5 weeks) and from 485 grams to 5245 grams (median 1530 grams), respectively. Table I summarizes the results of tobramycin peak and trough concentrations for the different GA-groups. As can be observed 19.1 % of peak and 32.8 % of trough levels were outside the desired therapeutic range. In the GA groups below 28 weeks and between 28 and 32 weeks the percentage of aberrant trough levels was particularly high, 48.8% and 38.3% respectively. On the basis of the scatterplots and set target serum tobramycin levels the dosing is recommended at 4 mg/kg with the following dosing intervals:

- GA < 32 weeks: 4 mg/kg every 48 hours
- 32≤ GA <37 weeks: 4 mg/kg every 36 hours
- GA ≥ 37 weeks: 4 mg/kg every 24 hours

For illustrative purposes, the curves predicted using the advised dosing regimen and the empirical Bayes' estimates were constructed and concentrations corresponding to the 5th, 50th and 95th percentile computed for the three dosing intervals-age groups (Fig. 1). Calculations were performed using SPSS for Windows (V6.1.2). Fig. 2 and Table II show the predicted peak and trough levels with these recommendations.

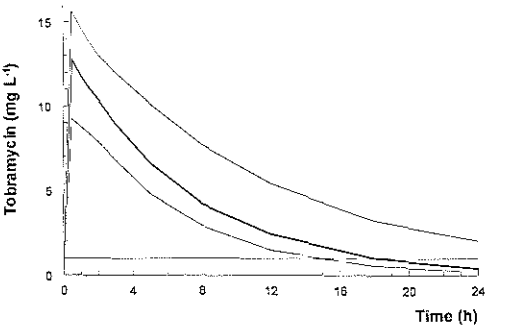
Table II. Predicted tobramycin concentrations using revised dosing recommendation

	Gestational age groups (weeks)									
Tobramycin (mg/L)	GA<28		28≤GA<32		32≤GA<37		GA ≥ 37		**TOTAL**	
trough ≤ 1	75	(91.5)	161	(96.4)	116	(90.6)	82	(88.2)	**434**	**(92.3)**
1 < trough ≤ 2	6	(7.3)	5	(3.0)	9	(7.0)	7	(7.5)	**27**	**(5.7)**
trough > 2	1	(1.2)	1	(0.6)	3	(2.3)	4	(4.3)	**9**	**(1.9)**
peak < 5	11	(13.4)	10	(6.0)	2	(1.6)	0	(0.0)	**23**	**(4.9)**
5 ≤ peak ≤ 10	69	(84.1)	149	(89.2)	92	(71.9)	6	(6.5)	**316**	**(67.2)**
peak > 10	2	(2.4)	8	(4.8)	34	(26.6)	87	(93.5)	**131**	**(27.9)**
TOTAL	**82**	**(17.4)**	**167**	**(35.5)**	**128**	**(27.2)**	**93**	**(19.8)**	**470**	**(100.0)**

numbers are number of patients, numbers in parentheses are percentages of total in group.
GA = gestational age

Figure 1. Predicted tobramycin concentration curves using revised dosing recommendation. A, 4 mg/kg every 24 hrs. B, 4 mg/kg every 36 hrs. C, 4 mg/kg every 48 hrs. Curves are 95th,50th and 5th percentiles.

A



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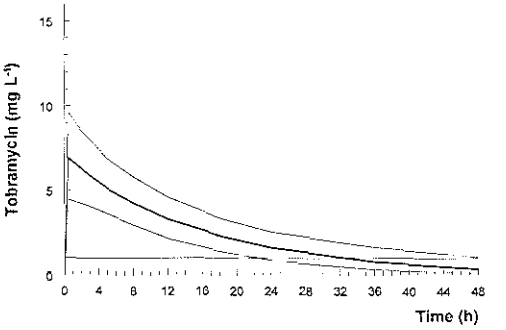

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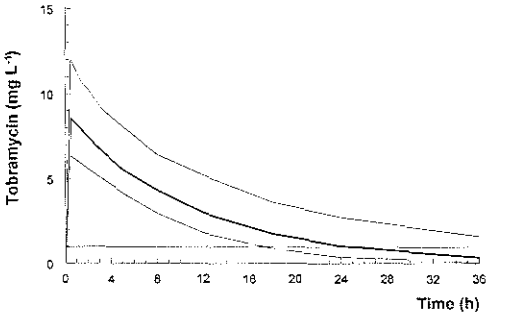
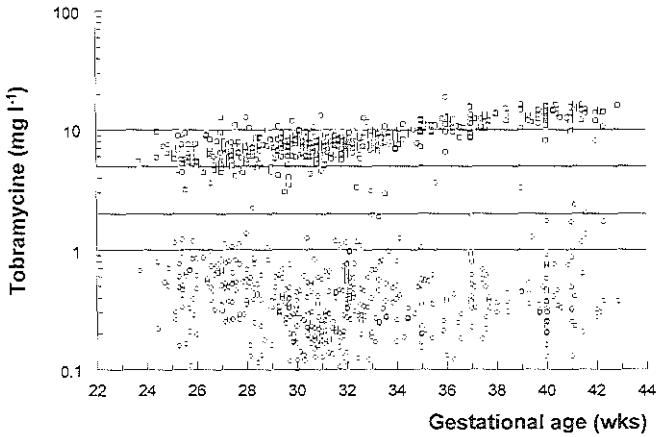


Fig. 2 clearly shows that predicted peak levels rise with gestational age. Predicted peak levels range from 2.9 to 18.7 mg/L, with a median of 8.0 mg/L. Predicted peak levels were below 5 mg/L in 4.9% of the newborns and above 10 mg/L in 27.9 % of the newborns. Median peak levels in the GA-groups were 6.1 mg/L < 28 weeks, 7.3 mg/L between 28 and 32 weeks, 8.7 mg/L between 32 and 37 weeks and 13.2 in the term group. Insufficient peak levels are found in 11 of 82 (13.4 %) of neonates with a GA < 28 weeks. Of these 11 newborns, 10 had predicted peak levels between 4 and 5 mg/L. Predicted trough levels ranged from 0.01 to 8.1 mg/L (median 0.36 mg/L).Trough levels were above 2 mg/L in 1.9% of all cases and between 1 and 2 mg/L in 5.7 %. As Fig. 2 shows, there was no relation between GA and trough levels. Figure 1 shows the predicted serum levels over time for the three GA-groups. The 50th percentile line of tobramycin serum levels dropped below 1 mg/L at approximately 18, 24 and 32 hours in the once every 24, 36 and 48 hour group respectively.

Figure 2. Predicted tobramycin peak (squares) and trough (circles) levels with revised dosing recommendation



Prospective study

Prospective evaluation was performed in 23 neonates. Their GA ranged from 24.4 to 42.1 weeks (median 32.5). Table III summarizes the results of observed tobramycin peak and trough concentrations for the different GA-groups using the recommended dosing regimen. Peak levels ranged from 2.9 to 13.5 mg/L with a median of 7.9 mg/L. Only one peak level is below 5 mg/L. Median peak levels in the GA-groups were 6.9 mg/L < 32 weeks, 7.3 mg/L between 32 and 37 weeks and 9.0 in the term group. Trough levels were between 0.1 and 5.7 mg/L (median 0.7 mg/L). Trough levels exceeding 1 mg/L were found in 7 cases. Of these, three patients had trough levels of 1.2 mg/L and one patient had a trough level of 1.3 mg/L. Median trough levels in the GA-groups were 0.7 mg/L below 32 weeks, 0.65 mg/L between 32 and 37 weeks and 0.95 mg/L in the term group.

Table III. Measured tobramycin concentrations using revised dosing recommendation

	Gestational age groups (weeks)							
Tobramycin mg/L)	GA<32		32≤GA<37		GA≥37		**TOTAL**	
trough ≤ 1	6	(85.7)	5	(62.5)	5	(62.5)	**16**	**(69.6)**
1<trough ≤ 2		-	2	(25.0)	2	(25.0)	**4**	**(17.4)**
trough > 2	1	(14.3)	1	(12.5)	1	(12.5)	**3**	**(13.0)**
peak<5	1	(14.3)		-		-	**1**	**(4.3)**
5≤ peak ≤10	6	(85.7)	7	(87.5)	6	(75.0)	**19**	**(82.6)**
peak > 10		-	1	(12.5)	2	(25.0)	**3**	**(13.0)**
TOTAL	**7**	**(30.4)**	**8**	**(34.8)**	**8**	**(34.8)**	**23**	**(100.0)**

Numbers are number of patients, numbers in parentheses are percentages of total in group
GA=gestational age

DISCUSSION

Earlier investigations concerning the pharmacokinetics of aminoglycosides and other drugs in neonates have shown that elimination half lives are longer in neonates, especially in preterm neonates[8-11, 24]. This is primarily the result of a higher percentage of body water and thus a larger volume of distribution and reduced clearance[25, 26]. Most dosing schedules for preterm and term neonates take this into account[7-12, 14]. We had the clinical impression that our use of GA-related dosing still led to serum concentrations which were frequently outside the desired range. The inventory of our own results over the past few years showed that about one third of the initial trough serum levels were too high, particularly in premature neonates, and that in view of these results a more appropriate dosing schedule should be found.

The limitation of dosing aminoglycosides in neonates lies in the long elimination half life, and therefore the only way to effectively reduce trough serum levels without compromising adequate peak levels is by further increasing the dosing interval. It is difficult to define the desired therapeutic range for aminoglycosides. Peak levels of >4 to 5 mg/L are generally accepted as necessary for antibacterial efficacy whith administration three times a day[18-21]; however questions are being raised about the underlying fundament of this assumption[3]. What is known, is that efficacy of aminoglycosides is related to peak level/MIC ratio and AUC[1, 21], and that in vitro ratios of 10:1 prevent emergence of aminoglycoside-resistant pathogens[22]. In the first week of life, the pathogens for which tobramycin is indicated as therapy are mainly acquired trough the birth passage. By far the most common pathogen in this group of gram-negative bacteria is *E. Coli*[27]. In a recent survey of the Dutch population, the MIC_{90} for *E. Coli* was found to be 1 mg/L[23], and although in theory a peak serum concentration of 10 mg/L would be optimal, a peak/MIC ratio of 5 can be considered to be effective.

The effect of serum concentrations on toxicity is even harder to quantify. High aminoglycoside peak levels do not increase nephrotoxicity because of drug-specific saturable uptake[28-30]. In several large meta-analytical studies toxicity seems to be related to high predose levels, indicating that trough levels are not low long enough to prevent renal accumulation[1, 2, 5, 19]. Commonly accepted trough level goals are < 2 mg/L, but for once-a-day administration most authors keep 1 mg/L as a safe limit[4, 17]. Another point in the discussion is that renal toxicity is mostly reversible, whereas ototoxicity is usually irreversible. Most authors suggest that ototoxicity is related to total dose and duration of therapy rather than to serum aminoglycoside levels, but the relation to aminoglycoside

serum levels remains unclear. This form of toxicity usually occurs in patients who have received either long, or repeated, courses of aminoglycosides[3].Reports about ototoxicity in neonates are contradictory. Some authors report no relation[31-33], whereas others did find a higher incidence[34-36]. Until conclusive evidence is given, it seems prudent to keep duration of tobramycin therapy as short as possible.

On the grounds of a peak/MIC ratio above 10 and the MIC_{90} of *E. Coli* in our population a peak tobramycin serum concentration as high as 10 mg/L is desirable from the efficacy point of view. A trough level below 1 mg/L will have to suffice until better data about toxicity are available.

Using population pharmacokinetics, we established a better dosing scheme to meet these criteria. This resulted in the following GA-related regimen:

- Gestational age $\leq$ 32 weeks: 4 mg/kg/48 hours
- Gestational age > 32 weeks but < 37 weeks: 4 mg/kg/36 hours
- Gestational age $\geq$ 37 weeks: 4 mg/kg/24 hours

With this regimen most predicted peak levels are in the required range in neonates with a GA above 32 weeks, with acceptable predcited trough values for almost all (see table II). In the GA-group below 28 weeks, predicted peak serum levels are arguably too low in 12 of 82 patients, but still 11 of these are between 4-5 mg/L. The prospective evaluation showed that serum peak levels are in the desired therapeutic range in all but one patients (see table III). Measured trough levels were mildly elevated in four patients and clearly too high in three, so there is a definite need for measuring trough serum levels before the second dose. This dosing regimen also makes redundant the need for a loading dose of aminoglycosides in prematures, as suggested by some[11, 17, 37] because high enough peak levels are achieved at the first dose. In addition, the practical advantage of this proposed schedule is a fixed starting dose per kilogram bodyweight, irrespective of GA.

A possible problem in the once every 48 h group is that tobramycin levels might be subtherapeutic for too long. Serum levels at 24h in this group (figure 1) show that most neonates are around 2 mg/L, which is still higher than the MIC of relevant micro-organisms, but drops below 1 mg/L after approximately 32 hours. In the prospective group of 23 patients serum trough levels did not fall too low. Furthermore tobramycin is always given in combination with amoxicillin in this group and the post-antibiotic effect, or post-antibiotic leukocyte enhancement effect, or sub-MIC effect, which will prevent

regrowth of bacteria at sub-MIC levels of tobramycin for another period of at least hours[38-40], so we consider this a safe dosing interval.

In conclusion, the information that we presented shows that acceptable therapeutic tobramycin peak and trough concentrations can be reached with a simple dosing schedule for three separate GA-groups in the first week of life. Trough levels according to our scheme are not toxic and probably not long enough below 1 mg /L to permit bacterial regrowth.

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Extended interval dosing of tobramycin in neonates: implications for therapeutic drug monitoring

Chapter

3.2

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Submitted

SUMMARY

Objective: To individualize tobramycin dosing regimens in neonates of various gestational ages using early therapeutic drug monitoring.

Methods: This study was performed in neonatal patients with suspected septicemia in the first week of life. All patients received tobramycin in a dose of 4 mg/kg/dose, as a 30' I.V. infusion, with a gestational age (GA) related initial interval of 48 hours (<32 weeks), 36 hours (32-36 weeks) and 24 hours (≥ 37 weeks). The target serum peak and trough serum concentrations were 5-10 mg/L and 0.5 mg/L, respectively. Serum trough samples as well as 1 and 6 hour samples were taken after the first dose. Tobramycin concentrations were used to obtain gestational age dependent population models with NPEM software. To investigate the effect of timing of sampling in a second group of patients, serum trough samples as well as 3 and 8 hour samples were taken after the first dose of tobramycin was administered. Serum trough concentrations were predicted using linear pharmacokinetics in both groups and by using the population models with Bayesian feedback of one or two serum concentrations in the second group. These predicted concentrations were compared to actual serum trough concentrations. The predictive performance of the 1-6h and 3-8h models and the population models were compared to a gestational age related model without therapeutic drug monitoring (TDM).

Results: A total of 247 patients were analyzed, 206 with 1-6h serum samples and 41 with 3-8h serum samples. Peak serum concentrations were above 5 mg/L in 90.8 % and trough serum concentrations above 1 mg/L in 25.5% of cases. The 3-8h linear model had a bias of –0.31 mg/L and a precision of 0.48 mg/L and performed significantly better than the 1-6h model. The best NPEM model had a bias of –0.11 mg/L and a precision of 0.45 mg/L. None of the models yielded a significant improvement of predictive performance over the model without TDM.

Conclusion: Routine early therapeutic drug monitoring does not improve the model based prediction of initial tobramycin dosing intervals in neonates in the first week of life.

INTRODUCTION

Neonatal sepsis is an important health care problem, especially in developing countries, with an estimated worldwide mortality of 1.5-2 million per year [1].

Although a trend for a decline in sepsis associated neonatal deaths in neonatal intensive care units (NICU's) has been reported, treatment of (suspected) neonatal sepsis remains a cornerstone in neonatal intensive care practice [2]. In recent years there is an increase towards gram-negative infections in the NICU[3, 4]. Culture proven early onset sepsis (occurring within 72 hours of birth) in very low birthweight infants (VLBW) is reported to occur in 1.9% of cases, but this does not reflect the need for antibiotic treatment in this patient group[3]. Diagnosing sepsis in these vulnerable patients is difficult. Neither blood culture, nor leukocyte count or CRP give conclusive evidence[5, 6]. In many VLBW infants early-onset sepsis is suspected on clinical grounds and antibiotic therapy is started and continued for 3-7 days. Initial empiric treatment with a combination of an aminoglycoside with penicillin, amoxicillin or a cephalosporin is common practice[7]. Optimization of aminoglycoside use in neonates warrants therapeutic drug monitoring (TDM) for both efficacy and toxicity reasons. Aminoglycoside efficacy is related to the ratio of peak serum concentration to the minimal inhibitory concentration (MIC) of the infecting microorganism and the area under the time versus concentration curve (AUC) [8]. If a peak MIC ratio of >10 is taken as essential, this means that adequate initial peak serum concentrations of 5-10 mg/L are warranted[9]. Toxicity is related to high pre-dose concentrations and serum trough concentrations should be in the range of 0.5–2 mg/L depending on the dosing interval[7, 10, 11]. Traditionally TDM for aminoglycosides is performed in steady state around the fourth dose. For several reasons this is not useful in neonates in the first week of life. Antibiotic courses in neonates are often discontinued after a few days when blood cultures and other tests remain negative. Because of prolonged half-life and reduced clearance in prematures intervals of 24-48 hours are advised in this patient group[9, 12, 13]. Large inter-individual differences remain and predictive performance of these proposed regimens might be improved by TDM. Traditional therapeutic TDM in this setting would not be performed in time to be of use. Early TDM directly after the first dose may improve treatment for the individual patient. There are two commonly used approaches to TDM in this setting. The first method is to take two serum samples in the elimination phase after the first dose and calculate the individual dose interval by using first order elimination kinetics. The timing of the first sample is normally 1h after start of infusion, but it has been suggested that this is too

early [14]. The second method is determining the individual interval using a population pharmacokinetic model with Bayesian feedback of one or two serum sample. The aim of our study was to explore the possibility of individualizing dosing interval in neonates of varying GA's during the first week of life. Both TDM strategies were analyzed. Furthermore the influence of timing of serum samples was studied. The predictive performance of these strategies was compared to our standard GA-related dosing regimen previously published[9].

PATIENTS AND METHODS

Patients

All neonates, in the first week of life, who were treated with tobramycin as part of their empiric treatment for suspected neonatal sepsis in the neonatal intensive care unit of the Sophia Children's Hospital between December 1996 and June 2000 were eligible for this retrospective study. Only infants whose paired 1-6 hour or 3-8 hour and trough serum tobramycin levels were available were included. Furthermore the trough serum concentration had to be sampled within one hour of the advised GA-related dosing interval of our previous study[9].

Parameters

GA, birthweight, Apgar scores and medication were noted in the patient files as a routine procedure. GA was determined on the basis of the mother's menstrual history, confirmed by early ultrasound examinations if available, and by physical examination with the use of the criteria of Dubowitz et al[15].

Administration and dosage regimen of tobramycin

Tobramycin was given in combination with penicillin G as empiric treatment for suspected neonatal sepsis. Patients with documented invasive bacterial infection received intravenous therapy as considered appropriate by the attending physician, usually at least 7 days. Treatment was discontinued after 72 h in patients with sterile cultures and without a focus of infection. Administration of tobramycin was performed in a 30-minute intravenous infusion in a dose of 4 mg/kg. The initial dosing interval was 48, 36 or 24 hours in neonates with a GA of <32 weeks, 32-36 weeks and ≥ 37 weeks, respectively [9].

Therapy adjustments were made at the discretion of the attending physician. All doses and times of administration were recorded routinely. Tobramycin serum samples were taken as part of routine therapeutic drug monitoring 1 and 6 hours after the first dose and just before the second dose.
After analysis of this patient group (1-6h group) serum sampling was changed to 3 and 8 hours (3-8h group) after the first dose and just before the second dose to investigate the effect of sample timing on serum trough concentration prediction.

Analytical Techniques

Concentrations of tobramycin were measured by a Fluorescence Polarization Assay using a TDxFLx (Abbott Diagnostic Division, Amstelveen, NL).
The coefficient of variation for this test in our laboratory is 8% at 0.3 mg/L and <5% from 1-20 mg/L.

Population modeling

Data from the 1-6 h group of patients were used to obtain a population model by way of a nonparametric expectation maximization algorithm developed by Schumitzky (NPEM program, USC*PACK clinical collection version 10.7, LAPK, Los Angeles, CA) employing all 3 available data points (1h, 6h and trough concentration) per patient. A total of three models were made, one for each different gestational age group (GA-group): <32 weeks, 32-36 weeks, ≥37 weeks. Models were described in terms of volume of distribution (V_d, L/kg) and elimination constant (K_{el}, h^{-1}). Serum concentrations were weighted by the reciprocal of its variance, fitted by the following equation:
$SD = 0.0599 + 0.0126C + 0.00438\ C^2$, where SD is the standard deviation of the assay and C represents the measured tobramycin serum concentration.
The population models were achieved in 2 steps. The front part of the NPEM program calculated individual parameter estimates for K_{el} and V_d in a one-compartment model by the iterative 2-stage Bayesian (IT2B) modeling approach. Parameter value boundaries used as priors for this step were arbitrarily set at 0 to 0.4 h^{-1} for K_{el} and 0.2 to 3 l/kg for V_d.
The parameter estimates were then used as input for the actual NPEM program resulting in mean population parameter estimates and SD's.

Data Analysis

The following tobramycin TDM strategies were compared for predictive performance

1. Linear pharmacokinetics (1-6h group and 3-8h group)

Tobramycin data were analyzed according to a one-compartment open model for the 1-6h group and the 3-8h group. Analysis for the 1-6h group will be described in detail. Analysis for the 3-8h group was similar. Based on the assumption that one and six hour as well as the trough serum concentrations were determined in the elimination phase of the drug, serum concentrations were used to calculate the elimination constant (K_{el}), the elimination half-life ($t_{½ß}$) and the time to reach a serum concentration of 0.5 mg/L (t_{target}) as follows: $K_{el} = \frac{\ln(c6) - \ln(c1)}{5}$, where C_6 and C_1 are serum concentrations (mg/L) at t_6 and t_1 respectively, $t_{½ß} = 0.693/K_{el}$, $t_{target} = \frac{-\ln(\frac{0.5}{C_1})}{K_e} + 1$.

The interval between the start of infusion and trough sampling [interval(h)] was determined.

The predicted serum trough concentration (C_{pred}) was calculated as follows:

$C_{pred} = C_1.e^{-Kel.(interval-1)}$

2. Population model and Bayesian feedback

The GA-related population model was used in conjunction with Bayesian feedback of either or both of the 3h and 8h serum concentrations to predict individual serum trough concentrations of the 3-8h group by way of the MW\Pharm software package (MW\PHARM, version 3.30, MediWare; Groningen, The Netherlands). Tobramycin trough serum concentrations were predicted in this way and compared using either or both of the 3h and 8h serum concentrations as feedback. The same procedure was not performed in the 1-6h group because population parameters were based on this group. Validation in the same patient group could lead to a false favorable performance[16].

3. GA-group model without TDM (no TDM group)

In our previous study we concluded that the initial tobramycin dosing interval in neonates in the first week of life would be optimal with 48, 36 or 24h in GA-groups <32, 32-36 and ≥37 weeks, respectively [9]. This was based on a desired trough serum concentration of

0.5 mg/L. If the actual trough serum concentration in the present study was taken within 1 hour of this GA-related interval, the predicted trough serum concentration was then defined as 0.5 mg/L. In this model no individual TDM information was used to predict trough serum concentrations.

Predictive performance and statistical evaluation

The predictive performance of all models was evaluated by comparing predicted serum concentrations with measured serum trough concentrations according to the method of Sheiner and Beal [16]. Bias was calculated as the mean prediction error (ME); the mean difference between measured and predicted concentration. This is a measure for the systematic component of error. Precision was calculated as the mean squared prediction error (MSE); the mean of the sum of squared differences between actual and predicted serum concentrations. The root mean squared prediction error is the squared root of MSE and converts the measure of precision back to concentration units. Relative predictive performance was determined by comparing differences and confidence intervals of differences of ME and MSE for models.
Statistical analysis was performed using SPSS 8. 0 statistical software (SPSS Inc., Chicago, USA). Significance for relative predictive performance was defined when the 95% confidence interval did not include zero. The Wilcoxon signed rank test and Mann-Whitney test were used as nonparametric tests.

RESULTS

Patient groups

In the 1-6h group a total of 379 patients had paired 1-6h serum concentrations and trough concentrations taken. Serum sampling times were aberrant or incomplete in 32 patients. An incorrect dose (< 3mg/kg or > 5 mg/kg) was given in 5 patients. In 136 patients the serum trough concentration was taken outside 1 hour of the GA-related interval. Thus a total of 206 patients were evaluated. In the 3-8h group serum sampling group 77 patients were included. Serum sampling time was aberrant or incomplete in 14 patients. An incorrect dose was given in 2 patients. Twenty patients had their serum trough concentration taken outside 1 hour of the GA-related interval. Forty-one patients were evaluated. Table I shows demographic variables of the 1-6 and 3-8 study group.

There are no significant differences for GA, birthweight Apgar score or postnatal age between 1-6h and 3-8h groups.

Table I: Demographic variables of the 1-6h and 3-8h study group

	Group 1-6h (n=206)	Group 3-8h (n=41)	Significance
Gestational age (weeks)	33.1 (24.4-42.1)	33.3 (25.9-40.3)	0.97 (NS)
Birthweight (grams)	2011 (580-4780)	1976 (765-4500)	0.78 (NS)
APGAR score 5 min	7.5 (1-10)	7.5 (2-10)	0.97 (NS)
Postnatal age (days)	0.97 (0-7)	0.93 (0-7)	0.80 (NS)

Data are mean(range)

Distribution of obtained serum concentrations

Table II shows obtained peak serum levels in the 1-6h group. In the 3-8h group no peak serum concentrations were determined. Peak concentrations ranged from 2.4-14.1 mg/L.

Table II: Tobramycin peak serum concentrations according to GA-group in the 1-6h group

	Gestational age groups							
Tobramycin peak serum concentration	**<32 weeks**		**32-36 weeks**		**≥37 weeks**		**TOTAL**	
< 5mg/L	14	(14.7)	4	(8.9)	1	(1.5)	**19**	**(9.2)**
5-10 mg/L	79	(83.2)	38	(84.4)	53	(80.3)	**170**	**(82.5)**
> 10 mg/L	2	(2.1)	3	(6.7)	12	(18.2)	**17**	**(8.3)**
TOTAL	**95**	**(100)**	**45**	**(100)**	**66**	**(100)**	**206**	**(100)**

Data are number of patients (percentage of total)

Mean serum peak concentrations (±SD) for GA-groups are 6.4±1.6, 7.2±1.8 and 8.2±1.9 mg/L for <32, 32-36 and ≥37 weeks, respectively. A total of 90.8% of peak concentrations was higher than the lower limit for presumed optimal efficacy (5 mg/L). Only 5 of 19 neonates with peak concentrations below 5 mg/L had concentrations below 4 mg/L.

Table III: Tobramycin trough serum concentrations according to GA-groups (both 1-6h and 3-8h group). Data in parentheses are percentages of total

	Gestational age groups							
Tobramycin trough serum concentration	**<32 weeks**		**32-36 weeks**		**≥37 weeks**		**TOTAL**	
< 0.5mg/L	60	(53.6)	17	(28.3)	12	(16.0)	**89**	**(36.0)**
0.5-1 mg/L	44	(39.3)	26	(43.3)	25	(33.3)	**95**	**(38.5)**
>1 mg/L	8	(7.1)	17	(28.3)	38	(50.7)	**63**	**(25.5)**
TOTAL	**112**	**(100)**	**60**	**(100)**	**75**	**(100)**	**247**	**(100)**

Data are number of patients (percentage of total)

Table III shows trough serum concentrations for the 1-6h and 3-8h group. Mean serum trough concentrations (±SD) for GA-groups are all higher than the target of 0.5 mg/L. Values (±SD) are 0.52 ± 0.33, 0.78 ± 0.46 and 1.07 ± 0.58 mg/L for <32, 32-36 and ≥37 weeks, respectively. Approximately one quarter (25.5%) of patients had serum trough concentrations higher than 1 mg/L, the highest percentage (50.7%) in the term group. Fourteen neonates had a trough serum concentration < 0.2 mg/L, half of these in the GA age group <32 weeks.

Population model

The results of the NPEM analysis of the three different GA-groups for the 1-6h group are shown in table IV. There are substantial differences between V_d and K_{el} for different GA-groups with a decrease of V_d and an increase of K_{el} in relation to gestational age. K_{el} increases with 53% and V_d decreases with 23% when comparing preterms < 32 weeks with term infants.

Table IV: Population pharmacokinetic parameter estimates of the 1-6h group

		N	K_{el} (h^{-1})	V_d (L/kg)
GA-models	GA < 32 weeks	95	0.064 ± 0.034	0.70 ± 0.17
	GA 32-36 weeks	45	0.066 ± 0.022	0.63 ± 0.15
	GA ≥ 37 weeks	66	0.098 ± 0.046	0.54 ± 0.11

GA = gestational age, K_{el} = elimination rate constant, V_d = volume of distribution. Data are means ± standard deviation

Predictive performance

Data for predictive performance of the investigated models in accurately estimating trough concentrations are shown in table V. These data represent all GA-groups.

Table V: Predictive performance of models in estimating serum trough concentration (n=41).

	BIAS	**PRECISON**	
Models used	Mean error (mg/L)	MSE* (mg^2/L^2)	Root MSE* (mg/L)
No TDM model (1-6h group)	-0.26	0.34	0.58
No TDM model (3-8h group)	-0.20	0.20	0.45
NPEM model *(3-8h serum concentration feedback)*	-0.30	0.21	0.46
NPEM model *(3h serum concentration feedback)*	-0.11	0.20	0.45
NPEM model *(8h serum concentration feedback)*	-0.24	0.16	0.42
Linear model *(1-6h group)*	-0.39	0.69	0.83
Linear model *(3-8h group)*	-0.31	0.23	0.48

*MSE = mean squared prediction error

Bias represents the systematic error in the model and was negative in all cases (–0.11 up to –0.39 mg/L). This means that all models underpredicted the actual trough serum concentration. Linear pharmacokinetic models had a more negative bias than comparable NPEM and no-TDM models. Bias of NPEM models using one serum concentration as feedback was lower than when using two. The measure for precision [root mean squared error (mg/L)] differed between 0.42 and 0.83 mg/L. Linear pharmacokinetic models had a worse precision than comparable NPEM and no-TDM models. Precision for NPEM models using one or two serum concentrations as feedback did not differ much and were comparable to precision of the no-TDM model. To investigate whether predictive performance was related to GA, data were also analyzed separately per GA-group (data not shown). Bias and precision for NPEM-, linear- as well as no-TDM models in the term

infant group were worse as compared to preterm infants < 32 weeks. The only exception was bias in the NPEM 3h model. The model with the best bias and precision was the no-TDM model for preterms < 32 weeks with a bias of –0.02 and a precision of 0.04 mg/L.

To determine the relative predictive performance, ratios for bias and precision between the no-TDM model and other models were calculated [16]. The predicted trough concentration with the no-TDM model was defined as 0.5 mg/L. Table VI shows results for this analysis. A positive ratio for bias or precision implies a lower predictive accuracy of the TDM model as compared to the no-TDM model.

Table VI: Relative predictive performance of linear and population models compared to no-TDM models in estimating serum trough concentration

	Bias (mg/L)		Precision (mg^2/L^2)	
Models used	Ratio	(95% CI)	Ratio	(95% CI)
1-6h linear*	0.12	(0.03,0.22) §	0.34	(0.01,0.70) §
3-8h linear	0.11	(0.001,0.22) §	0.02	(-0.08,0.13)
NPEM model† *(3-8h serum concentration)*	0.09	(-0.003,0.19)	0.007	(0.09,0.11)
NPEM model† *(3h serum concentration)*	-0.09	(-0.24,0.05)	-0.007	(-0.13,0.12)
NPEM model† *(8h serum concentration)*	0.04	(-0.07,0.14)	-0.05	(-0.16,0.06)

* Relative to 1-6h no TDM model, † Relative to 3-8h no TDM model, § $p<0.05$

Predictive performance of the no TDM model is significantly better for bias (1-6 and 3-8h models) and precision (1-6h model) than the linear model. NPEM models using 3h and/or 8h Bayesian serum feedback had a predictive performance comparable to the no-TDM strategy. The NPEM 3h model had a slightly better bias and precision without reaching significance. Analysis of linear pharmacokinetic models showed that the 3-8h model had a superior precision ($p<0.01$) and a comparable bias to the 1-6h model.

Since one of the aims of TDM was to predict aberrant trough concentrations, we also tested whether the best population model can accurately predict undesirable trough levels in individual patients. Undesirable trough serum concentrations were defined as <0.2 mg/L or > 1.0 mg/L.

Results for this analysis are shown in table VII. As can be seen the 3h model accurately predicted only 3 out of 10 undesirable trough serum concentrations.

Table VII: predicted trough serum concentrations with NPEM 3h model versus measured serum trough concentrations

	Predicted troughs			
Measured trough	< 0.2 mg/L	0.2-1.0 mg/L	> 1.0 mg/L	**TOTAL**
< 0.2 mg/L	-	1	-	**1**
0.2-1.0 mg/L	6	21	4	**31**
> 1.0 mg/L	1	5	3	**9**
TOTAL	**7**	**27**	**7**	**41**

Data are number of patients

Covariate analysis

Covariate analysis was performed to study the influence of perinatal asphyxia and exposure to other medication on serum trough concentrations. The 5' Apgar score (AS5) as a measure of asphyxia showed a negative correlation with trough serum concentration for the ≥ 37 week group ($p < 0.01$). All term neonates with an AS5 below 5 had increased serum trough concentrations. Serum trough concentration were lower in neonates with a GA < 32 weeks who were antenatally exposed to corticosteroids ($p<0.01$). No correlation with antenatal or postnatal exposure to indomethacin was found, though it is important to realize that only 14 neonates received postnatal indomethacin before serum sampling. Start of therapy was within 48h from birth in 228 patients and no relation to postnatal age could be demonstrated.

DISCUSSION

Once daily dosing of aminoglycosides in adults has become common practice during the last decade. Several large studies have shown that extended interval dosing has been associated with an increase of clinical response rate and a decrease of oto- and nephrotoxicity[10, 17-20]. The need for longer dosing intervals has also been established in neonates, though a difference in toxicity between ODD and MDD has not been demonstrated yet in this group[9, 12, 13, 21, 22]. These extended intervals are GA related.

Earlier investigations concerning the pharmacokinetics of aminoglycosides and other drugs in neonates have shown that elimination half-lives are longer in neonates, especially in preterm infants[23-27]. This is primarily due to both reduced clearance and the higher percentage of body water resulting in a larger volume of distribution[28, 29]. Extended interval dosing has implications for desired serum concentrations and methods of aminoglycoside TDM. Monitoring of aminoglycosides is based on the observation that outcome is improved with peak serum concentrations of 4-5 mg/L[30, 31] and toxicity is reduced with trough serum concentrations of < 2 to 4 mg/L[32, 33]. Combined with the fact that peak/MIC ratios of 5-10 are essential in preventing emergence of aminoglycoside resistant bacteriae, this has led to the common TDM goals of peak and trough serum concentrations of 5-10 mg/L and < 2mg/L, respectively[8, 34]. With extended interval dosing there might be a need for other trough concentration goals. Some authors have suggested to maintain the trough standard of 2mg/L in ODD and only reduce dose or extend dose intervals when troughs > 2 mg/L are encountered[11, 35]. If a trough of < 2 mg/L is accepted with a ODD regimen however, exposure to aminoglycosides in terms of AUC could be 2.5 times as high as that with conventional multiple daily dosing[36]. There is clinical evidence that this goal could lead to an increase of nephrotoxicity[11, 37]. It therefore seems more prudent to set trough concentration goals at 0.5 - 1.0 mg/L. In the present study we confirmed our earlier finding that a dose of 4mg/kg, irrespective of GA leads to adequate peak serum concentrations in >90 % of cases[9].

Several methods have been advocated to individualize aminoglycoside treatment in relation to these goals. A method being used in adults uses a peak and mid-interval serum concentration which allows the calculation of AUC or elimination half-life with the use of linear pharmacokinetics[11, 20, 36, 38]. These calculated values might then be used for adjusting the dose or dosing interval of the administered aminoglycoside. In the present study we investigated whether use of early TDM using this approach would improve prediction of individual dose intervals over a model with no TDM from an earlier study [9]. Our data show that obtaining 1h and 6h serum concentrations after the first dose as advocated by Begg [38]and using linear kinetics yields a poor prediction of tobramycin trough serum concentrations and thus of individualized dose interval. Since some have suggested that this might be due to a prolonged distribution phase we changed sampling times to 3 and 8h[14]. This led to a statistically significant improvement of precision. However prediction based on the original GA-related model, using no TDM, is superior for bias and precision to this type of monitoring. For practical purposes linear kinetics are therefore not useful

in this setting. A second method uses population pharmacokinetic parameter estimates [39]. These estimates with Bayesian feedback of one or more serum concentrations have been shown to adequately predict aminoglycoside serum concentrations in adults[39]. Individualization of dosing regimens in neonates with Bayesian feedback has been studied by some authors[13, 40, 41]. Bias and precision in these studies ranged from -0.11 to -0.372 mg/L for bias and 0.359 -0.6 mg/L for precision for gentamicin[13, 40] and -0.12 mg/L for bias and 3.69 mg/L for precision for amikacin[41]. These values are comparable to the best Bayesian feedback model in this study, where a bias of -0.11 mg/L and a precision of 0.45 mg/L are found (table V).

The introduction of Bayesian feedback in this study, using the best model, slightly improves bias, but is not statistically superior to the no TDM model. Furthermore use of this NPEM model only selects 3 out of 9 patients with high trough concentrations and thus is not of much practical use in pinpointing individuals at risk for prolonged exposure to tobramycin. Use of the no TDM model leads to a substantial number of relatively high trough serum concentrations since approximately 25% of trough concentrations were > 1 mg/L. In this study neonates with serum concentrations taken outside the 1h limit of the GA–related interval were left out of the analysis. Since it is possible that the difference in timing of the interval is related to more extreme pharmacokinetic parameters in the group not included, we also looked at the total groups of neonates (342 in the 1-6 group, 61 in the 3-8h group). No significant differences for bias and precision of both linear and NPEM models are found between the study groups and the total groups (data not shown).

Several studies have looked at covariates for explaining the large interindividual variation of aminoglycosides in neonates. As in other studies this study shows a GA related increase in K_{el} and decrease of V_d [42-44].

This study confirms the negative correlation between the AS5 and serum trough concentration seen before [43, 44]. The relation to term neonates can be expected because the AS5 is a better predictor of hypoxia and concomitant renal failure in term than preterm newborns [45]. The decrease of serum trough concentration in preterms with prenatal exposure to corticosteroids might be a reflection of increased intra-uterine maturation of kidney function [46]. In contrast to other studies no relation to PNA or administration of indomethacin is found [42-44, 47]. This is probably due to the fact that 92% of neonates received tobramycin within 48h of birth in combination with the fact that indomethacin is often started on day 3.

How should these results be translated to dosing and therapeutic drug monitoring of aminoglycosides in neonates?

Since adequate initial peak serum concentrations were found in both this and our earlier study, routine measurement of peak serum concentrations does not seem to be warranted[9]. A direct relation between serum concentrations and toxicity has not been shown in neonates. Toxicity seems to be related to high trough concentrations and duration of therapy in adults[19, 48]. The currently accepted opinion is that aminoglycoside induced toxicity does not seem to be associated with short courses of antibiotics (<5 days). It is possible that a prolonged period of aminoglycoside concentrations below MIC might permit bacterial regrowth. Monitoring trough serum concentrations before the second dose might allow us to prevent potential bacterial regrowth (low concentrations of the aminoglycoside) or toxicity (high concentrations of the aminoglycoside). However it remains questionable if this trough concentration has any value in predicting the amount of tobramycin exposure after the second, third or fourth dose of the aminoglycoside[26].

Although we were not able to predict trough concentrations adequately enough, others have indicated the possibility of predicting steady state peak and trough concentrations in the first week of life, with a single serum sample taken after the first dose[13, 41]. Neither of these studies looked specifically at trough serum concentrations or compared their strategies to not performing TDM. Given the fact that bias and precision in these studies is comparable to ours it is unlikely that predictive performance in these studies would be superior to not using TDM. In conclusion, our results indicate that routine early TDM is not useful in neonates in the first week of life. Prediction of individual tobramycin dosing intervals can not be improved by the use of 1-6h or 3-8h serum concentrations after the first dose. Neonates in the first week of life should be started on the proposed dosing schedule of 4 mg/kg dose with a GA related interval of 24,36 or 48h [9]. TDM should only be performed routinely in neonates receiving tobramycin for longer than 5 days for toxicity reasons. Patients with renal failure as well as patients with obvious neonatal asphyxia (e.g. AS5 < 5) should be monitored more closely.

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Vancomycin population pharmacokinetics in neonates

Chapter

4

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SUMMARY

Background: Recently the value of vancomycin therapeutic drug monitoring as well as the required therapeutic range has been subject of debate, resulting in new recommendations. This study was performed to incorporate these new insights in an up-to date dosing scheme for neonates of various gestational ages.

Methods: A retrospective study with prospective validation. 108 newborn infants with suspected central line related septicemia during the first month of life received vancomycin 30 mg/kg/day divided into 2 doses regardless of gestational or post-conceptional age. Trough and peak vancomycin serum concentrations were determined before and after the third dose. Vancomycin data were analyzed according to a one-compartment open model with use of NONMEM population pharmacokinetic software. Model parameters were evaluated and then used to simulate vancomycin dosing for different dose and dose interval combinations. Targets were a trough concentration between 5 and 15 mg/L and a peak below 40 mg/L. In the prospective study, the optimal scheme was tested in 22 patients.

Results: Of the 108 patients, 34.3% of measured trough- and 17.6% of peak concentrations were outside the desired therapeutic range. The model best fitting the data included clearance and volume per kg and was independent of gestational age (GA). Simulation of various dosing schemes showed that a dosing schedule of 30 mg/kg/day, irrespective of GA, in three doses was optimal, and this scheme was prospectively tested. Mean trough concentrations before the second dose were 8.2±2.2 mg/L versus a predicted trough of 8.9±2.5 mg/L. No peak levels higher than 40 mg/L were found.

Conclusions: the use of the proposed schedule leads to adequate vancomycin trough serum concentrations and there is no need for routine monitoring of peak serum concentrations.

INTRODUCTION

The immunologically incompetent premature neonate is especially susceptible to invasive Gram-positive infections through invasive procedures such as central venous lines. *Staphylococcus aureus* and coagulase-negative staphylococci account for up to 31% of nosocomial infections in newborn infants[1]. Vancomycin is the first-choice antibiotic for treatment of these infections in neonates. This glycopeptide antibiotic, which is bactericidal through inhibition of cell wall synthesis, has been used in pediatric patients, including neonates, since 1959[2]. Historically, vancomycin dosing has been titrated to obtain peak serum concentrations between 20-40 mg/L and serum trough concentrations of 5-10 mg/L. These therapeutic goals are widely used in pediatrics and neonatology. There are, however, no controlled clinical trials that show a relation between serum concentrations and clinical response.

Vancomycin reportedly has potential oto- and nephrotoxic side effects. These side effects however are rare, especially after removal of impurities from preparations in the 1960s. Ototoxicity is characterized predominantly by transient tinnitus and hearing loss[3] and has not been described in neonatal patients. Nephrotoxicity has been reported incidentally[4-8], especially when given in combination with an aminoglycoside. Recent research showed no relation between peak serum concentrations > 40 mg/L and nephrotoxicity in neonates[9], although a relation to very high concentrations over > 60 mg/L is suggested[8]. Therefore recent papers have discussed the necessity of therapeutic drug monitoring of vancomycin[10-13]. In light of these and other papers it seems to be more clinically relevant to look at serum trough concentrations as the main determinant of effective therapy[14, 15]. Some studies suggest that minimum trough levels of at least 10 mg/L should be obtained for efficient therapy[16]. As yet no prospective clinical trials have investigated this hypothesis, and no dosing schemes based on target trough concentrations have been described in neonates. The aim of our study was to retrospectively investigate population pharmacokinetics of vancomycin in infants. To that purpose we simulated various dosing schedules to determine which dosing scheme would be optimal. Finally we prospectively evaluated this optimal dosing scheme to determine its value in clinical practice.

PATIENTS AND METHODS

Study design

Retrospective study with prospective validation.

Patients

All neonates with a postnatal age of less than 29 days who were treated with vancomycin, in the neonatal intensive care unit of the Sophia Children's Hospital between August 1992 and December 1997 were eligible for this study. Infants were only included if their paired peak and trough serum vancomycin concentrations were available.

Parameters

All parameters were abstracted from the patient files. Gestational ages (GAs), birthweights and weights at start of antibiotic therapy were recorded. GAs were determined on the basis of the mother's menstrual history, confirmed by early ultrasound examinations if available, and by physical examination with the use of the criteria of Dubowitz et al [17].

Administration and dosage regimen of vancomycin

Vancomycin was given as empiric treatment for suspected neonatal sepsis with a line-related focus, or after confirmation of a positive blood culture with coagulase-negative staphylococci. Patients with culture proven invasive bacterial infection received at least 7 days of intravenous therapy. Administration of vancomycin was performed in a 1h i.v. infusion with the following dosing regimen: 30 mg/kg /day divided in 2 doses, irrespective of GA.
All doses and times of administration were recorded routinely. Trough and peak blood samples were taken before and 1h after completion of the third dose. Dosage adjustments were made according to the outcome, with the intention to keep trough concentrations between 5 and 10 mg/L and peak concentrations between 20 and 40 mg/L.

Analytical Techniques

Concentrations of vancomycin were measured by a Fluorescence Polarization Assay using a TDxFLx (Abbott Diagnostic Division, Amstelveen, NL). The coefficient of variation for this test in our laboratory was 5.1% at 7 mg/L and 2.9% at 35 mg/L.

Data Analysis + Dosage recommendations and simulations

Vancomycin data were analyzed according to a one-compartment open model, assuming the data were attributable to the third dose after birth, using NONMEM population pharmacokinetics software (NONMEM version V, NONMEM project group, University of California, San Francisco, CA) with a number of different models; all were one compartment models with a constant coefficient of variation intra- and inter-individual error model. First order conditional estimation (FOCE) was applied in all cases. Individual empirical Bayes parameter estimates were generated to examine possible covariate relationships. The different models were compared using the minimum value of the objective function (likelihood-ratio test). Model parameters (including residual variability) were used to simulate vancomycin dosing in 100 subjects for different dose and dose interval combinations. The target trough concentration was 5-15 mg/L and peak concentrations preferably below 40 mg/L.

Prospective study

The predictive performance of the proposed dosing regimen was evaluated prospectively in patients receiving vancomycin according to the dosing recommendation mentioned in the results: 30 mg/kg/day divided in 3 doses.
Vancomycin trough serum concentration was determined prior to the second dose, to ascertain adequate therapy after the first dose. Furthermore peak and trough serum concentrations were determined before and 1h after the fifth dose to detect possible accumulation of vancomycin. The fifth dose was chosen because in the model as described, this was the dose at which a steady state was reached.

RESULTS

Retrospective study

Vancomycin trough and peak concentrations were obtained in 115 neonates. Data for seven neonates were indicated as aberrant because results were not identifiable as being either peak or trough concentrations and removed from the data set. Results mentioned are of the remaining 108 neonates.

Their GAs and birth weights ranged from 24 to 41 weeks (median age, 28.9 weeks) and from 485 to 4625 g (median weight 1002 g), respectively. Postnatal age and weight at start of therapy ranged from 3 to 27 days (median age, 14 days) and from 510 to 4410 g (median weight, 1045 g), respectively. Figure 1 and 2 summarize the results of vancomycin peak and trough concentrations for the various post-conceptional age (PCA) groups.

As shown, 22.2% of peak concentrations and 34.3% of trough concentrations were outside the desired therapeutic range. 17.6% of trough concentrations was below 5 mg/L.

Population pharmacokinetic analysis was performed with different models in which GA, PCA, clearance, volume, clearance per kg and volume per kg were used as parameters. The best description of the data was found in the model using clearance per kg and volume per kg. In this model empirical Bayes estimates of clearance and volume did not correlate with weight measures; the r^2 (squared correlation coefficient) between volume and gestational and post-conceptional age measures was less than 0.1 indicating that less than 10% of the variability could be explained by age. Population pharmacokinetic parameters for this model were a clearance of 0.057 ± 0.0018 l/hr/kg ((inter-individual variability 31%) and a volume of distribution (Vd) of 0.43 ± 0.013 l/kg ((inter-individual variability 25%). For descriptive purposes an additional model parameterized in terms of clearance/kg and half-life was constructed. The population average half-life was 6.0 h with a standard error of 0.27 h and a coefficient of variation of 34%. Evaluation of these models indicate that vancomycin population pharmacokinetics in neonates is best described using clearance per kg and volume per kg. If dosed per kg this means that the expected concentration profile is independent of weight or age e.g. with the same Cmax and half-life and therefore the same Cmin. Deviations from this profile are due to inter-individual differences and not caused by differences in covariates.

Fig. 1: Peak concentrations in the retrospective study group

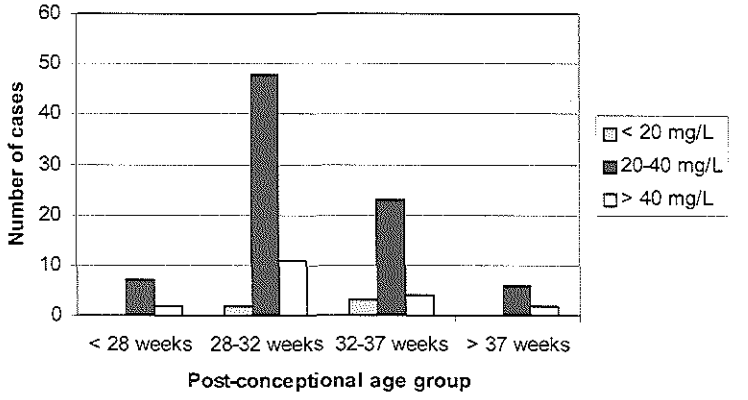
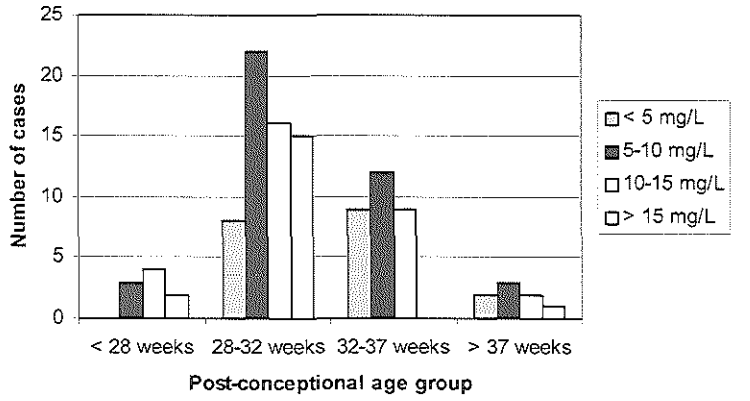


Fig. 2: Trough concentrations in the retrospective study group



Using these pharmacokinetic parameters several vancomycin dosing regimens were simulated to determine which dosing schedule would be optimal in view of the target trough concentration. Results show that once daily administration of 30 mg/kg would lead to an average peak concentration after the first dose of 59.9 mg/L and a trough (at 24 h) of 3.7 mg/L. Other simulated dose and dose interval combinations are shown in figure 3 and 4. As can be seen twice daily dosing of 15 mg/kg/dose leads to an unacceptable percentage of undesired trough and peak concentrations, whereas 20 mg/kg

twice daily, results in too much accumulation, indicating that these regimens are not optimal. Thrice daily dosing leads to accumulation when giving 12 mg/kg/dose and to a substantial percentage (27%) of ineffective initial trough concentrations when giving

Fig. 3: Predicted trough concentrations with tested dose regimens. Numbers are percentage of predicted trough concentrations < 5 mg/L

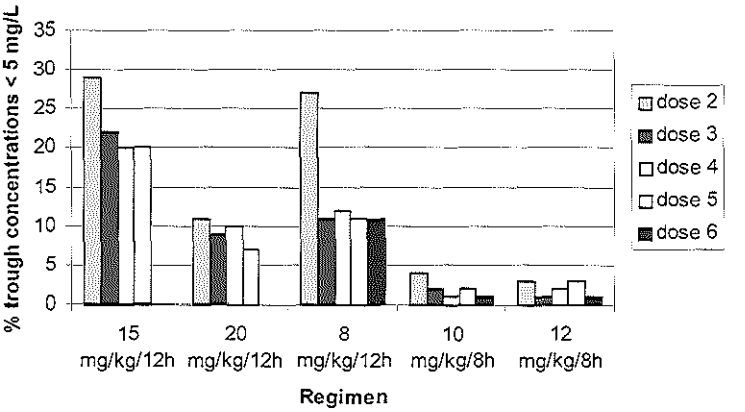
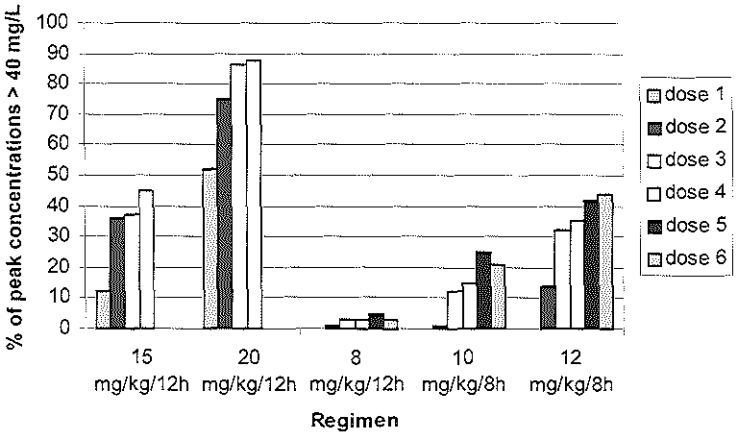


Fig. 4: Predicted peak concentrations with tested dose regimens. Numbers are percentage of predicted peak concentrations > 40 mg/L



8 mg/kg/dose. The best regimen tested was 10 mg/kg/dose, in which less than 5% of predicted trough concentrations is below 5 mg/L and steady state is reached after dose 5. Predicted peak concentrations after the 5th dose range from 22.4 to 50.0 mg/L (mean 34.3 ± 7.7 mg/L). Trough concentrations with this regimen range from 4.3 to 15.0 mg/L (mean 8.9 ± 2.5 mg/L) before the second dose and 5.0 to 30.6 mg/L (mean 15.7 ± 6.3 mg/L) before the 5th dose.

Prospective study

The application of the vancomycin 10 mg/kg/8h regimen was prospectively tested in 22 patients. Table I shows the demographic variables for the prospective study group in relation to the retrospective group. Postnatal age was significantly lower in the prospective group (p=0.026), compared to the retrospective group. GA, birth weight, weight at start of therapy and PCA were not significantly different.

Table I: demographics of retrospective and prospective group

	Retrospective group Range (median)		Prospective group Range (median)		p-value (one way ANOVA)
Gestational age (weeks)	24-41	(28)	25-42	(29)	0.488
Birthweight (grams)	485-4625	(1002)	770-3500	(1102)	0.625
Actual weight (grams)	510-4410	(1045)	730-3420	(1160)	0.469
Postconceptional age (weeks)	26-42	(31)	27-43	(31)	0.832
Postnatal age (days)	3-27	(14)	7-21	(11)	0.026

Table II shows the results of vancomycin serum concentrations. As shown, 95.5% of initial trough concentrations was in the desired therapeutic range. Vancomycin trough concentrations before the second dose ranged from 3.4 to 12.5 mg/L (mean 8.2± 2.2) mg/L). Trough concentrations before the 5th dose ranged from 4.6 to 20.6 mg/L (mean 12.3 ± 4.1 mg/L). Trough concentrations before the 5th dose were significantly (paired samples T-test, p< 0.001) higher than before the second dose. Both before the second and 5th dose only one trough concentration was below 5 mg/L. Vancomycin trough serum concentration before the 5th dose was higher than 15 mg/L in four cases, with serum concentrations of 15.3, 17.8, 20.6 and 20.6 mg/L respectively.

Table II: Vancomycin serum concentrations (prospective study)

Vancomycin serum concentration	Trough 1	Trough 5	Peak 5
< 5 mg/L	1 (4.5 %)	1 (4.5 %)	
5-15 mg/L	21 (95.5 %)	17 (77.3 %)	
> 15 mg/L	0 (0.0 %)	4 (18.2 %)	
<20 mg/L			3 (13.6 %)
20-40 mg/L			19 (86.4 %)
> 40 mg/L			0 (0.0 %)
TOTAL	**22 (100 %)**	**22 (100 %)**	**22 (100 %)**

Numbers are the number of patients with vancomycin serum concentrations within specified range before dose 1 and 5 and after dose 5. Numbers in parentheses are the percentages of the total in the group.

Peak concentrations after the 5th dose ranged from 16.6 to 34.5 mg/L (mean 25.8 ± 5.0 mg/L). There was no significant relation between serum concentrations at any of the three sample points and GA, postnatal age (PNA) or post-conceptional age (PCA)

DISCUSSION

Historically vancomycin dosing in neonates, similar to aminoglycosides, has been subject to therapeutic drug monitoring for two reasons: toxicity and clinical effect.

Apart from the infusion related histamine like reaction (red-man syndrome), due to impurities in the earlier preparations of vancomycin in the sixties, side-effects are relatively rare [10]. Proof of vancomycin related nephro- or ototoxicity in adults is circumstantial and probably only true for a selected high-risk population [18]. Furthermore this toxicity was described using the old formulation of vancomycin. Ototoxicity has not been described in neonates. Vancomycin-related related nephrotoxicity is rare and no relation between nephrotoxicity and serum concentrations has been found [9, 19]. The few neonates with nephrotoxicity all had documented very high serum concentrations, so it seems prudent to keep peak serum concentrations below an arbitrary threshold of 40 mg/L, but this in itself is insufficient reason for therapeutic drug monitoring.

Efficacy of vancomycin related to serum concentrations has been under debate. Several mainly in vitro studies have been performed to determine which pharmacodynamic

parameter correlates with efficacy of vancomycin. Serum bactericidal titers of 1:8 or more (corresponding to serum concentrations of > 12 mg/L) were associated with clinical cures in children [20]. In vitro models have shown no correlation between killing rates and vancomycin concentrations higher than 2-8 mg/L [15, 21]. In animals, outcome of endocarditis was related to vancomycin trough serum concentrations [22]. Although it is not possible to draw a definite conclusion as to which pharmacodynamic parameter is best correlated to efficacy, these studies indicate that keeping the trough level above the MIC is necessary to obtain clinically good results. Monitoring of vancomycin serum concentrations should be focussed mainly on keeping adequate trough concentrations. For this study goals were set at trough concentrations of 5-15 mg/L and peak concentrations preferably below 40 mg/L.

Using population pharmacokinetics we first established the parameters that best described our retrospective data. In contrast to most other studies [4, 23-31], these parameters included clearance per kg and volume per kg, but not PCA. Given the fact that each patient only contributes two data points, the dependence of for instance Cl/kg on GA would have to be strong to be detected by the method used. This strong dependence was not found; in our data there is only a trend towards higher serum concentrations at lower GA. Elimination half-life and clearance of most drugs is longer in preterm neonates, partly due to a higher percentage of body water. However this factor and the postnatal increase of renal function[32] change considerably in the first postnatal week, during which vancomycin is seldomly given. Furthermore most (81.8 %) of our patients were antenatally exposed to intra-uterine corticosteroid administration, which diminishes the GA-dependent difference in metabolism of different antibiotics [33, 34]. These factors might explain the mitigated GA effect in our retrospective data. The independence of PCA was confirmed in the prospective study.

Reported mean clearance, Vss and $t_{½\beta}$ for neonates and infants ranges from 0.036 to 0.1 L/kg/hr, 0.44 to 0.97 L/kg and 3.0 to 12.0 hrs, respectively[19]. Clearance and Vd found for our model were comparable to the other neonatal population pharmacokinetic study of Seay et al. [35]: CL of 0.057 ± 0.0018 vs 0.018 to 0.059 L/kg/hr, Vd 0.43 ± 0.013 vs 0.50 L/kg, though in this study CL was corrected for GA < 32 weeks and dopamine use, explaining the wide range. Elimination half-life in our population was quite different: $t_{½\beta}$ 6.0 ± 0.27 hrs compared to 13.4-33.7 hrs, and more in concordance with the range mentioned in other studies [19]. Based on the result of these parameters, and the desired therapeutic range mentioned before, we tested several possible vancomycin dosing

schemes (table II), of which 10 mg/kg/dose, three times a day led to a minimization of undesirable high peak- and especially too low trough serum concentrations. Prospective evaluation showed that indeed only 4.5% (1 of 22) of initial trough serum concentrations was below 5 mg/L, assuring effective therapy from the start. No potentially toxic peak serum concentrations were found. In 4 patients serum trough concentrations before the fifth dose were arguably too high, but still not higher than 20.6 mg/L. We did not simulate constant rate infusion of vancomycin which was recently advocated[24]. Though this seems a logical approach to obtain desirable steady state vancomycin serum concentration, there are several reasons why we do not consider this a feasible option. One of the main treatment modalities for line related infections is removing the central venous line. This would make continuous dosing of vancomycin unpractical in this group of patients in whom venous access is not always easy. Furthermore drug interactions, though not found in this study[24], are a potential hazard. Last but not least, the time relation between vancomycin serum concentrations and microbial kill rates does not warrant such a cumbersome dosing method.

In concurrence with recent literature and given our results there is no obvious need to monitor peak vancomycin serum concentrations in neonates without a strong suspicion of renal insufficiency [12, 13, 36], though this is contradicted by one author [37].

In conclusion we have shown that the application of a pharmacokinetic population model, built on retrospective data, is useful in determining a practical dosing scheme. Prospective validation shows that vancomycin dosing in neonates can be simplified to a GA-independent schedule of 10 mg/kg/8h and leads to adequate vancomycin trough serum concentrations before the second dose, without potentially toxic peak serum concentrations. Our data indicate a need for routine monitoring of trough, but not of peak vancomycin concentrations in neonates.

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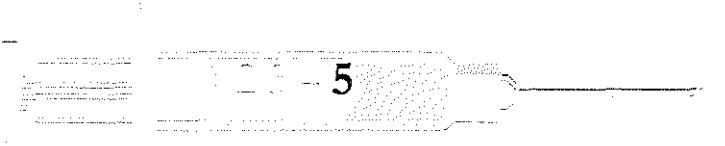
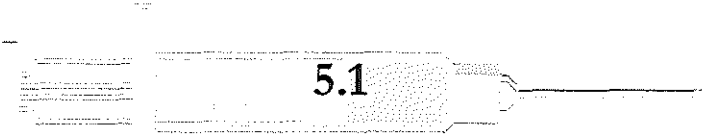
Ototoxicity related to neonatal use of tobramycin and/or vancomycin

Chapter



A pilot case control follow-up study on hearing in children treated with tobramycin in the newborn period

Chapter



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Submitted

SUMMARY

Objective: To assess the occurrence of hearing loss in children due to neonatal exposure to long courses of tobramycin and/or high tobramycin serum concentrations.

Methods: This was a pilot case-control study in 3-4 year old children. Data on tobramycin administration were abstracted from the patient files of an earlier study. Patients exposed in the neonatal period to either long courses (> 7days) or high serum concentrations of tobramycin constituted the study group. The control group consisted of patients without tobramycin exposure. Patients were matched for other risk factor according to criteria of the Joint Committee on Infant Hearing. All patients underwent the following investigations: otoscopy and pneumatic otoscopy, followed by impedance audiometry, to exclude middle ear effusion. Click-evoked oto-acoustic emissions (ce-OAE) as well as distortion product oto-acoustic emissions (dp-OAE), tested at f2 frequencies ranging from 1-10 kHz, were measured to assess hearing. All patients with abnormal ce-OAE results underwent brainstem electric response audiometry (BERA) as well. Since aminoglycoside ototoxicity is usually bilateral, results were compared per patient and not per ear.

Results: A total of 29 patients were tested. Eleven patients were excluded due to middle ear effusion. Data for 18 patients were analyzed. In the tobramycin treated group (n=9) both ce-OAE and dp-OAE (at all tested frequencies) were not detectable in 6 ears of 3 patients. All other patients had normal ce-OAE's as well as normal dp-OAE's in this frequency range. Difference between the tobramycin treated and control group for OAE as well as dp-OAE showed a trend (p=0.08). In all three patients with undetectable emissions BERA confirmed a cochlear loss of 60-70 dB at 3 kHz in both ears. These three patients had the longest total exposure to tobramycin: 20-24 days and 84-92 mg/kg, respectively. No relation to either peak or trough serum concentrations could be detected.

Conclusion: There was no statistical relation between hearing loss and tobramycin exposure, probably due to sample size. Our results do indicate a need for a case-control follow-up study of hearing in neonates exposed to long courses of aminoglycosides.

INTRODUCTION

Bacterial infections play an important role in the morbidity and mortality of preterm neonates[1]. Aminoglycosides are effective against most gram-negative infections in infants and play an important role in the initial empiric treatment of neonatal septicemia.

The most important specific adverse effects of aminoglycosides are nephro- and ototoxicity. The incidence of nephrotoxicity is not well known, but seems to be considerably lower in preterm infants than in adults[2].

Aminoglycosides accumulate in the lymphatic fluid of the inner ear and are potentially cochleo- and vestibulotoxic. Outer hair cells, inner hair cells and spiral ganglional neurons are damaged in a process of excitotoxic cell death due to enhancement of glutamate N-methyl-D-aspartate (NMDA) receptor activity[3]. Cochlear hearing loss can be divided in acute reversible and chronic irreversible ototoxicity with a total reported incidence of 0 to 47% in adults[4]. Hearing loss is mainly bilateral and starts in the high frequency range above 5 kHz, but is also found in lower frequencies in serious cases[5]. Reported occurrence of aminoglycoside induced ototoxicity in neonates is low in the range of a few percent[6, 7]. The risk of developing clinically significant hearing problems in neonates treated shorter than one week seems to be small[6, 8, 9]. Suggested risk factors associated with aminoglycoside induced ototoxicity are elevated peak serum concentrations and duration of therapy, but the actual relation is unclear[6, 9-11]. As in adults, aminoglycoside extended dose intervals are recommended for neonates[12-14]. These schedules advise doses of 3.5-4 mg/kg with intervals of 24-48h for gentamicin and tobramycin with concomitant higher peak serum concentrations and lower troughs. Several studies did not find a relation between these new dosing regimens and ototoxicity, but since the incidence is low, study size was possibly too small to detect a difference[12, 15, 16].

There are many pitfalls in relating neonatal hearing loss to aminoglycoside use. Numerous risk factors for neonatal hearing loss have been identified. Perinatal infections, meningitis, prematurity, hyperbilirubinemia, birthweight < 1500 grams, asphyxia, respiratory distress syndrome, mechanical ventilation, antibiotics, and diuretics have all been incriminated[9]. Potentiation of aminoglycoside induced hearing loss due to loop diuretics has been described[17]. Furthermore delayed onset of hearing loss, possibly related to aminoglycoside use has been described[9, 18-20]. Hence aminoglycoside induced hearing loss in neonates should be studied in the light of these concomitant factors.

The aim of the present pilot study was to assess the occurrence of hearing loss due to neonatal high-risk exposure to aminoglycosides, defined as long courses and/or high

serum concentrations. For this purpose 3-4 year old children, exposed in the neonatal period to either long courses (> 7days) or high serum concentrations (peak > 12 mg/L, trough > 2 mg/L) of tobramycin were compared to patients without tobramycin exposure, but matched for other risk factors of hearing loss.

PATIENTS AND METHODS

Study design

The present study was a pilot case-control study of hearing loss conducted in children of 3-4 years old admitted to the NICU of the Sophia Children's Hospital during their the neonatal period.

Patients were selected from an earlier study on the emergence of antibiotic resistance due to antibiotic use[21]. In that study (study period December 1996 – December 1997), neonates admitted to one ward received initial treatment for suspected septicemia with a combination of penicillin-G or flucloxacillin and tobramycin. In the other ward the initial treatment consisted of amoxicillin or flucloxacillin with cefotaxime. All other treatment protocols for these wards were equal. Patients from that study were divided in two groups; those who had received tobramycin during admission (eligible for study group), and those who had only received other antibiotics (eligible for control group).

This study was approved by the institutional review board and patients were only included after informed consent from a parent or guardian was obtained.

Study group

All patients whose records indicated that they had received tobramycin were reviewed. Patients with an increased risk of tobramycin related toxicity were identified. Risk factors were defined as prolonged exposure (> 7 days), elevated peak serum concentrations (> 12 mg/L) or elevated trough serum concentrations (> 2 mg/L). All patients with one or more of these risk factors whose current address could be traced were approached and, if informed consent was obtained, included.

The dosage regimen used in the period 1996-1997 was as follows. Tobramycin (4 mg/kg) was administered as a 30-minute intravenous infusion. The initial dosing interval was 24,

36 or 48 hours in neonates with a gestational age of <32 weeks, 32-37 weeks and ≥ 37 weeks, respectively. Therapy adjustments were made at the discretion of the attending physician. All doses and times of administration were recorded routinely. Tobramycin serum samples were taken as part of routine therapeutic drug monitoring 1 and 6 hours after the first dose and just before the second dose.

Control group

For each patient in the study group matched controls were identified in the non-tobramycin group. Patients were matched for risk factors for neonatal hearing loss, according to criteria of the Joint Committee on Infant Hearing[22]. These were defined as: family history of hereditary childhood sensorineural hearing loss, in utero infections (e.g. toxoplasmosis, herpes), craniofacial anomalies and other syndromes related to hearing loss, birthweight < 1500 g, hyperbilirubinemia requiring exchange transfusion, bacterial meningitis, Apgar scores of 0-4 at 1' or 0-6 at 5', mechanical ventilation for more than 5 days, use of loop diuretics and use of vancomycin. The first matched control without middle-ear effusion in whom measurement of oto-acoustic emissions was technically possible, was included in the study.

Data collection and audiologic testing

Risk factors for hearing loss in the intervening period between neonatal admission and time of audiologic testing were assessed by interviewing the parents. Exposure to other ototoxic drugs, meningitis and head trauma as well as familial hearing loss were excluded. Frequency of otitis media with or without antibiotics, paracentesis or ear operations were recorded.

Investigators were blinded to the antibiotic history of the patient. All patients underwent the following investigations. The tympanum was visualized using a binocular Zeiss OPMI-9 microscope. Pneumatic otoscopy was used to assess mobility of the tympanic membrane. Test results per ear were labeled as normal (aerated), containing fluid, or a diminished mobility. Tympanometry was performed using a clinical impedance device (GSI33, Grason Stadler). Tympanograms were defined as type A, B or C according to Jerger, where type A is normal [23].

Otoscopy and tympanograms were used to determine the validity of test results of the otoacoustic emissions. In cases with an abnormal tympanogram and/or abnormal

otoscopic result the absence of otoacoustic emissions was considered as due to a middle ear dysfunction.

Otoacoustic emissions were measured using an ILO96 OAE-instrument (OtoDynamics, UK). Click-Evoked otoacoustic emissions were recorded, with the standard non-linear click sequence stimulation, at a level of 82 dBSPL, with the real time low pass filtering enabled during recording, and a response pass band filter between 700 Hz and the instruments upper frequency limit (6.25 kHz). Distortion Product Oto Acoustic Emissions (dp-OAE) were also recorded at the 2f1 – f2 frequency, with primary levels of 60 and 55 dBSPL for f1 and f2, respectively. F2 frequencies of 1kHz- 10 kHz were used with a resolution of 3 points per octave. Scoring on absence or presence of the ce-OAE's and dp-OAE's was done subjectively by an expert judge (author # 2).

All patients without middle ear dysfunction, but with abnormal ce-OAE results underwent brainstem electric response audiometry (BERA) as well.

Statistical analysis

Statistical analysis was performed using SPSS 8. 0 statistical software (SPSS Inc., Chicago, USA). Comparison between patients exposed to tobramycin and matched controls was performed using a paired samples t-test.

RESULTS

A total of 59 patients had received tobramycin for ≥ 7days and/or were exposed to tobramycin serum concentrations outside of the desired therapeutic range. The current address of 30 could be traced of whom 12 responded. These twelve patients constituted the tobramycin group. A total of 17 patients were tested in the control group. Eleven patients were excluded of whom three were exposed to tobramycin . In these 11 patients no dependable otoacoustic emissions could be recorded, despite retesting, apparently due to middle ear effusion. Eighteen patients were included, 9 patients with exposition to tobramycin and 9 patients without. Individual demographic variables for these patients are listed in table 1. All patients were intubated or had a nasopharyngeal tube for more than 5 days. No patient had a bilirubin level necessitating exchange transfusion. Patient 1 and 2 are matched for GA, but not for birthweight.

A total of 33 out of 36 ears were evaluable with ce-OAE and dp-OAE. Three patients (one with tobramycin) had middle ear effusion in one ear.

In the tobramycin treated group both ce-OAE and dp-OAE were not detectable in 6 ears of 3 patients. Results for dp-OAE showed no emissions in the range of 1-10 kHz for both ears. All other patients had normal ce-OAE's as well as normal dp-OAE's in this frequency range. Difference between the tobramycin treated and control group for ce-OAE as well as dp-OAE showed a trend ($p=0.08$), but did not reach statistical significance. In all three patients with undetectable emissions, BERA confirmed the abnormalities. In all three patients a cochlear loss of 60-70 dB at 3 kHz in both ears was found. These three patients had the longest total exposure to tobramycin: 20-24 days and 84-92 mg/kg, respectively. No relation to either peak or trough serum concentrations could be detected.

Patient 5 had a right sided grade III intraventricular hemorrhage. Patients 7 and 9 are heterozygote twins. Patient 9 showed bilateral periventricular leucomalacia on cerebral sonography.

Table 1: Individual parameters of patients included in the study

Patient	Tobramycin: total exposure (days)[1]	Tobramycin: total exposure (mg/kg)	Maximum peak[2]	Maximum trough[3]	Vancomycin exposure (days)	Furosemide exposure (mg/kg)	Gestational age (weeks)	Birth-weight (grams)	AS 5[4]
1	8	8	7.5	1.6	0	0	31 0/7	995	7
2					0	2	31 6/7	2010	9
3	8	8	11.1	0.3	0	0	28 0/7	950	9
4					0	0	27 6/7	1190	7
5	20	8	9.8	0.7	0	4	27 0/7	1040	5
6					0	0	29 4/7	1235	6
7	22	14	7.7	1.1	13	0	26 1/7	1050	9
8					6	2	27 0/7	1130	7
9	24	15	7.8	0.6	0	1	26 1/7	960	7
10					0	2	24 6/7	670	10
11	6	6	14.8	0.9	0	0	37 5/7	3155	9
12					0	0	37 0/7	3800	9
13	16	12	9.7	0.6	0	2	25 5/7	1130	7
14					0	0	29 3/7	1450	8
15	16	9	8.2	1.1	22	22	37 0/7	2840	10
16					30	30	39 6/7	4000	10
17	14	7	14.0	2.3	4	4	28 1/7	860	8
18					1	1	28 1/7	1140	8

[1] Longest consecutive treatment (days), [2] Maximu peak serum concentration, [3] Maximum trough serum concentration, [4] 5' Apgar score

DISCUSSION

The relation between administration of aminoglycosides and ototoxicity has been under discussion since early reports in 1945[24]. Studies in adults have suggested an influence of absence of sufficiently low trough concentrations for adequate periods, but there is little evidence for a relation between peak or trough serum concentrations and ototoxicity[5, 11, 25, 26]. In adults as well as neonates a relation between ototoxicity and total dose and duration of therapy is postulated[8-10]. Treatment for longer than 10 days is considered to be a risk factor[5, 8]. Reported aminoglycoside ototoxicity in neonates is low, also with present day extended interval dosing[7, 12, 16, 27]. No relation between serum concentrations of aminoglycosides and ototoxicity has been demonstrated. Also, hearing screening in neonates did not show an increase in occurrence of hearing loss in aminoglycoside exposed patients[28-31]. These studies did not however look specifically at serum concentrations. On the other hand several authors have demonstrated that, mainly reversible, abnormalities on BERA can be seen early on in aminoglycoside treated infants, indicating alteration of the central transmission of auditory brainstem responses[10, 18, 32, 33]. Furthermore it has been demonstrated in adults and suggested in neonates that aminoglycoside related ototoxicity can occur weeks to months after discontinuation of treatment[5, 18-20]. In the study by Kawashiro et al., all patients with this type of hearing loss were exposed to 7-14 days of treatment with gentamicin and/or amikacin, next to other risk factors[19]. To capture the possibility of delayed hearing disturbance we investigated the patient group most at risk for aminoglycoside related ototoxicity at the age of 3-4 years. Since aminoglycoside ototoxicity is mostly bilateral we chose not to compare patients to matched controls per ear but per patient. Although no statistical significance was found, possibly due to the small number of patients, it is worrisome that three patients in this high-risk group had moderate to severe cochlear hearing loss. No relation to either peak or trough serum concentration could be detected. Also hearing loss, which if induced by aminoglycosides was expected to be mainly in the higher frequency range, was apparent at all frequencies in our patients. This could imply that these were more severe cases of aminoglycoside induced hearing loss, where damage has progressed beyond the basal turn of the cochlea. There was a striking relation to total exposure; the three patients with hearing loss had the highest overall exposure to tobramycin (duration as well as in mg/kg) of the 9 patients studied. This result is in concordance with several other studies[8-10]. Borradori and co-workers performed a case-control study in 8 neonates with sensorineural hearing loss, all exposed to more than 10 days of aminoglycoside treatment

and found a significant relation to cumulative dose and total treatment duration of aminoglycosides[9]. In the study by Chayasirisobhon et al., neonates treated with gentamicin for ≥ 10 days were at a significant greater risk of having abnormalities on BERA than neonates exposed to < 10 days of treatment[8]. Patients in both studies were not matched for all other risk factors. Therefore abnormalities might have been related to underlying co-morbidity. An earlier study also found a correlation between wave V latencies on BERA with the total dose of aminoglycosides administered[10]. Hearing was evaluated within 2 days of end of treatment. Therefore abnormalities might have been reversible.

Since two of our patients with hearing loss were twins, familial hearing abnormalities, possibly of mitochondrial origin could be the cause[34]. Family history for hearing loss was negative and permission for mitochondrial DNA analysis was refused.

How should we perceive the seemingly conflicting evidence of aminoglycoside related ototoxicity in newborns? On the one hand screening programs found no relation between exposure to aminoglycosides and hearing loss[28-31]. Also, with the present extended dose intervals, aminoglycoside related ototoxicity is rare [12, 16]. On the other hand several studies in relatively few patients, including this one, found a relation to duration of therapy. In most of these studies neonates had been exposed to more than 10 days of aminoglycoside treatment[8-10]. Furthermore, there are reports where delayed onset of hearing loss in infants is described[9, 18-20]. It is possible that hearing screening performed in infants before discharge might be too early to detect this type of hearing loss. A second reason might be that aminoglycoside induced hearing loss starts at higher frequencies which are not detected by routine hearing screening methods. These factors imply that aminoglycoside related hearing loss could be underreported. The main limitation of this pilot study is the small number of patients included. Our results do indicate a need for a case-control follow-up study in neonates exposed to aminoglycosides for longer than 10 days compared with patients matched for other risk factors related to hearing loss.

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Newborn hearing screening: tobramycin and vancomycin as risk factors for hearing loss

Chapter

5.2

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Submitted

SUMMARY

Objective: to investigate the chance of detecting hearing loss with neonatal hearing screening in relation to exposure to tobramycin and vancomycin expressed in terms of duration of therapy and serum concentrations.

Methods: Automated Auditory Brainstem Response (A-ABR) hearing screening was performed in all neonates with at least one risk factor as defined by the Joint Committee on Infant hearing. Data on administration of tobramycin, vancomycin and furosemide as well as available serum concentrations were abstracted from patient files of neonates who underwent hearing screening between November 1998 and November 2000. Exposure to these drugs was quantitated in terms of total dose, duration of therapy and, where possible, serum concentrations and related to the result of hearing screening using logistic regression. In patients failing hearing screening, exposure to ototoxic medication was assessed in the light of other risk factors for hearing loss.

Results: A total of 625 patients were analyzed. Forty-five neonates failed hearing screening. Tobramycin, vancomycin and furosemide were used in 508, 130 and 174 patients, respectively.
Exposure to vancomycin and tobramycin in terms of treatment duration, total dose or serum concentrations was not related to failure to pass A-ABR screening. Exposure to both antibiotics in the same patient, as well as combination with furosemide treatment, was also not related to a failure to pass hearing screening. In none of the patients with serum concentrations outside the therapeutic range, exposure to ototoxic medication was the most likely risk factor for hearing loss.

Conclusion: No quantitative or qualitative relation between exposure to tobramycin or vancomycin and a failure to pass hearing screening was found. Routine TDM of vancomycin and tobramycin in neonates for ototoxicity reasons is not helpful in detecting patients at risk for clinically important hearing loss.

INTRODUCTION

Congenital neonatal hearing loss has a reported prevalence of 0.1-0.3%[1, 2]. Neonates admitted to a neonatal intensive care unit (NICU) have a higher risk of developing sensorineural hearing loss of approximately one in every hundred[3]. Late recognition leads to impaired acquisition of language and speech[4]. Therefore, early detection of impaired infant hearing is important, and consequently many neonatal hearing screening programs have been developed. The methods most often used are Oto-Acoustic Emissions (OAE) and Automated Auditory Brainstem Response (A-ABR)[5]. A-ABR is a very reliable screening method with a reported sensitivity of 100% and specificity of over 95% [6-8].

Screening programs in the NICU follow one of two strategies. Screening is either performed in all neonates[6, 9] admitted to a NICU or in neonates with certain risk factors previously described[3]. Risk factors are among others: family history of hearing loss, perinatal infections, meningitis, birthweight < 1500 gms, hyperbilirubinemia, asphyxia, respiratory distress syndrome, mechanical ventilation, diuretics and antibiotics, especially aminoglycosides and vancomycin[10]. These potentially vestibulo- and cochleotoxic antibiotics are frequently used for early (aminoglycoside) and late onset (vancomycin) neonatal septicemia. Vestibulotoxicity is difficult to determine in neonates, so reports on ototoxicity are limited to hearing loss. Vancomycin reported ototoxicity, mainly based on case reports, is < 2% in adults. Data from the current literature do not show a relation between vancomycin related ototoxicity and serum concentrations, and it is not clear whether ototoxicity should be attributed to vancomycin or other confounding factors[11]. Little is known about vancomycin ototoxicity in neonates. The few studies addressing this issue did not find ototoxicity[12, 13].

Aminoglycoside induced ototoxicity in adults usually occurs in patients who have received either long, or repeated, courses of aminoglycosides[14]. A relation between high serum concentrations and toxicity has been suggested, but not demonstrated. There are still many gaps in our knowledge on the relation between aminoglycoside use and hearing loss during infancy. Even though some studies show a relation to administration of aminoglycosides, it remains difficult to separate the effect of aminoglycoside use from other confounding factors[15-17]. No clear relation was found to peak and trough concentrations and most of these studies did not correct for concomitant risk factors. Concurrent treatment with furosemide and vancomycin was associated with hearing loss[18, 19]. Furthermore these studies were performed in neonates receiving aminoglycosides several times daily, while over the last few years dosing intervals, similar

to dosing regimens in adults, have been extended[20, 21]. Ototoxicity should be studied in the light of these new regimens. It is also clear that this toxicity has to be seen against the background of other risk factors. Although several recent screening studies in neonates have not shown aminoglycoside administration to be an important risk factor, no study has looked specifically at serum concentrations and duration of therapy[1, 22-24]. The aim of the present study was to explore the risk of detecting hearing loss with neonatal hearing screening in relation to exposure to tobramycin and vancomycin expressed in terms of duration of therapy and serum concentrations.

PATIENTS AND METHODS

Study design

The present study was conducted in neonates admitted to the NICU of the Sophia Children's Hospital from November 1998 to November 2000.

Inclusion criteria

All neonates who underwent A-ABR screening were included in this study. A-ABR screening was performed in neonates with the following risk factors, as noted on a chart by the attending physician: positive family history for hearing loss, positive serology for toxoplasmosis, rubella, cytomegalo virus or herpes virus, craniofacial abnormalities, birthweight below 1500 grams, hyperbilirubinemia necessitating exchange transfusion, cerebral complications, a 1' APGAR score below 5 or a 5' APGAR score below 7, mechanical ventilation longer than 5 days, syndromal abnormalities.

Data collection

Parameters

Gestational age (GA), birthweight, indication(s) for A-ABR screening, test date and result were abstracted from A-ABR case record forms. Apgar scores were abstracted from the patient files. Information on administration of potential ototoxic medication (tobramycin, vancomycin and furosemide) was abstracted from the computerized hospital medication ordering system by two independent investigators and cross-checked.

Administration and dosage regimen of tobramycin, vancomycin and furosemide

Administration of tobramycin was performed in a 30-minute i.v. infusion in a dose of 4 mg/kg. The initial dosing interval was 24, 36 or 48 hours in neonates with a GA of <32 weeks, 32-36 weeks and ≥ 37 weeks, respectively. Tobramycin serum samples were taken as part of routine therapeutic drug monitoring 1 and 6 hours (October 1998-February 2000) or 3 and 8 hours (from March 2000 onwards) after the first dose and just before the second dose (all), at the discretion of the attending physician. Thus tobramycin peak concentrations were only available for the first period. Vancomycin was administered in a dose of 10 mg/kg in a 1h infusion with an interval of 8h irrespective of GA. Trough and peak serum sampling was performed around the fourth dose, with the trough just before and the peak 1h after completion of the infusion. Therapy adjustments were made at the discretion of the attending physician. All doses and times of administration were recorded routinely. Duration of therapy and maximum peak and trough concentrations were noted for vancomycin.
Exposure to furosemide in mg/kg was also noted.

Auditory testing

Hearing screening was performed with an automated auditory brain stem response device (ALGO-1 E, Natus Medical Inc., California, USA) by specialized nurses. This device measures responses to a monoaural 35 dBnHL click stimulus. Artefact rejection for ambient noise and myogenic activity is automatic. ALGO-1 E displays a pass when the internal algorithm reaches a likelihood ratio ≥ 160 in discriminating between response + noise, noise and no response[5] . The algorithm criteria were defined by comparison with conventional ABR. Sensitivity for this test (compared to conventional ABR as the gold standard) is 100%, specificity ranges from 96 to 98.7% [8, 25-27].
Patients with refers from A-ABR testing were scheduled for a retest and a further retest on second failure to pass. All patients failing this sequence of testing were referred for further conventional testing.

Statistical analysis

Statistical analysis was performed using SPSS 8. 0 statistical software (SPSS Inc., Chicago, USA).

The Wilcoxon signed rank test and Mann-Whitney test were used as nonparametric tests. All variables were entered as single covariates for logistic regression. For the purpose of analysis certain continuous variables were categorized, to enable valid inclusion of non-exposed patients. Tobramycin: exposure as longest consecutive duration of treatment (days): 0, 1-5, >5; total exposure (mg/kg): 0, ≤ 20 mg/kg, > 20 mg/kg; peak serum concentrations: 0 (no exposure), ≤ 12 mg/L, > 12 mg/L; trough serum concentrations 0 (no exposure), ≤ 2, > 2 mg/L. Vancomycin: exposition in days of treatment: 0, 1-7 and > 7 days; peak serum concentrations: 0 (no exposure), ≤ 40 mg/L, > 40 mg/L; trough serum concentrations 0 (no exposure), ≤ 15 mg/L, > 15 mg/L Furosemide exposition: 0, 1-10 and >10 mg/kg. If a relation between serum concentrations and ototoxicity exists, this is expected with higher exposure. For this reason, univariate logistic regression with serum concentrations as continuous variable was also performed in a subgroup of patients with serum tobramycin and vancomycin peak and trough concentrations exceeding arbitrary limits: tobramycin 8 mg/L and 1 mg/L, respectively and vancomycin 30 mg/L and 10 mg/L, respectively.

Multiple logistic regression was performed on combination therapy of tobramycin, vancomycin and furosemide.

RESULTS

Patient characteristics

During the study period a total of 1197 patients were admitted to the NICU. A total of 669 patients were eligible for ALGO screening. In 44 patients complete A-ABR screening was not performed; 28 patients died before screening, 11 parents refused cooperation in screening and in 2 repeat A-ABR screening has not been performed. In three patients A-ABR screening failed for technical reasons. All three underwent conventional ABR and tested as normal. Data for 625 patients were analyzed.

Demographic parameters are shown in table 1. There were no differences in GA and birthweight between patients passing or failing A-ABR screening. There is a significant difference in age at final A-ABR screening between neonates who pass or fail hearing screening, inherent to the method of screening.

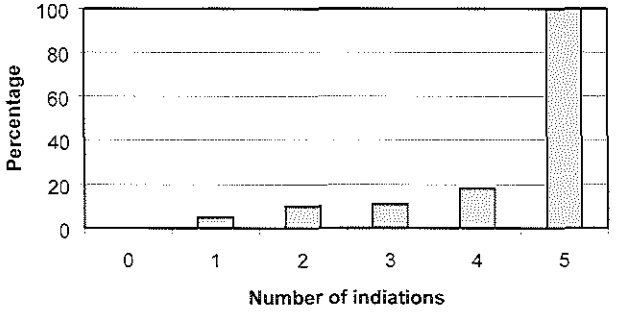
Table1: Demographics for study group. Numbers are medians and ranges

	Total (n=625)		Pass (n=580)		Refer (n=45)		p-value (Mann-Whitney)
Gestational age (weeks)	32.0	(24-43)	31.9	(25.3-43.0)	32.1	(24–41.9)	0.56
Birthweight (grams)	1480	(520-5800)	1478	(520-5800)	1550	(545 – 4050)	0.89
Age at A-ABR (days)	19	(0-286)	18	(0-286)	47	(1-255)	<0.001

Statistical relations between ototoxicity and risk factors

Figure 1 shows the distribution of number of risk factors per patient. Thirteen neonates had no indication, but were tested by request of the parent, because they were part of a twin pregnancy of which the other half had to be screened. There was one indication for testing in 377 neonates, two indications in 178 neonates and 3 or more indications in 57 neonates.

Fig.1: Risk of failing A-ABR screening in relation number of indications for screening



In univariate analysis, the number of indications per patient for testing was the single most important risk factor (p=0.0007). This is also illustrated by figure 1. Given the influence of the number of indications as well as the significant difference in postnatal age at final screening, univariate analysis of other variables was corrected for these two factors. Table 2 shows all inclusion criteria for A-ABR screening and the relative risk for failure of this screening. These numbers are only valid in a population with at least one risk factor. The presence of craniofacial abnormalities was the most important single risk factor in failing A-ABR screening. No other single risk factor attained significance.

Table 2: Distribution of risk factors in infants with normal and abnormal A-ABR screening. P-values are from univariate analysis, corrected for number of indications and age at A-ABR screening

Risk factor	A-ABR normal (n=580)		A-ABR refer (n=45)		P-value	Odds ratio (95% CI)
Family history	12	2.1%	3	6.7%	0.18	2.5 (0.6-9.9)
TORCH*	8	1.4%	1	2.2%	0.81	1.3 (0.2-11.0)
Craniofacial	10	1.7%	5	11.1%	0.0014	6.9 (2.1-22.6)
Birthweight<1500	304	52.4%	22	48.9%	0.04	0.5 (0.2-1.0)
Hyperbilirubinemia	17	2.9%	3	6.7%	0.19	2.4 (0.6-9.2)
Cerebral complications	63	10.9%	8	17.8%	0.84	1.1 (0.4-2.8)
Apgar score 1'<5/5'<7	223	3.8%	17	37.8%	0.62	0.8 (0.4-1.7)
Mechanical ventilation>5 days	172	29.7%	19	42.2%	0.65	0.8 (0.4-1.8)
Syndrome	23	4.0%	4	8.9%	0.24	2.0 (0.6-6.2)

* Positive serology for toxoplasmosis, rubella, cytomegaly virus or herpes.

Table 3 shows details on exposure to ototoxic medication in the study group. As can be seen 508 patients received tobramycin, 130 patients vancomycin and 174 patients furosemide. Total exposure to tobramycin was 15.3 ± 11.1 and 20 ± 19.6 mg/kg for neonates passing and failing hearing screening, respectively. For vancomycin, total exposure was 234 ± 159 and 375 ± 273 mg/kg respectively for groups of neonates who passed or failed hearing screening. These data were not statistically different between both groups. Table 3 also shows ototoxic medication as risk factor for failure of A-ABR screening. Potential ototoxic medication, defined in terms of duration of treatment, total exposure and aberrant peak and trough serum concentrations was analyzed using logistic regression. As can be seen exposure to vancomycin and tobramycin in terms of treatment duration and total dose was not significantly related to failure to pass A-ABR screening. Exposure to both antibiotics in the same patient, as well as combination with furosemide treatment, was also not related to hearing loss. Peak and trough serum concentrations of both vancomycin and tobramycin were not associated with an increased risk of failing A-ABR screening. In the subgroup of patients with higher peak- or trough serum concentrations of vancomycin or tobramycin, entered as continuous variable in logistic regression, no relation to failing A-ABR screening was detected (p-value ranging from 0.13 - 0.67).

Table 3: Univariate analysis of ototoxic medication as risk factor for failure of A-ABR screening. P-values and odds ratios are corrected for number of indications and age at A-ABR screening

	Total	Pass	Refer	n	p-value	Odds ratio (95% CI)
Tobramycin	508	473	35	625	0.19	0.6 (0.3-1.3)
Total exposure (mg/kg)> 20 mg/kg	102	93	9	625	0.28	0.8 (0.4-1.3)
Longest sequential treatment > 5 days	135	125	10	625	0.18	0.7 (0.4-1.2)
Peak concentration > 12 mg/L	6	5	1	449	0.23	0.6 (0.3-1.4)
Trough concentration > 2mg/L	9	8	1	393	0.55	0.8 (0.4-1.7)
Vancomycin	130	119	11	625	0.52	0.8 (0.4-1.7)
Vancomycin exposure > 7 days	54	8	62	625	0.89	1.0 (0.6-1.5)
Peak concentration > 40 mg/L	4	3	1	593	0.45	0.7 (0.3-1.7)
Trough concentration > 15 mg/L	26	25	1	604	0.22	0.7 (0.3-1.3)
Furosemide	174	158	16	625	0.49	0.8 (0.4-1.6)
Total exposure > 10 mg/kg	25	21	4	625	0.90	1.0 (0.5-1.7)
Tobramycin + vancomycin	122	111	11	625	0.67	0.8 (0.4-1.8)
Tobramycin + furosemide	154	140	14	625	0.44	0.7 (0.4-1.6)
Tobramycin + vancomycin + furosemide	66	59	7	625	0.73	0.8 (0.3-2.1)

n = number of patients included in analysis

Relation of ototoxicity to antibiotic use in individual patients

Tobramycin

Patients with potential ototoxic serum concentrations failing A-ABR screening were analyzed. Only one of the six patients with a peak tobramycin concentration > 12 mg/L failed hearing screening and conventional ABR showed bilateral hearing loss of 50-60 dB with a cochlear component at follow-up. This patient was born a premature (GA 26 weeks) and had low Apgar scores (6/7) as well as periventricular leucomalacia. Furthermore he also received vancomycin for 35 days with normal serum concentrations . Only one out of three peak serum concentrations of tobramycin in this patient was > 12 mg/L.

Nine patients had trough serum concentrations > 2 mg/L. One of these nine patients failed A-ABR screening. This patient had bilateral hearing loss with a cochlear component of 50-60 dB. This patient received only 2 doses of tobramycin however and also had neonatal asphyxia and delayed motor development. Since there is evidence that

trough concentration goals should be set to 0.5-1.0 mg/L with extended interval dosing of aminoglycosides, we also looked at the 56 patients with trough serum concentrations exceeding 1.0 mg/L[28, 29]. In this group, three neonates failed hearing screening. One patient, with a trough > 2 mg/L, is described above. Of the two others, the first patient, who had severe perinatal asphyxia, was exposed to only one dose of aminoglycosides. The second patient, exposed to 7 doses (2 courses, longest consecutive treatment 7 days), had severe bilateral hearing loss without a clear cause.

Eleven patients received tobramycin for ≥ 10 consecutive days; two failed A-ABR screening. The first patient had slight cochlear loss (20-25 dB) in one ear and was also exposed to 12 days of vancomycin treatment. The second patient had severe bilateral hearing loss with no reproducible responses on ABR pointing at auditory neuropathy and/or severe loss of ear sensitivity to sound. This patient was not exposed to vancomycin, but had 3 other risk factors; birthweight <1500 grams, 1' Apgar score of 5 and abnormalities on cerebral ultrasound.

Vancomycin

Four patients had peak vancomycin concentrations > 40 mg/L. One of these patients (peak 42.3 mg/L) had bilateral severe hearing loss. This patient, who had a grade III intraventricular hemorrhage and sepsis/meningitis, died later.

One of 26 patients with vancomycin trough concentrations exceeding 15 mg/L failed hearing screening. This patient (trough 17.7 mg/L), who had neonatal septicemia and periventricular leucomalacia, was exposed to 12 days of vancomycin and 27 days of tobramycin. He had mild (20-25 dB) cochlear loss in one ear. There was a rise in serum creatinin to 112 μmol/L prior to this serum trough concentration.

Sixteen patients were exposed to vancomycin for more than 14 days, with 3 failures on hearing screening. One patient had grade III intraventricular hemorrhage as well as abnormalities on MRI compatible with kernicterus. The second and third patient received 3 doses of tobramycin as well, with normal serum concentrations for both antibiotics. The second patient had 5 indications for A-ABR screening, including bilateral grade III IVH and posthemorrhagic hydrocephalus. The third patient, with neonatal periventricular leucomalacia and hydrocephalus, had bilateral cochlear hearing loss (50 dB).

DISCUSSION

The relation between use of tobramycin or vancomycin and neonatal hearing loss is not well defined. In this study we investigated the effect of exposure to tobramycin and vancomycin, both commonly used in the NICU, on failing hearing screening. Ototoxicity has been related to use of both antibiotics and therapeutic drug monitoring (TDM) has been advocated. Commonly accepted TDM goals for tobramycin are peak and trough serum concentrations of 5-12 mg/L and < 2mg/L, respectively. A clear relation of serum concentrations of aminoglycosides to ototoxicity has not been demonstrated in adults or neonates[10, 14-16, 30]. Possible reduction of nephro- and ototoxicity with extended interval dosing of aminoglycosides was a major reason for implementation of this regimen in adults and neonates during the last decade. A definite reduction in the number of patients with aminoglycoside related ototoxicity has not been demonstrated however[31, 32]. These new extended dosing interval regimens have implications for therapeutic drug monitoring. To prevent prolonged exposure, it has been suggested that trough concentration goals should be reduced to 0.5-1 mg/L in adults with once daily dosing[28, 29]. In this study we evaluated the effect of our extended interval dosing regimen on occurrence rate of failure to pass A-ABR screening. As in several other screening studies, we could not detect an overall difference of prevalence in hearing loss between patients exposed or not exposed to aminoglycoside treatment.[1, 22, 24, 33]. None of these studies showed detailed information on administration regimens and/or serum concentrations, which might be important since other studies in neonates suggest that ototoxicity might be related to total duration of therapy and high peak and/or trough serum concentrations[10, 15, 16, 34]. No relation to any of these determinants was found in the present study. Several studies have demonstrated that extended interval dosing in neonates is relatively safe[16, 21, 35]. These studies have focussed on screening neonates exposed to aminoglycosides without matching for concomitant risk factors. Borradori et al. found a relation between hearing loss and aminoglycoside treatment in a matched control study in 8 children with hearing loss[10]. Their study found a relation between cumulative dose and treatment days, but not with serum concentrations. However, patients were not matched for all risk factors (e.g. hyperbilirubinemia, days on ventilatory support). In this study, there was no patient exposed to tobramycin in whom aberrant tobramycin serum concentrations where the most likely cause of hearing loss.

TDM goals for vancomycin are peak and trough concentrations of 20-40 mg/L and < 15 mg/L, respectively. There is circumstantial evidence relating high peak

concentrations to ototoxicity, but a relation between vancomycin related ototoxicity and serum concentrations has not been demonstrated in adults[11, 36]. Information in neonates is very scarce but did not demonstrate vancomycin related hearing loss[13, 37].

In this study no relation was found between exposure to vancomycin and hearing loss. Only one patient with peak concentrations exceeding 40 mg/L failed A-ABR screening, but as described before, this patient had sever neurologic deficit including meningitis and IVH which have been shown to induce hearing loss[38, 39]. All three patients with high trough vancomycin concentrations and failure to pass A-ABR screening had central nervous system abnormalities (e.g. IVH, hydrocephalus) associated with hearing loss[38, 40]. Incidence of ototoxicity is said to be higher in patients receiving both aminoglycosides and vancomycin[18, 19, 36]. The present study, however showed no relation between failure to pass A-ABR screening and concomitant use of tobramycin and vancomycin. Several authors have indicated that the potential ototoxic effect of aminoglycosides is potentiated by loop diuretics and/or vancomycin[10, 41, 42]. In the present study we did not find this association, not even when the patient group with highest exposure to both furosemide and tobramycin was compared to the rest (data not shown).

There are three limitations to this study. First, this study describes hearing screening in an at risk population, with at least one risk factor for hearing loss. One has to be careful to translate results to the total group of newborns admitted to a NICU. However a high percentage of all patients admitted to the NICU and exposed to either tobramycin (60%) or vancomycin (84%) were included in this study. Second, click-evoked A-ABR screening detects hearing loss in the 2-4 kHz frequency range, which is clinically important for speech and language development. Aminoglycoside related hearing loss starts in the higher frequency ranges, above 8 kHz, but is also found in lower frequencies in serious cases[43]. It is possible that some neonates in this study had hearing loss in this range, which can not be investigated with current routine techniques in this age group. Because cochlear damage induced by aminoglycoside use is stationary over the years, the long-term clinical importance of this high-frequency hearing loss is doubtful.

Third, delayed onset of hearing loss in infants has been described[10, 44]. In the present study, 77% of patients exposed to tobramycin for more than 10 days underwent hearing screening at least two weeks after cessation of therapy. It is conceivable however, that hearing screening was performed too early in some patients to detect hearing loss and it might be necessary to reassess hearing in neonates with prolonged exposure to aminoglycosides.

Given these limitations, this study still has important implications for TDM. Aberrant serum concentrations for tobramycin and vancomycin in the present study were not associated with failing hearing screening, and adherence to TDM goals did not preclude hearing loss. There was no patient in this study in whom aberrant serum concentrations were a likely cause of hearing loss. This leads us to conclude that routine TDM of vancomycin and tobramycin in neonates is not helpful in detecting patients at risk for clinically important hearing loss.

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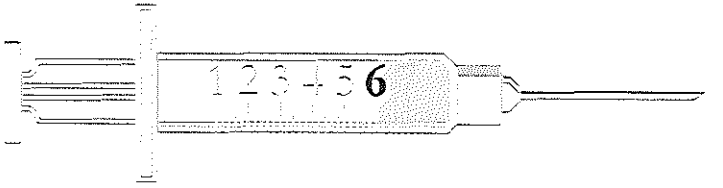
General discussion and summary

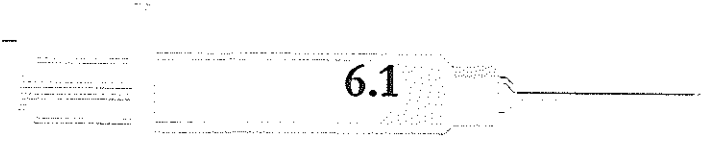
(samenvatting)

Chapter



General discussion

Chapter



INTRODUCTION

Neonatal sepsis remains one of the main causes of mortality and morbidity of newborn infants admitted to a neonatal intensive care unit[1]. Sepsis in this age group can be divided in early onset, defined as within the first 3-4 days of life and late-onset, occurring after 4 days[1].

Group B streptococcus and gram negative organisms such as *Escherichia coli* and *Haemophilus influenza* are the most common causative organisms for early-onset sepsis[2]. Especially in the United States an increase of gram-negative neonatal infections has been noted due to prenatal administration of antibiotics[3]. Late onset neonatal sepsis is related to the increased use of invasive procedures such as central venous lines and includes as major pathogens gram-positive organisms from the skin: *S. epidermidis* and *S. aureus*[4, 5]. The spectrum of pathogens in these two different age groups has led to commonly accepted empiric antibiotic strategies. Early onset sepsis is treated with a combination of a penicillin with either a third generation cephalosporin or an aminoglycoside. Empiric treatment of late onset sepsis often starts with a combination of flucloxacillin and either a third generation cephalosporin or an aminoglycoside, with a switch made to vancomycin when culture results and resistance patterns indicate a need for change[4, 6, 7].

Treatment with vancomycin as well as aminoglycosides has historically been subject to therapeutic drug monitoring (TDM), and many dosing regimens in neonates have been constructed, with the aim to adhere to currently accepted therapeutic ranges.

Several interconnecting aspects surrounding dosing of these antibiotics in newborns have to be taken into account to optimize the therapeutic effect of these drugs. Goals for TDM have to be determined based on the relation between serum concentrations and efficacy/toxicity. On the basis of target concentrations and pharmacokinetic behavior of these drugs in neonates a dosing regimen must be developed. During treatment efficacy and toxicity have to be monitored. Monitoring can not be limited to taking well timed serum concentrations.

In the following sections the different aspects of TDM, dosing and monitoring of efficacy and toxicity will be discussed in detail.

THERAPEUTIC DRUG MONITORING: WHEN AND WHY ?

In general, routine TDM is only rational when the use of the drug has the following characteristics:

1. Availability of an assay for the drug with an adequate assay error.
2. Clinical effect or toxicity of the drug is difficult to determine or has a delayed presentation.
3. A large interindividual variation in pharmacokinetic behavior between patients.
4. A good correlation between serum concentrations and effect or toxicity.
5. Use of TDM should appropriately predict subsequent serum concentrations in the same patient.

Aminoglycoside and vancomycin use in newborns fulfill several of these prerequisites. Good quality serum assays for both tobramycin and vancomycin exist. The most widely used routine method, Fluorescence Polarization Assay, has adequate performance characteristics. In both drugs clinical effect as well as toxicity are difficult to determine. Toxicity, especially ototoxicity, can have a delayed presentation, as will be discussed later. As described in chapter 1, there are large interindividual differences in the pharmacokinetics of vancomycin and aminoglycosides (including tobramycin) in newborns. Of the points mentioned above, two remain unclear. There is still a lot of uncertainty on both a good correlation between serum concentrations and effect or toxicity and the usefulness of TDM to predict subsequent serum concentrations in the same patient. These issues will be discussed in the following paragraphs.

TDM of aminoglycosides: correlation between serum concentrations and effect

Since aminoglycosides have a narrow therapeutic window, any discussion regarding the dosing regimens of these drugs should take into account both efficacy and toxicity. Given the differences between individual aminoglycosides, this implies that concentrations and dose recommendations discussed in the following sections are valid for gentamicin, tobramycin and netilmicin. Because of the pharmacokinetic and pharmacodynamic characteristics of amikacin values regarding this drug have to be multiplied by three.
Efficacy of aminoglycosides is related to both peak serum concentration to minimal inhibitory concentration (MIC) ratio (Peak/MIC) and area under the time versus concentration curve (AUC/MIC) in clinical and experimental studies[8-10]. It seems that

peak/MIC ratio's of 5-10 are desirable for clinical efficacy. Given the sensitivity of gram-negative organisms in our NICU, this ratio translates to desirable peak serum concentrations of ≥ 5 mg/L for our setting. The study presented in chapter 3.1 shows that this goal is reached in more than 90% of patients with the proposed dosing regimen. Based on desirable ratio's and pharmacokinetic behavior of aminoglycosides in neonates, higher doses at longer intervals (24h for adults, 24-48h in neonates) have been advocated over the last 10 years. Studies in adults as well as neonates have failed to detect an increase of efficacy with this strategy[11-16]. The studies in chapter 3 have shown the need for extending dose interval up to 48h for premature neonates. However, no conclusion on resulting efficacy can be drawn from our studies.

TDM of aminoglycosides: correlation between serum concentrations and toxicity

Nephrotoxicity of aminoglycosides is related to the quantity of aminoglycosides stored in the proximal tubular cell[17]. Since aminoglycosides show drug saturable uptake into these cells, nephrotoxicity can occur when the time period of low trough concentrations is too short to prevent accumulation[18]. Although aminoglycoside induced nephrotoxicity has been related to high serum trough concentrations in studies in humans, exact information on the relation to serum concentrations is lacking. Aminoglycoside induced nephrotoxicity in neonates is rare, especially if the treatment period does not exceed 7 days. No relation between serum concentrations and glomerular filtration rate (GFR) disturbances has been demonstrated in newborns[15, 16, 19]. However, reversible tubular dysfunction, resulting in a decreased capacity to form concentrated urine as well as electrolyte loss, is seen more often[20, 21].

Mainly based on toxicity characteristics, extended interval dosing has been advocated for adults and neonates. Extended interval dosing has implications for TDM goals. Although desired peak serum concentrations of circa 5 mg/L would still suffice, trough concentration goals should be lower. Aiming at a trough concentration of 2 mg/L would lead to a 2.3 times higher total exposure (AUC)[22]. Trough concentration goals of 0.5-1 mg/L seem to be more appropriate. For amikacin these goals should be multiplied by three. Most studies, however, are still using trough concentration goals associated with multiple daily dosing (2 mg/L). Several clinical and meta-analysis studies in adults have shown nephrotoxicity to be equal or less with single daily dosing as compared to multiple daily dosing[11-14]. In neonates no difference in nephro- or ototoxicity between once-daily dosing and multiple daily dosing has been demonstrated[15, 16]. This suggests that

nephrotoxicity in newborns is either infrequent and a difference is therefore undetected in the relatively small studies or that maturational dependent differences in nephrotoxicity exist.

Ototoxicity of aminoglycosides in man has been related to total dose and duration of therapy with no clear relation to high serum concentrations[23]. A causal relation between ototoxicity and exposure to aminoglycosides in neonates has not been proven. Most studies comparing aminoglycoside treated infants to non-treated patients did not detect permanent hearing loss[24-26]. Routine hearing screening in infants exposed to aminoglycosides has failed to detect a significant relation to hearing loss[27, 28]. These studies did not quantify exposure in terms of serum concentration and/or duration of therapy. In chapter 5.2 we studied 625 neonates undergoing routine neonatal hearing screening and could not demonstrate a relation to exposure to tobramycin. Neither duration of therapy, nor high serum concentrations or simultaneous treatment with other ototoxic drugs was found to be a significant risk factor. Also, in patients failing A-ABR screening, aberrant serum concentrations were not found to be the most likely explanation for hearing loss. There are reports however which describe delayed-onset hearing loss with a possible association to prolonged (>7-10 days) exposure to aminoglycosides[29-31]. This hearing loss could be missed by routine hearing screening, which is often performed shortly after discontinuation of the drug. In chapter 5.1 we also studied possible late effects of aminoglycoside exposure on hearing in a matched case-control study, by examining 3-4 year old children who had been treated with tobramycin as neonates. Although no statistical significance was found, possibly due to the small sample size, the finding of three infants with moderate to severe hearing loss, all of them exposed to >14 days of aminoglycoside is worrisome. Minimizing exposure to tobramycin in terms of duration of therapy should be the aim.

Our studies thus show that aberrant tobramycin serum concentrations do not detect patients at risk for hearing loss, and adherence to TDM goals does not preclude hearing loss. From that point of view, the fourth point mentioned above as a prerequisite to perform TDM, is not fulfilled.

In summary, efficacy is related to peak concentration to MIC ratio's, but improvement of efficacy with extended interval dosing has not been demonstrated. Clinically important nephro- an ototoxicity are rare in neonates in courses shorter than 7 days, and there is no clear relation to serum concentrations. The importance of routine TDM in the first week of life for efficacy and toxicity reasons is questionable.

TDM of aminoglycosides: predictive performance

Predictive performance of TDM of aminoglycosides in newborns needs careful attention. Routine TDM for aminoglycosides is normally performed around the third or fourth dose, and is based on the assumption that steady state is more or less attained. For several reasons this assumption is not valid in neonates in the first week of life. Antibiotic courses in neonates are often discontinued after a few days when blood cultures and other tests remain negative. Since extended interval (24-48h depending on GA) dosing of aminoglycosides is now general practice, steady state would not be reached before discontinuation of the drug, and TDM would therefore not be performed in time to be of use. One solution to this problem is to predict initial individual dosing interval using population pharmacokinetic parameters with Bayesian feedback of early serum concentrations. In this thesis (chapter 3.2) we hypothesized that, given the large inter-individual pharmacokinetic differences within GA-groups, early TDM, directly after the first dose, may improve these predictions for the individual. A prerequisite is however that a model incorporating early TDM data is superior in predicting subsequent serum concentrations to a model based on the population parameters alone. Data in adults have shown that population estimates with Bayesian feedback of one or more serum concentrations can adequately predict aminoglycoside serum concentrations[32]. In neonates this is not so clear. Two studies in neonates showed that serum concentrations can be predicted from an early sample using a population approach with Bayesian feedback[33, 34]. Neither study looked specifically at predicting trough serum concentrations, which is necessary to individualize dose interval. Also, they did not compare their feedback model to a strategy without TDM [33, 34]. We investigated the Bayesian approach as well, looking solely at predicting trough serum concentrations, and found a predictive performance comparable to the other two studies. We could not however detect an increase of predictive performance of this method over our original population based, gestational age related dose advice without using TDM. Patients at risk for prolonged exposure to aminoglycosides in our study were not detected by this method. Hence, the results of our study indicate that routine early therapeutic drug monitoring does not improve the model based prediction of initial tobramycin dosing intervals in neonates in the first week of life.

In conclusion, routine early TDM of aminoglycosides in newborns in the first week of life does not seem to be very useful from the viewpoint of toxicity. Furthermore early TDM does not adequately predict subsequent trough serum concentrations. We therefore

propose that routine TDM of aminoglycosides is not needed in the first 7 days of life. An exception should be made for patients with renal failure, patients with obvious neonatal asphyxia (e.g. 5' Apgar score < 5), and patients exposed to drugs or situations which are known to significantly alter pharmacokinetic behavior (e.g. indomethacin, ECMO).
In these difficult to manage patients use of a population model with feedback of repeated serum concentrations as well as serum creatinin might be useful in guiding therapy.

TDM of vancomycin

TDM of vancomycin: correlation between serum concentrations and effect

For vancomycin the correlation between serum concentrations and efficacy or toxicity is not clearly defined. Several, mainly in vitro, studies have shown no relation between killing rates of bacteria and increasing vancomycin concentrations above the MIC[35, 36]. In animals, outcome of endocarditis was related to vancomycin trough serum concentrations[37]. Clinical studies in neonates have shown a wide range of serum concentrations associated with resolution of infection, but causal relation was not studied[38, 39]. Although it is not possible to draw a definite conclusion, these studies indicate that keeping the trough level above the MIC is necessary to obtain clinically good results. This implies minimal serum concentrations are needed of approximately 4-5 mg/L, when considering MIC's and protein binding. These findings are not reflected in currently accepted TDM goals however, where peak and trough serum concentrations of 20-40 mg/L and 5-10 mg/L respectively, are generally accepted.

TDM of vancomycin: correlation between serum concentrations and toxicity

Vancomycin can cause reversible nephrotoxicity in humans. Toxicity has been related to trough concentrations > 10 mg/L, but it remains unclear whether elevated serum concentrations are the cause or consequence of renal dysfunction. Ototoxicity has been described in incidental cases where patients were exposed to high peak serum concentrations, but a relation between vancomycin induced ototoxicity and serum concentrations can not be ascertained from available literature. Although there are studies relating both nephro- and ototoxicity to vancomycin in combination with an aminoglycoside, there is little evidence for vancomycin alone. In neonates, data are limited even more and very scarce. Several hearing screening studies did not detect vancomycin

related ototoxicity[27, 28]. In the study presented in chapter 5.2, we investigated the effect of qualitative and quantitative exposure to vancomycin on hearing screening in infants. This was the first study to relate serum concentrations of vancomycin to hearing screening. We could not detect a significant relation of any denominator of vancomycin use to hearing loss found with neonatal hearing screening.

From the above, it can be concluded that there is no clear relationship between serum concentrations and toxicity, and TDM for toxicity reasons is not substantiated by current literature or studies presented in this thesis. TDM might be warranted for efficacy and should focus on adequate serum trough concentrations.

TDM of vancomycin: predictive performance

Adequate predictive performance for TDM of vancomycin in neonates has been established. In contrast to aminoglycosides, subsequent vancomycin serum concentrations can be reasonably well predicted with use of 2 serum samples in a Bayesian feedback model[40]. Additional feedback concentrations are needed approximately every 14 days[40].

In conclusion, TDM requirement criteria for vancomycin are doubtful. Neither efficacy nor toxicity show a clear relation to serum concentrations; the relation of efficacy to trough serum concentrations does not seem to exist above concentrations exceeding the MIC. Although there are substantial inter-individual differences in pharmacokinetic behavior they are not important in the context of pharmacokinetic-pharmacodynamic relations. In the light of these factors routine TDM of vancomycin, with both peak and trough concentrations, is not warranted for either efficacy or toxicity reasons. A case can be made for routine monitoring of serum trough concentrations for efficacy reasons. Intensified TDM should only be performed in patients in whom an alteration of pharmacokinetic behavior (e.g. renal failure) is likely.

DOSING REGIMEN: HOW MUCH AND HOW OFTEN ?

Although TDM goals, as discussed above, for both vancomycin (trough > 5 mg/L) and aminoglycosides (peak 5-12 mg/L, trough < 1 mg/L) are disputable, they have implications for drug dosing in neonates. When designing a dosing regimen, these goals together with pharmacokinetic characteristics have to be taken into account.

Aminoglycoside dosing

Pharmacokinetics of aminoglycoside antibiotics in neonates, as other drugs, are gestational age related. Premature neonates have a larger volume of distribution and lower clearance. As in adults once daily dosing has been advocated in neonates. Based on these considerations several dosing regimens have been suggested and tested for neonates[15, 33, 41, 42]. The dose recommendation of our study presented in chapter 3.1 (4 mg/kg) is similar to these studies, where dose advice ranges from 2.5-5 mg/kg for neonates of varying gestational ages, mostly with an interval of 24h. Some of these studies however vary dose to keep the dosing interval the same, which is counterproductive in the face of efficacy[15, 42]. Lower doses in these studies led to subtherapeutic peak concentrations in 15-47% of patients[15, 33]. Our studies in chapter 3 have shown that dosing tobramycin at 4.0 mg/kg for neonates of all GA's in the first week of life leads to peak serum concentrations > 5 mg/L for more than 90% of patients. The need for dosing intervals exceeding 24h in preterm infants has been established, but not evaluated by others[15, 42]. Results from our studies have indicated a need for extending dose intervals up to 48h in neonates with a GA< 32 weeks. Use of our GA related dose interval showed that in preterms< 32 weeks, mean trough serum concentrations were still 0.52 mg/L after 48h. Results from recent studies, including ours, clearly indicate that aminoglycoside doses of approximately 4 mg/kg with a GA-related interval are warranted. There is no need for a loading dose with this regimen.

Vancomycin dosing

The pharmacokinetic profile of vancomycin in neonates is mainly determined by postconceptional age (PCA) and renal function, although a relation to other factors like birthweight and gestational age (GA) is described.

As with aminoglycosides, dose advises in most studies so far try to adhere to traditional monitoring goals. Studies based on PCA and/or serum creatinin have shown to achieve serum concentrations within that therapeutic range[43-46]. Dose recommendations range from 10-20 mg/kg with an interval of 8-36h. In contrast to others we showed in chapter 4.1 that, if serum goals are set at trough concentrations > 5mg/L, vancomycin can be dosed at 10 mg/kg every 8h in all neonates in the first month of life, irrespective of PCA. This regimen leads to >95% of trough serum concentrations above 5 mg/L. The lack of association to PCA in our study is partly explained by two factors. First, very few patients received vancomycin in the first week of life, the period where volume of distribution,

clearance and renal function show the largest change. Second, the majority of our patients (81.8 %) were antenatally exposed to corticosteroids, which has been shown to diminish the GA-dependent difference in renal clearance of other antibiotics[47]. The dosing advise presented in this thesis, namely 10 mg/kg every 8h for all neonates in the first month of life, has the practical advantage of being simple. Since dosing errors are frequently seen in infants, this practical dosing scheme might be important in the clinical setting[48].

Finally, therapy with both tobramycin and vancomycin should be seen in relation with disease and immunological status of the patient. Although extended dose intervals are generally desirable, diseases in which microbiological relation to serum concentration or pharmacokinetic behavior are very different (e.g. endocarditis, renal failure, exposure to indomethacin, ECMO) or in immunologically compromised patients, treatment should be individualized, as will be described in the following paragraph.

MONITORING OF EFFICACY AND TOXICITY

Monitoring efficacy of antibiotic treatment in the first week of life is difficult. Culture proven early onset sepsis occurs in approximately 2% of VLBW infants, but there are limitations to blood cultures in neonates and single blood cultures can be false negative[2, 49, 50]. Furthermore increasing prenatal treatment of mothers with antibiotics obscures culture results in newborns. In our study population culture proven early-onset sepsis was approximately 3%, in other words, for every patient with a positive blood culture, 30 others were treated as well. Early detection of neonatal sepsis remains difficult. Laboratory tests are unspecific and clinical signs can be ambiguous. Neonatal sepsis is suspected in many VLBW-infants on clinical grounds and antibiotic treatment is frequently started and discontinued after 48-72h when blood cultures and results of other tests remain negative[1]. Given the infrequent occurrence of positive blood cultures, these can not be used as marker for antibiotic efficacy. Laboratory data, including C-reactive protein, complete blood count and ratio of immature to total neutrophils lack sufficient sensitivity to detect neonatal sepsis, and can therefore not be used to evaluate efficacy[51]. The same counts for clinical features (e.g. respiratory rate, skin color) which can either not be quantified or lack a clearly defined relation between predictor and outcome.

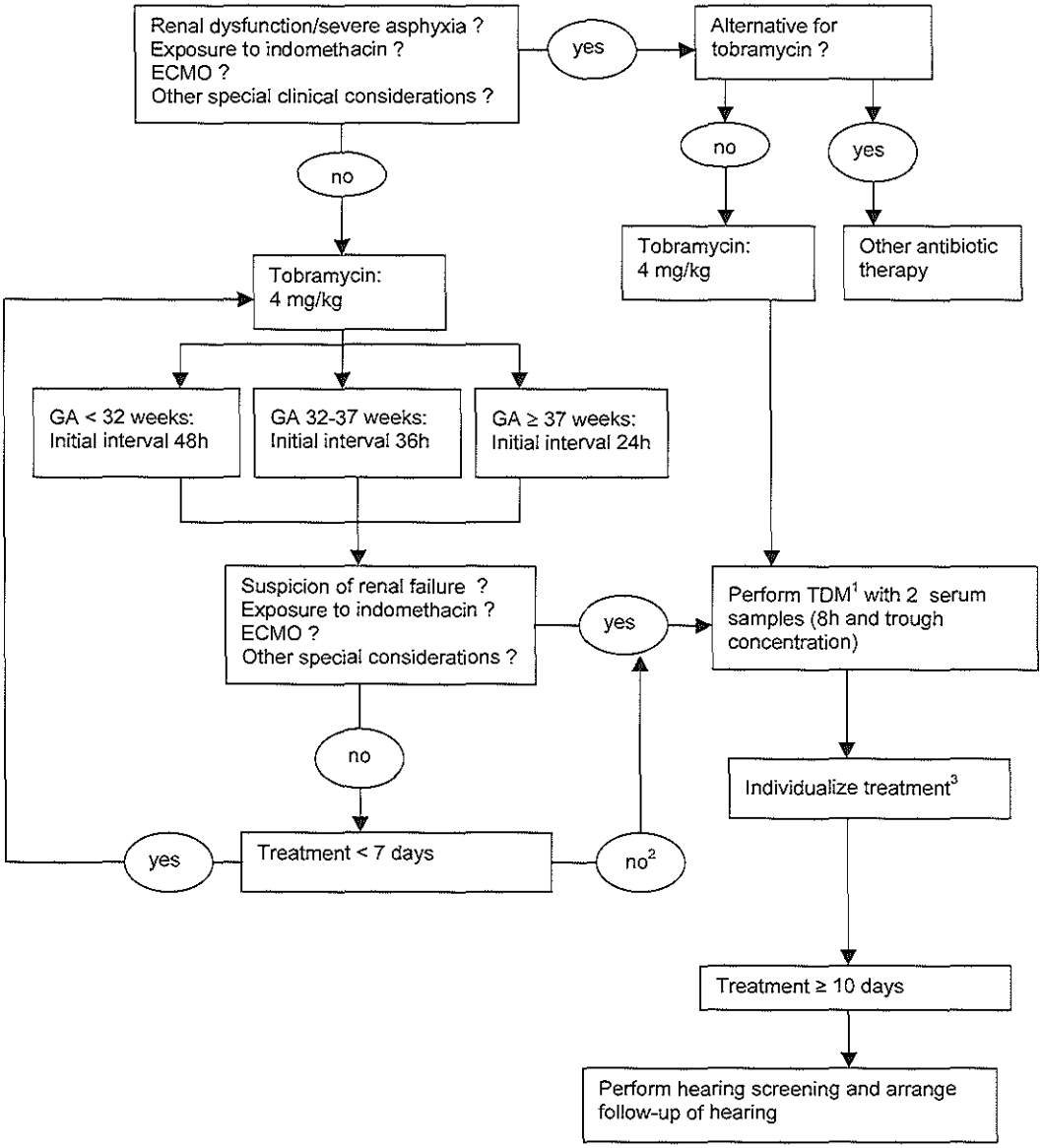
Monitoring efficacy in late-onset sepsis is slightly better. Up to 31% of VLBW infants have late onset culture proven sepsis[5]. In our series, 30% of neonates treated with vancomycin had positive blood cultures. Even though this number is higher, it still is difficult to monitor efficacy this way. Treatment of central line associated sepsis often includes removal of the intravascular catheter and it is hard to separate the antibiotic effect from other treatment modalities used. Again traditional clinical and laboratory parameters are not useful. It is clear from the point of view of efficacy that there is a need for a more evidence-based approach of suspected neonatal sepsis. Markers like interleukin-1 receptor antagonist, interleukin-6 and interleukin-8 have shown promising results in the early detection of neonatal sepsis[52, 53]. It is clear that a reduction in unnecessary treatment of neonatal suspected sepsis is needed before the question of antibiotic efficacy can be addressed.

Toxicity is another matter. As discussed in the section on TDM, monitoring serum concentrations alone will not prevent aminoglycoside and vancomycin associated nephro- and ototoxicity. Since TDM is not enough, the obvious way to augment monitoring nephrotoxicity for aminoglycosides as well as vancomycin is assessing renal function. Both antibiotics are almost totally renally excreted and a decrease of renal function is directly reflected in accumulation of the drug. Although glomerular function in neonates in the first week of life can not be reliably predicted from a single serum creatinin concentration, repeated measurements are indicative of renal function[54]. In neonates at risk for, or with overt renal failure (e.g. asphyxia), clinically detectable by oliguria and/or a rise of serum creatinin, administration of aminoglycosides should be seriously reconsidered. If no good antibiotic alternatives for aminoglycosides are available, dose interval should be extended on the basis of repeated serum drug monitoring.

Results from hearing screening studies in newborns have indicated that aminoglycoside and/or vancomycin ototoxicity has not been proven, especially in the face of short courses of treatment. The Achilles heel of this conclusion is the reports on delayed onset hearing loss associated with prolonged exposure to aminoglycosides, as several studies including ours indicate[29-31]. Although routine follow-up of hearing screening in infants treated with aminoglycosides and/or vancomycin is not necessary, a case can be made for retesting infants exposed to more than 10-14 days of therapy of aminoglycosides.

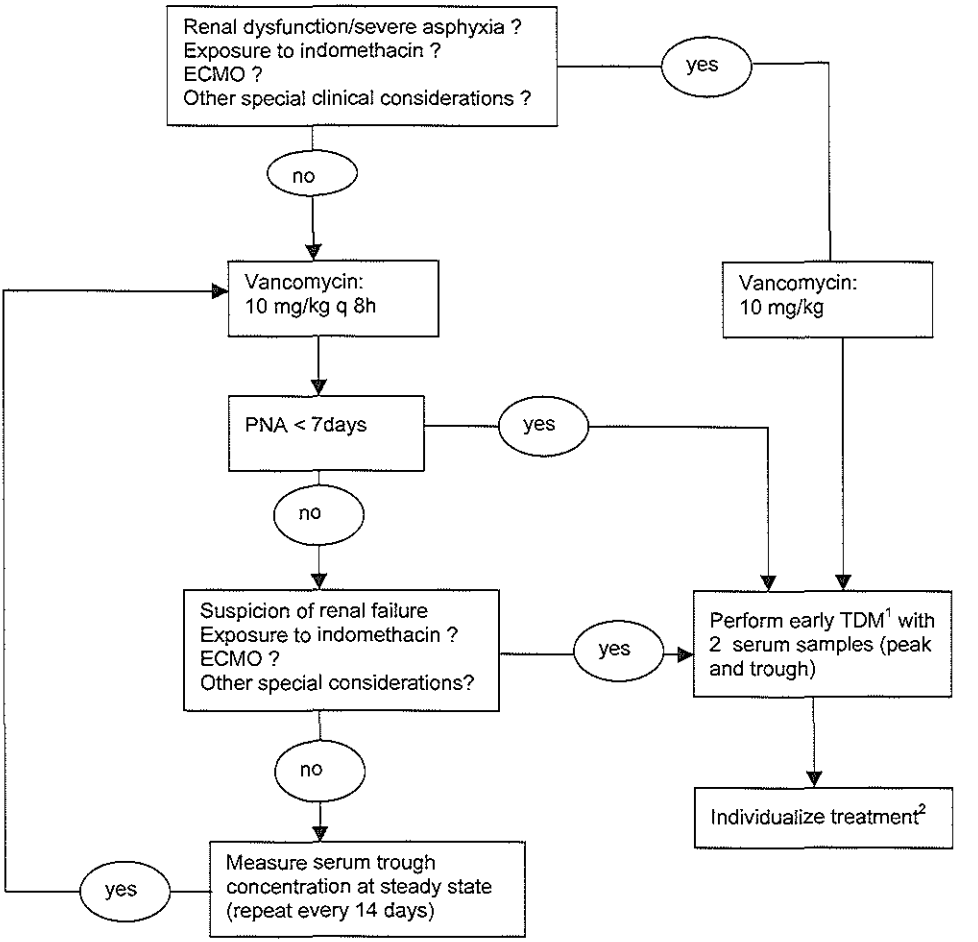
In conclusion, based on current literature and the findings of this thesis, we propose the following simplified strategy for dosing and monitoring tobramycin and vancomycin in neonates (Fig. 1 and 2).

Fig. 1: Dosing and TDM strategy for tobramycin in newborns in the first week of life



[1] Before the next dose.
[2] Evaluate necessity of continuing tobramycin.
[3] Individualize treatment, preferably on the basis of a population model with serum concentrations and renal function as feedback.

Fig. 2: Dosing and TDM strategy for vancomycin in the first month of life



[1] Early is before the next dose.
[2] Individualize treatment, preferably on the basis of a population model with serum concentrations and renal function as feedback.

Limitations of the studies

Results from our studies have important implications for dosing and therapeutic drug monitoring of tobramycin and vancomycin in neonates. It is necessary to judge these results in the light of certain limitations of our studies.

The major limitation of our studies analyzing pharmacokinetics of vancomycin and tobramycin is the retrospective nature. The TDM data used in our studies were taken from a large section of patients exposed to these drugs in everyday NICU practice. Results therefore depict the total spectrum of inter-individual variability encountered in this population. Implicitly, conclusions of these studies can be used in the same heterogeneous group. However, although only patients were included in whom timing of drug administration as well as serum sampling was recorded, these data may not have the same precision as when prospectively recorded. Furthermore a general limitation to studies in neonates is the actual amount of drug given. In newborns, for aminoglycosides, this can be up to 20% lower than the prescribed dose, due to dilution and other processes involved in administration[55]. This might lead to an overestimation of inter-individual pharmacokinetic differences. Since NONMEM accounts for unexplained variability of both these issues, this is not a major drawback with population modeling[56]. Also, it must be stressed that both proposed dosing regimens have been prospectively validated in the studies presented in this thesis.

In the study describing predictive performance of TDM, only patients were selected in whom trough serum sampling was performed at the GA-related interval, to facilitate a comparison with a regimen without TDM. This might have led to a selection bias; patients not included in the analysis might have had more extreme pharmacokinetic parameters. We investigated this assumption by analyzing the total study group. Predictive performance in the total group was not significantly different from the study group.

There are three limitations to our hearing screening study. First, this study describes hearing screening in an at risk population, with at least one risk factor for hearing loss. One has to be careful to translate results to the total group of newborns admitted to a NICU. However a high percentage of all patients admitted to the NICU and exposed to either tobramycin (60%) or vancomycin (84%) were included in this study. Only one patient not included in this study was exposed to more than 7 days of tobramycin treatment. Second, click-evoked A-ABR screening detects hearing loss in the 2-4 kHz frequency range, which is clinically important for speech and language development. Aminoglycoside related hearing loss starts above 8 kHz and it is thus possible that some

neonates in this study had hearing loss in this high-frequency range. Because cochlear damage induced by aminoglycoside use is stationary over the years, the long-term clinical importance of this high-frequency hearing loss is doubtful. Third, delayed onset of hearing loss in infants has been described. In the study in chapter 5.2 a large percentage of patients exposed to tobramycin for more than 10 days underwent hearing screening at least two weeks after cessation of therapy. It is conceivable however, that hearing screening was performed too early in some patients to detect hearing loss and it might be necessary to reassess hearing in neonates with prolonged exposure to aminoglycosides.
The main limitation of our matched case-control study in 3-4 year old children exposed to tobramycin as neonates is the relatively small number of patients included. All patients whose address was traceable were approached, of whom approximately half responded. Also a few patients were not measurable due to chronic middle ear effusion.

Future perspectives

Although this thesis addresses several features of tobramycin and vancomycin use in neonates, further research into selected issues is needed.

- Efficacy of aminoglycosides in neonates can not be addressed effectively as long as more than 95% of treated neonates have negative blood cultures. Research into clinical, biochemical and other markers that can adequately select patients at risk for invasive bacterial infections is needed.
- For vancomycin, studies relating clinical efficacy to serum concentrations as well as MIC's will have to be performed.
- Although the safety of extended interval dosing of aminoglycosides in newborns is comparable to that of multiple daily dosing, a clinical advantage has not been conclusively shown. These advantages are mainly extrapolated from adult and animal studies. Further prospective double blind studies in large numbers of neonates are needed to assess this theoretical advantage.
- Ototoxicity related to aminoglycoside use in newborns has not been proven for short courses. The alarming case reports of delayed onset hearing loss in neonates with prolonged exposure to aminoglycosides warrants further investigation.
- Since mitochondrial point mutations are associated with aminoglycoside related ototoxicity, genetic studies in infants with unexplained hearing loss and exposure to aminoglycosides will have to be performed.
- This thesis describes aminoglycoside use in the first week of life. There is a lack of data on the change of aminoglycoside pharmacokinetics in the period between one week and one month. This gap will have to be filled, especially for VLBW-infants, to determine dosing intervals in this period.
- The role of TDM of vancomycin and aminoglycosides, preferably in conjunction with Bayesian feedback, will have to be redefined in the light of changing serum concentration goals.

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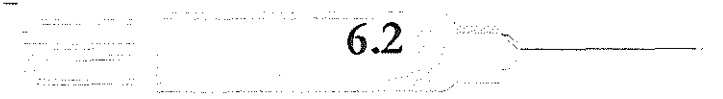
Summary

(Samenvatting)

Chapter



SUMMARY

This thesis describes pharmacokinetic and pharmacodynamic aspects of tobramycin and vancomycin in the neonatal intensive care unit.

Chapter 1 provides an overview of current knowledge on use of both antibiotics in the neonatal setting, and describes the aims of the studies.

Chapter 1a describes the use of the four main aminoglycosides (gentamicin, tobramycin, netilmicin and amikacin) in neonates. Special attention is given to the influence of gestational age and patient-related factors, such as exposure to ECMO, indomethacin and corticosteroids. The recent shift towards longer dosing intervals of aminoglycosides in adults, which has also been noted in neonates has implications for dosing and TDM of these drugs in neonates.

Chapter 1b summarizes the literature on use of vancomycin in the NICU. The relation between pharmacokinetic behavior and PNA as well as renal function is described in detail. The recent discussion regarding the validity of current therapeutic range targets as well as necessity of routine determination of peak serum concentrations in adults are put in a neonatal context. The implications for drug dosing as well as TDM in newborns are discussed.

Chapter 1c denotes the objectives of the studies presented in this thesis:

1. Explore the use of parametric and nonparametric population modeling of tobramycin in the setting of routine therapeutic drug monitoring in a NICU.
2. Determine a gestational age related extended interval dosing of tobramycin in neonates.
3. Investigate the potential of individualizing drug therapy by way role of early TDM of tobramycin in neonates.
4. Determine the need for gestational age or postconceptional age related dosing of vancomycin in the NICU.
5. Evaluate the occurence of hearing loss caused by neonatal exposition to vancomycin and/or tobramycin.

In ***chapter 2*** we compared two population modeling methods, nonlinear mixed effect modeling (NONMEM) and nonparametric expectation maximization (NPEM), using routinely obtained therapeutic drug monitoring data of tobramycin in neonates. NONMEM and NPEM were found to be dissimilar in population estimates. The source

of these differences was investigated by creating models in which the error algorithm used by NONMEM emulates that of NPEM and vice versa. We determined that differences in range estimates of pharmacokinetic parameters between NONMEM and NPEM are largely determined by the method of incorporating error patterns in both programs. Use of both modeling techniques are synergistic in adequately describing population pharmacokinetics.

Chapter 3 describes the development of a neonatal dosing regimen of tobramycin and the place of early TDM in individualizing treatment in neonates in the first week of life.
In *chapter 3.1* a tobramycin dosing schedule was established for neonates of various gestational ages, with use of a population pharmacokinetic method. In a study in 470 newborns in the first week of life, paired peak and trough serum concentrations were analyzed according to a one-compartment open model using NONMEM population pharmacokinetic software. Using population estimates the following dosing regimen was recommended: GA below 32 weeks: 4 mg/kg/48 hours, GA between 32 and 36 weeks: 4 mg/kg/36 hours, GA above 36 weeks: 4 mg/kg/24 hours. This dosing advice was prospectively tested in a group of 26 patients. Measured concentrations were within the desired therapeutic range for more than 90% of patients. This study taught us that dose intervals in newborns in the first week of life are GA-related and should be longer than generally recommended.
In *chapter 3.2* we looked at the possibility of refining individual treatment as recommended in chapter 3.1 by way of linear pharmacokinetics or a population model with Bayesian feedback. Tobramycin concentrations of 206 patients were used to obtain gestational age dependent population models with NPEM software. A second group of 41 patients with different sampling times was studied as well. Serum trough concentrations were predicted using linear pharmacokinetics in both groups and by using the population models with Bayesian feedback of one or two serum concentrations in the second group. These predicted concentrations were compared to actual serum trough concentrations. The predictive performance of these models was compared to the GA-related model in chapter 3.1 without TDM.
None of the evaluated models yielded a significant improvement of predictive performance over the model without TDM. This study showed us that early therapeutic drug monitoring does not improve the model based prediction of initial tobramycin dosing intervals in neonates in the first week of life.

In ***chapter 4*** we performed a study on vancomycin pharmacokinetics in neonates in the first month of life. In the same way as in the study on tobramycin, routinely sampled peak and trough serum concentrations in steady state of 108 newborns were analyzed with NONMEM, according to a one-compartment open model. The model best fitting the data included clearance and volume per kg and was independent of GA. Simulation of various dosing schemes showed that a dosing schedule of 30 mg/kg/day, irrespective of GA, in three doses was optimal, and this scheme was prospectively tested in 22 patients. Mean trough concentrations were comparable to predicted trough concentrations. No peak levels higher than 40 mg/L were found. The conclusion of our study was that the proposed dosing regimen leads to adequate vancomycin trough serum concentrations. There is no need for routine monitoring of peak serum concentrations.

Chapter 5 describes ototoxicity in relation to administration of tobramycin and/or vancomycin.

In *chapter 5.1* we tested the effect of neonatal tobramycin use on hearing loss in 3-4 year old children. This study was a pilot case-control study where neonates who had received tobramycin during their admission where compared to newborns who had only received other antibiotics. Nine NICU graduates with a high risk profile for aminoglycoside induced hearing loss (prolonged exposition to tobramycin and/or high serum concentrations) were matched for other potential risk factors for hearing loss with nine control infants. Hearing was evaluated by means of oto-acoustic emissions and, if necessary, brainstem evoked response audiometry. Although there was no statistical difference, three of nine tobramycin treated children had moderate to severe cochlear hearing loss compatible with aminoglycoside ototoxicity. These three infants were all exposed to tobramycin for longer than 14 days and there was no relation to high serum concentrations. All control patients had normal hearing. Our results suggest that tobramycin ototoxicity is related to duration of therapy rather than serum concentrations. Hearing screening of infants with prolonged exposure to tobramycin is warranted.

In *chapter 5.2* we investigated the effect of administration of vancomycin, tobramycin and furosemide on hearing in 625 neonates. This group of newborns underwent routine automated auditory brainstem response screening as part of neonatal follow-up on the basis of previously described risk factors. The relation between administration of the aforementioned ototoxic drugs and a failure to pass hearing screening was investigated. No statistical relation of hearing loss to exposition to these drugs, described in terms of total exposure as well as aberrant serum concentrations, was found. In individuals failing

to pass hearing screening, a causal relation between exposure to ototoxic medication and hearing loss could not be demonstrated. The results of our study indicated that aminoglycoside- and vancomycin related ototoxicity is rare. TDM of these drugs was not helpful in detecting newborns at risk for hearing loss.

Chapter 6 described the results of our studies in context with the literature. We discussed the implications of our findings for dosing regimens and TDM of tobramycin and vancomycin in neonates. We provided a flow-chart for management of these two drugs in the NICU setting. Limitations of our studies were pointed out and directions for future research were given.

SAMENVATTING

Dit proefschrift beschrijft farmacokinetische en farmacodynamische aspecten van het gebruik van tobramycine en vancomycine in de neonatale intensive care unit (NICU).

Hoofdstuk 1 geeft een overzicht van de huidige kennis op het gebied van het gebruik van beide antibiotica in de neonatale setting en beschrijft de doelstellingen van de studies.
Hoofdstuk 1a beschrijft het gebruik van de 4 belangrijkste aminoglycosiden (gentamicine, tobramycine, netilmicine en amikacine) bij pasgeborenen. Hierbij wordt speciale aandacht gegeven aan de invloed van zwangerschapsduur en patiëntgerelateerde factoren zoals blootstelling aan extracorporele membraan oxygenatie, indomethacine en corticosteroïden. De recente verschuiving naar langere doseringsintervallen van aminoglycosiden bij volwassenen, die ook bij neonaten gezien wordt, heeft implicaties voor dosering en therapeutische monitoring van deze geneesmiddelen bij pasgeborenen.
Hoofdstuk 1b vat de literatuur op het gebied van het gebruik van vancomycine bij pasgeborenen samen. De relatie tussen farmacokinetisch gedrag en postnatale leeftijd alsook nierfunctie wordt in detail beschreven. De recente discussie in de volwassen literatuur aangaande de validiteit van de huidige doelen voor therapeutische vancomycine spiegels, alsmede de noodzaak van het routinematig meten van piek serumconcentraties, wordt besproken in de neonatale context. De gevolgen voor dosering en spiegelbepalingen worden bediscussieerd.
Hoofdstuk 1c geeft de doelstellingen weer van de studies in dit proefschrift:

1. Onderzoek het gebruik van parametrisch en non-parametrisch populatiemodelleren van tobramycine tegen de achtergrond van het routinematig monitoren van dit geneesmiddel op een NICU.
2. Bepaal een zwangerschapsduur afhankelijk doseringsinterval voor tobramycine bij pasgeborenen.
3. Onderzoek de mogelijkheden van het individualiseren van de neonatale behandeling met tobramycine door middel van vroege bepaling van geneesmiddel spiegels.
4. Bepaal de noodzaak tot het hanteren van een zwangerschapsduur of postconceptioneel gerelateerde dosering van vancomycine bij pasgeborenen op een NICU.
5. Evalueer het vóórkomen van gehoorverlies veroorzaakt door neonatale blootstelling aan vancomycine en/of tobramycine.

In ***hoofdstuk 2*** is met gebruik van routinematig verkregen geneesmiddelspiegels van tobramycine bij neonaten een vergelijking gemaakt tussen twee methodes voor het modelleren van populaties; nonlinear mixed effect modeling (NONMEM) en nonparametric expectation maximization (NPEM). NONMEM en NPEM bleken te verschillen in populatie schattingen. De bron van deze verschillen werd onderzocht door modellen te creëren waarin NONMEM het fouten algoritme dat gebruikt wordt door NPEM simuleert en vice-versa. Wij stelden vast dat de verschillen in schattingen van de spreiding van farmacokinetische parameters met name bepaald worden door de manier waarop beide programma's fouten algoritmen inbouwen. Gebruik van beide populatie-modellen is synergistisch in het adequaat beschrijven van populatie farmacokinetiek.

Hoofdstuk 3 beschrijft de ontwikkeling van een neonataal doserings schema van tobramycine. Tevens wordt de plaats bepaald van het vroeg bepalen van serumspiegels bij neonaten in de eerste levensweek ten behoeve van individualisering van de behandeling.
In *hoofdstuk 3.1* wordt met behulp van een populatie farmacokinetisch model een doseringsschema vastgesteld voor neonaten met een verschillende zwangerschapsduur. De gepaarde piek- en dalconcentraties van 470 neonaten in de eerste levensweek werden geanalyseerd met gebruikmaking van een 1-compartiments model in NONMEM. Met behulp van de populatieschattingen werd het volgende doseringsschema geadviseerd: zwangerschapsduur < 32 weken: 4 mg/kg/48 uur, zwangerschapsduur tussen 32 en 36 weken: 4 mg/kg/36 uur, zwangerschapsduur ≥ 37 weken: 4 mg/kg/24 uur. Dit doseringsadvies werd prospectief getest in een groep van 26 patiënten. Gemeten serum concentraties lagen binnen het gewenste therapeutische bereik bij meer dan 90% van de patiënten. Deze studie leerde ons dat doseringsintervallen van pasgeborenen in de eerste levensweek zwangerschapsduurafhankelijk zijn en langer dienen te zijn dan algemeen aangenomen.
In *hoofdstuk 3.2* hebben we gekeken naar de mogelijkheid van het verfijnen van de individuele behandeling, zoals aanbevolen in hoofdstuk 3.1, door middel van het gebruik van lineaire farmacokinetiek of een populatiemodel met Bayesiaanse terugkoppeling. De tobramycine concentraties van 206 patiënten werden gebruikt in NPEM om zwangerschapsduur afhankelijke populatiemodellen te verkrijgen. Een tweede groep van 41 patiënten met andere afname tijdstippen van serumconcentraties werd eveneens bestudeerd. De dalspiegels werden voorspeld met behulp van lineaire farmacokinetiek in beide groepen en met de populatiemodellen met Bayesiaanse terugkoppeling van één of twee serumspiegels in de tweede groep. Deze voorspelde concentraties werden vergeleken

met de gemeten dalconcentraties. De voorspellende waarde van deze modellen werd vergeleken met het zwangerschapsduur afhankelijke doseringsschema van hoofdstuk 3.1 zonder gebruik van serum concentraties. Geen van de onderzochte modellen gaf een significante verbetering van de voorspellende waarde boven het model van hoofdstuk 3.1, waarbij geen gebruik wordt gemaakt van serumconcentraties. Deze studie liet ons zien dat routinematig vroege bepaling van serumconcentraties geen toegevoegde waarde heeft voor het voorspellen van de initiële tobramycine doseringsintervallen van pasgeborenen in de eerste levensweek.

In ***hoofdstuk 4*** verrichtten wij een studie naar de farmacokinetiek van vancomycine bij pasgeborenen in de eerste levensmaand. Zoals bij de studie naar tobramycine, werden routinematig verkregen steady-state top- en dalspiegels van 108 neonaten geanalyseerd met NONMEM, volgens een één-compartimentsmodel. Het best passende model bevatte klaring en volume per kilogram en was onafhankelijk van de zwangerschapsduur. Simulatie van verschillende doseringsschema's liet zien dat een dosering van 30 mg/kg/dag, verdeeld over 3 doses, onafhankelijk van de zwangerschapsduur, optimaal was. Dit schema werd prospectief onderzocht bij 22 patiënten. De gemiddelde dalspiegels waren vergelijkbaar met de voorspelde waarden. Er weren geen piekspiegels boven de 40 mg/L gevonden. De conclusie van onze studie was dat het voorgestelde doseringsschema leidt tot adequate vancomycine dalspiegels. Er is geen noodzaak tot routinematig bepalen van vancomycine piekspiegels.

Hoofdstuk 5 beschrijft ototoxiciteit in relatie tot de toediening van tobramycine en/of vancomycine.
In *hoofdstuk 5.1* testten we het effect van het gebruik van tobramycine in de neonatale periode op het vóórkomen van gehoorverlies bij 3-4 jaar oude kinderen. Deze studie was een pilot case-control studie waarin neonaten die tobramycine hadden gekregen gedurende hun opname werden vergeleken met pasgeborenen die alleen andere antibiotica hadden gekregen. Negen ex-neonaten met een hoog risico profiel voor aminoglycoside gerelateerd gehoorverlies (langdurige blootstelling aan tobramycine en/of hoge serum spiegels) werden gematched met negen controle patiënten voor andere potentiële risicofactoren voor gehoorverlies. Het gehoor werd geëvalueerd met oto-acoustische emissies en, zonodig, hersenstam respons audiometrie. Alhoewel er geen statistisch significant verschil was, hadden 3 van de 9 kinderen die behandeld waren met aminoglycosiden een matig tot ernstig cochleair gehoorverlies, compatibel met

aminoglycoside ototoxiciteit. Deze drie kinderen waren allen langer dan 14 dagen blootgesteld aan tobramycine en er was geen relatie met serumspiegels. Alle controlepatiënten hadden een normaal gehoor. Onze resultaten suggereren dat tobramycine ototoxiciteit sterker gerelateerd is aan de duur van de behandeling dan aan serumspiegels. Gehooronderzoek van neonaten met langdurige blootstelling aan tobramycine is noodzakelijk.

In *hoofdstuk 5.2* onderzochten wij het effect van toediening van vancomycine, tobramycine en furosemide op het gehoor bij 625 neonaten. Deze groep kinderen onderging routinematige gehoorsscreening met de geautomatiseerde auditieve hersenstam respons methode (A-ABR) als onderdeel van neonatal follow-up op basis van eerder beschreven risicofactoren. De relatie tussen toediening van de hiervoor genoemde ototoxische geneesmiddelen en het "niet slagen" voor de gehoorsscreening werd onderzocht. Er werd geen statistische relatie gevonden met blootstelling aan deze geneesmiddelen, uitgedrukt in termen van totale blootstelling alsmede afwijkende serumspiegels. Ook kon bij individuele patiënten geen causaal verband worden aangetoond tussen blootstelling aan deze ototoxische medicamenten en het "niet slagen" voor de gehoorsscreening.

De resultaten van deze studie gaven aan dat aminoglycoside- en vancomycine gerelateerde gehoorsschade zeldzaam is. Routinematig bepalen van serumspiegels hielp niet bij het detecteren van pasgeborenen met een verhoogd risico op gehoorverlies.

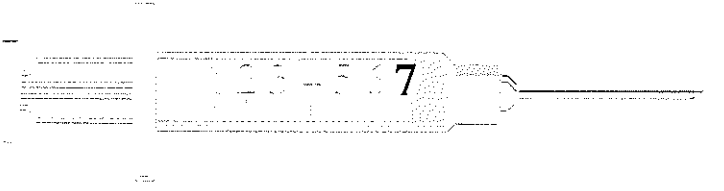
Hoofdstuk 6 beschrijft de resultaten van onze studies in samenhang met de literatuur. De implicaties van onze bevindingen ten aanzien van doseringsschema's en routinematig bepalen van serumspiegels van tobramycine en vancomycine bij pasgeborenen werden bediscussieerd. Er werd een stroomdiagram gepresenteerd voor het praktisch hanteren van deze twee geneesmiddelen in de neonatale setting. De beperkingen van onze studies werden beschreven en suggesties voor toekomstig onderzoek werden gedaan.

Dankwoord

Curriculum vitae

List of publications

Chapter



Dankwoord

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Curriculum Vitae

De auteur werd geboren op 22 februari 1960 te Rijswijk (Z-H). In 1978 behaalde hij het VWO-diploma aan de Christelijke Scholengemeenschap Rijswijk. In dat najaar begon hij met de studie geneeskunde aan de Rijksuniversiteit Leiden. Het artsexamen werd behaald in 1987. Na het vervullen van de dienstplicht was hij achtereenvolgens AGNIO verloskunde/gynaecologie en AGNIO kindergeneeskunde in het Reinier de Graafgasthuis te Delft. Op 1 april 1991 begon hij zijn opleiding tot kinderarts, eveneens te Delft (opleider : dr. P.J.C. van der Straaten). Vanaf 1 april 1992 werd de opleiding voortgezet in het Sophia Kinderziekenhuis te Rotterdam (opleider : prof. dr. H.K.A. Visser). Op 1 januari 1996 werd hij ingeschreven in het specialistenregister als kinderarts. Op 1 april 1996 startte hij zijn opleiding tot kinderarts-intensivist op de afdeling Intensive Care pediatrie van het Sophia Kinderziekenhuis. (hoofd : drs. E. van der Voort). Na twee jaar werd deze opleiding afgerond en werd hij geregistreerd als subspecialist. Vanaf 1 juli 1998 is hij als staflid verbonden aan de subafdeling Intensive Care Pediatrie van het Sophia Kinderziekenhuis (hoofd : prof. dr. H.A. Büller).

Tot zijn aandachtsgebieden behoort geneesmiddelonderzoek op de kinderleeftijd en hij is betrokken bij nationaal en internationaal onderzoek op dit gebied. Het onderzoek dat heeft geleid tot dit proefschrift werd uitgevoerd op de afdeling Intensive Care Neonatologie van het Sophia Kinderziekenhuis.

Tevens is hij intensief betrokken bij de lokale, regionale en landelijke scholing ter verbetering van de acute opvang van het ernstig zieke kind.

Hij is getrouwd met Patricia Geers en heeft 3 kinderen, Amy (1985), Ted (1988) en Liselotte (1995).

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