Population pharmacokinetics of antibiotics to prevent group B streptococcal disease: from mother to neonate

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Chapter 10

The pharmacokinetics of clindamycin in pregnant women in the peripartum period.

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Abstract

Objective: This study was undertaken to describe the pharmacokinetics of intravenously administered clindamycin in pregnant women.
Study Design: Pregnant women were recruited who needed treatment with clindamycin in the prevention of neonatal group B streptococcal disease or of endocarditis (900 mg every 8 hours and 600 mg every 6 hours respectively). Following delivery, both arterial and venous umbilical cord blood samples were obtained. Clindamycin concentrations were determined with the use of high-pressure liquid chromatography. Nonlinear mixed-effects modeling was performed in NONMEM.
Results: The pharmacokinetics of 7 patients was best described by a three-compartment model. Clearance and volume of distribution at steady state were 10.0 L/h and 6.32 x 10^3 L, respectively. Using a 900 mg dosing regimen in the average pregnant women, the ratios of the area under the concentration time curves in maternal serum over the Minimum Inhibitory Concentration (MIC) were 64, 32, and 8 for assumed protein binding of 60%, 80% and 95% respectively. Concentrations in umbilical cord were lower compared to the maternal concentrations.
Conclusion: Concentration-time profiles in maternal serum are likely to be adequate for the average pregnant woman. In contrast the observed concentrations in arterial umbilical cord blood indicate that the current dosing regimen may not be adequate to prevent the neonate from group B streptococcal disease.

Introduction

In pregnant women, clindamycin is used for various clinical conditions related to the mother or the neonate, especially when penicillin-allergy is encountered. Clindamycin is active against gram-positive cocci and anaerobic bacteria. In pregnant women at risk for endocarditis, it may be used to protect against infective endocarditis during labor[1]. Clindamycin is also one of the alternative drugs to protect neonates at risk for invasive group B streptococcal (GBS) disease[2]. Especially concerning the prevention of GBS disease, the use of antibiotics during labor has increased after the implementation of the culture-based prevention strategy in many countries[2].

During pregnancy and labor important physiological changes occur that may modify the pharmacokinetics of drugs. In non-pregnant individuals it has been shown that clindamycin distributes widely over the body, but that it does not adequately cross the blood-brain-barrier, even in case of bacterial meningitis[3]. It is metabolized and subsequently excreted into the urine and bile. The protein binding in non-pregnant humans ranges between 62% to 94%[4-8]. Because clindamycin is recommended in the prevention of both maternal and neonatal infection, the pharmacokinetics during labor in the mother and the transfer of the drug over the placental barrier are important.

Pharmacokinetic studies during labor face considerable ethical and logistical difficulties, limiting the opportunity for the collection of blood samples. These limitations may be overcome by the application of innovative approaches to the analysis of sparse data. Specifically, Non-Linear Mixed Effects Modeling (NONMEM) allows weighted analysis of data from both patients with large datasets and patients with small or incomplete datasets[9,10]. Moreover, by studying the population as a whole, the influence of specific circumstances on the individual PK parameters can be assessed using covariate analysis[11,12]. A more detailed background of population modeling can be found elsewhere[13,14]. The objective of this study is to describe the pharmacokinetics of intravenously administered clindamycin in pregnant women in the perinatal period and the transfer of clindamycin over the placental barrier.

Material and Methods

Patients

In the period between February 7, 2005 and February 28, 2007, all women with a gestational age of more than 26 weeks who needed antibiotic treatment with clindamycin were eligible for this study. Following the local guidelines, all women with proven or unknown *Streptococcus agalactiae* carriage were treated with

antibiotics when pregnancy was complicated by one of the following factors: preterm premature rupture of the membranes, rupture of the membranes for >18 hours, prematurity, fever (>37.8° C), bacteriuria in current pregnancy and a previous child with invasive GBS disease. The choice of the antibiotic for this study was dictated by the local guidelines, which recommend clindamycin in case of penicillin allergy in the prevention of GBS disease. Patients with an increased risk on endocarditis received clindamycin approximately 1 hour before delivery. Clindamycin is the antibiotic of first choice in the prevention of endocarditis, following local hospital guidelines.

The study was approved by the Medical Ethics Committee of the Medical Center Haaglanden, The Hague. Written informed consent was obtained from all patients. Women were excluded from the study when (1) they had been treated with oral or intramuscular antibiotics within 2 days before starting the therapy, (2) were unwilling to comply with the requirements of the study, (3) were known to be allergic to clindamycin, or (4) received co-medication that exhibits known interaction with clindamycin. All patients were at least 18 years of age.

All patients received a standard work-up that included a medical history and, biochemical and hematological examination at the onset of the study. Furthermore blood pressure, pulse, oral temperature, and body weight were recorded before the antibiotic administration.

Drug administration and blood sampling

Before the administration of clindamycin two intravenous catheters were placed, one in each arm. Clindamycin was administered according to local guidelines in the hospital using the first catheter. The dose of 600 mg, as prescribed in the prevention of endocarditis, was administered over 20 minutes (12 ml/mL NaCl 0.9%) every 6 hours. The dose of 900 mg, as used in the prevention of GBS disease, was administered over 30 minutes (9 mg/mL NaCl 0.9%) every 8 hours. The exact duration of infusion was recorded.

Blood samples of 2 mL were collected from the second catheter in the contralateral arm at timed intervals beginning at 1 min after the start of the infusion and, at 10 and 20 min (600 mg infusion) or 15 and 30 min (900 mg infusion). After completion of the infusion, sampling was scheduled at 3, 6, 10, 16 and 36 minutes, and afterwards every 30-45 minutes until the next antibiotic dosage. Blood samples were collected when possible, taking into consideration the physical and emotional inconvenience to the woman. The exact sampling times were recorded. Immediately after birth, both arterial and venous umbilical cord blood was obtained.

Blood samples were placed immediately on ice, allowed to clot and processed within one hour after collection. The samples were centrifuged at 1200 g for approximately 10 min. The supernatants were transferred into plastic storage tubes and frozen at -70˚ C until analysis.

Clindamycin HPLC

Samples were extracted by adding 0.05 ml NaOH (1.2 M) containing 40 mg/L propanolol as the internal standard. After vortexing, 2.6 ml dichloromethane (Sigma, The Netherlands) was added, and after vortexing, centrifuged during 5 min at 1500 g. The supernatant was removed and 2 ml pipetted in a new vial. The contents were dried at 40° C, airflow 5 L/min and solved in 0.2 mL potassiumdihydrophosphate, pH 4.6.

Clindamycin concentrations in 0.05 mL were determined by HPLC (Shimadzu, Den Bosch, NL). A reverse phase method (0.066 M pH 4.6 potassiumdihydrophosphate with 20% acetonitril), a C18 column (Bester, Amstelveen, NL) with a UV-VIS detector, wavelength 200 nm, temperature 40° C was used. The runtime was 10 min, injection volume 0.05 ml, flow 1 ml/min. A standard curve of clindamycin (Sigma-Aldrich, NL) was determined during each run. The lower limit of detection and quantification was 0.1 mg/L and was linear up to 50 mg/L. Higher concentrations were determined by diluting the samples. The between sample between day coefficient of variation (CV) was < 5%.

Pharmacokinetic analysis

Pharmacokinetic parameters were estimated by means of Non-Linear Mixed Effect Modeling (NONMEM). The model was implemented in the NONMEM ADVAN5 subroutine and the analysis was performed using the FOCE method with INTERACTION. All fitting procedures were performed with the use of the Compaq Visual FORTRAN standard edition 6.6 (Compaq Computer Cooperation, Euston, Texas, USA) and NONMEM® software package (version VI, release 1.2, ICON Development Solutions, Ellicott City, Maryland, USA).

To determine the basic structural pharmacokinetic parameters various 2- and 3-compartment models were tested. Model selection and identification of variability were based on the evaluation of the mean objective function value (OFV), pharmacokinetic parameter point estimates, and their respective confidence intervals, and goodness-of-fit plots. For differences between two structural models, the OFV with a pre-specified level of significance of $p<0.001$ was used (corresponding to a difference in OFV of at least 10 points). NONMEM minimizes an objective function in performing nonlinear regression analysis. To detect systematic deviations in the model fits, the goodness-of-fit plots were visually inspected. Data of individual observations versus individual or population predictions should be randomly distributed around the line of identity. The weighted residuals versus time or population predictions should be randomly distributed around zero. Population values were estimated for the parameters clearance (CL), the volumes of distribution (V) and intercompartmental clearances (Q).

Individual estimates for pharmacokinetic parameters were assumed to follow a log-normal distribution. Therefore an exponential distribution model

was used to account for inter-individual variability. Possible correlation between inter-individual variability coefficients on parameters was estimated and if present accounted for in the stochastic model (NONMEM Omega block option).

Selection of an appropriate residual error model was based on the evaluation of OFV and inspection of the goodness-of-fit plots. A proportional error model, additive error model and a combined proportional-additive error model were tested to describe the residual variability between the observed concentrations and those predicted by the model. The residual error term contains all the error terms, which cannot be explained and refers to, for example, measurement and experimental error and structural model misspecification.

To refine the model covariate analysis was also performed. The estimated pharmacokinetic parameters, on which a random effect has been identified, were plotted independently against the covariates bodyweight, body mass index, gestational age, oral temperature, the amount of edema and singleton or twin pregnancy to determine whether this influenced the pharmacokinetics. Covariate analysis was performed by forward addition of each candidate covariate into the model structure until no further improvement of goodness of fit was observed. A significance level of 0.05 was selected (corresponding to difference in OFV of 3.84 points). A further criterion for acceptance of covariate effects was that the estimated 95% confidence interval of the covariate effect did not overlap with zero. Contribution of each covariate to the final model was confirmed by backward deletion of each covariate from the model to account for possible interaction between covariates. Residual intra- and inter-individual variabilities were visually evaluated. The volume of distribution at steady state (V_{ss}) was calculated following standard procedures[15].

The accuracy of the final population model for the entire population was established using the bootstrap option in NONMEM, consisting of repeated random sampling with replacement from the original data. This resampling was repeated 100 times. The estimated parameters from the bootstrap analysis were compared to the estimates from the original data.

The mean pharmacokinetic estimates of the final model derived from the PK analysis in NONMEM were used in Berkeley Madonna (version 8.3.5, Berkeley Madonna Inc, University of California, USA) to simulate the mean concentration-time profiles after a 600 mg and 900 mg clindamycin dose in pregnant women during labor. These maternal concentrations were used to calculate ratios of the umbilical cord concentrations and simultaneous maternal concentrations. Furthermore, ratios of the area under concentration curve for the free-drug in maternal serum for 24 hours over the MIC (fAUC$_{0\text{-}24h}$/MIC) were calculated, taking into account various percentages of clindamycin protein binding. For clindamycin the ratio for the total drug concentration should be at least 147, whereas for free-drug concentrations this ratio should be at least 27 [16].

Results

In total, seven patients were included. Of these patients four received clindamycin in the prevention of neonatal GBS disease and two to prevent the mother from endocarditis. One patient needed antibiotics to prevent both neonatal GBS disease and endocarditis. The physician decided to treat her with clindamycin using the dosing regimen for GBS prevention. Six patients with singleton pregnancies were in labor. One patient was treated because of preterm premature rupture of the membranes (PPROM) and had a twin pregnancy. The characteristics of the study population are presented in table 1. All patients receiving clindamycin as

Data	**Units**	**Mean**	**SD**	**Range**
Maternal age	Y	36.1	4.24	31.3-41.8
Gestational age	wk	38.3	3.01	34-42.3
Body mass index	kg/m^2	32.1	5.36	22.1-39.1
Weight	kg	86.1	14.2	59.5-104.8
Creatinin	umol/L	55.9	20.3	38-100
Ureum	mmol/L	3.39	1.17	1.8-5.6
Uric acid	mmol/L	0.31	0.086	0.18-0.43
AF	U/L	495	601	168-1794
ASAT	U/L	22.6	6.78	15-34
ALAT	U/L	10.9	5.27	5-18
γGT	U/L	10.7	6.85	5-25
LDH	U/L	351	74.6	247-445
Systolic blood pressure	mmHg	118	17.8	100-150
Diastolic blood pressure	mmHg	65	7.64	50-70
Pulse	/ min	84.6	11.1	74-108
Temperature	° C	36.9	0.23	36.7-37.4

Table 1 *Population characteristics (n=7).*

prevention of GBS were healthy. Of the three patients receiving clindamycin for endocarditis prophylaxis one had a minor stenosis of the mitral valve, the second had a prosthetic aortic valve, a prosthetic mitral valve and had been operated in the past on the tricuspidal valve. The third patient had a dysfunction of the aortic valve and autoimmune thrombocytopenic purpura (AITP), what had resulted in a splenectomy.

A total of 177 samples were included in the study. Two samples were excluded because the concentrations were <0.1 mg/L. In one patient it was not possible to place the two catheters each on a separate arm. Both catheters were placed on the right arm. The catheter used for the clindamycin infusion was flushed after the clindamycin administration and occluded. The samples collected during the clindamycin infusion in this patient were excluded from the analysis. In one patient with endocarditis prophylaxis, only four samples could be obtained due to obstruction of the sampling catheter. In three out of the seven patients results of an infusion in the postpartum period only for study purposes were also included. One patient receiving clindamycin as GBS prophylaxis received a postpartum dose of 600 mg, because she only agreed with a postpartum dose when the time interval between delivery and the last dose was short.

From all six patients in labor both arterial and venous umbilical cord blood samples were taken. The individual arterial and venous concentrations, the time-interval between the collection of the samples and the start of the antibiotic infusion

Patient	Clindamycin dose (mg)	Time between start infusion and sampling (h)	Time between maternal peak-concentration and sampling (h)	Arterial cord concentration (mg/L)	Venous cord concentration (mg/L)	Maternal concentration (mg/L)*
1	900	1.85	1.38	2.5	1.1	5.08
2	900	4.27	3.67	0.9	1.0	1.88
3	900	1.45	0.85	4.0	3.3	6.01
4	600	4.53	4.15	1.1	1.1	0.69
5	900	10.5	10	0.1	0.1	0.19
6	600	0.95	0.65	1.7	2.7	3.03

* Maternal concentrations were determined by simulation using Berkeley Madonna with the mean parameter estimates from the final PK model.

Table 2 *Clindamycin concentrations in arterial and venous umbilical cord blood and in maternal blood.*

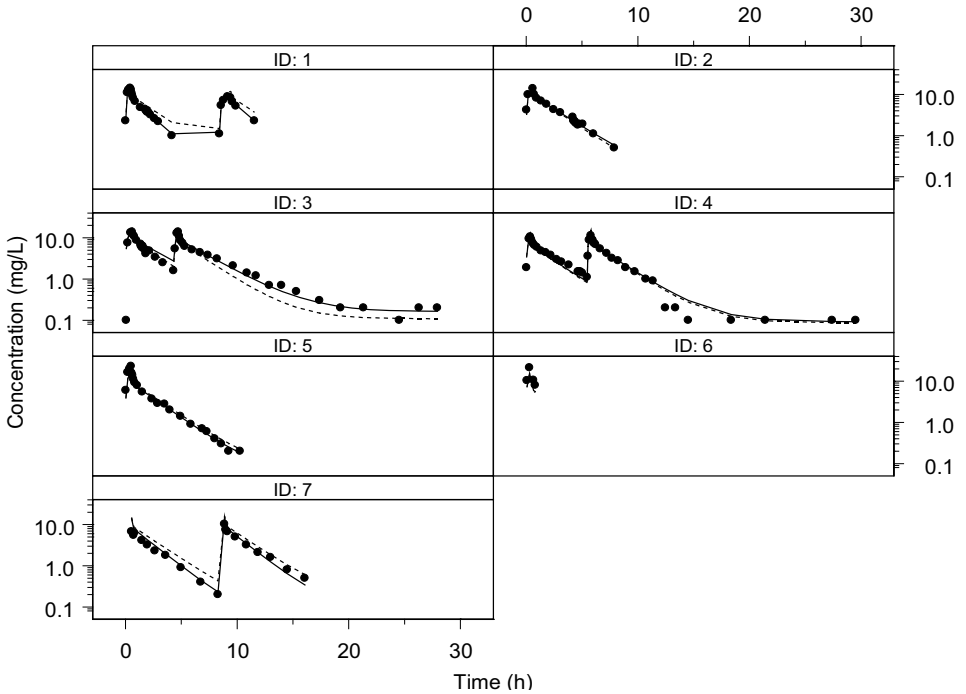

Figure 1: *individual plots with the final 3-compartment model. The black dots correspond with the individual datum points. The line represents the individual estimate and the dotted line the population estimate.*

and the time of the antibiotic peak-concentration are shown in table 2. Ratios of the venous umbilical cord blood concentrations and simultaneous maternal blood concentrations ranged from 0.22 to 0.89, with one outlier of 1.59. Ratios of arterial umbilical cord blood concentrations and simultaneous maternal blood concentrations ranged from 0.48 to 0.67 with one outlier of 1.59.

Various pharmacokinetic models were tested. Implementation of a 3-compartment model instead of a 2-compartment model improved the model-fit. The OFV decreased with 190 points, indicating that the 3-compartment model described the data better than the 2-compartment model. Using the 2-compartment model the concentration-time profiles in the two patients included for a prolonged period after the postpartum clindamycin infusion were not described adequately (figure 2A). Implementation of the 3-compartment model improved the model-fit, as is seen in figure 2B. Improvement of the model-fit using a 3-compartment model was also seen in the goodness-of-fit plots.

Considering the change in OFV, the visual inspection of the individual plots and the goodness-of-fit plots as well as the estimates of the pharmacokinetic parameters with their respective CVs, a three-compartment open model best

described the data. The residual error was best described by a proportional error model. Inter-individual variability was explained by variation in the parameters CL, V_3 and the residual error (54% on CL, 4.0% on V_3 and 55% CV on the residual error). None of the covariates could improve the model-fit. The V_{ss} was calculated to be 6.32 x 10^3 L and the gamma-phase $t_{1/2}$ was 2.6 h. The final estimates of the pharmacokinetic parameters and their respective CVs and 95% confidence intervals are presented in table 3. Due to the limited number of patients the parameter estimates for the inter-individual variability were not statistically significant. The individual plots and the plots of the observed concentrations versus the predicted concentrations are shown in figure 1 and 3 respectively.

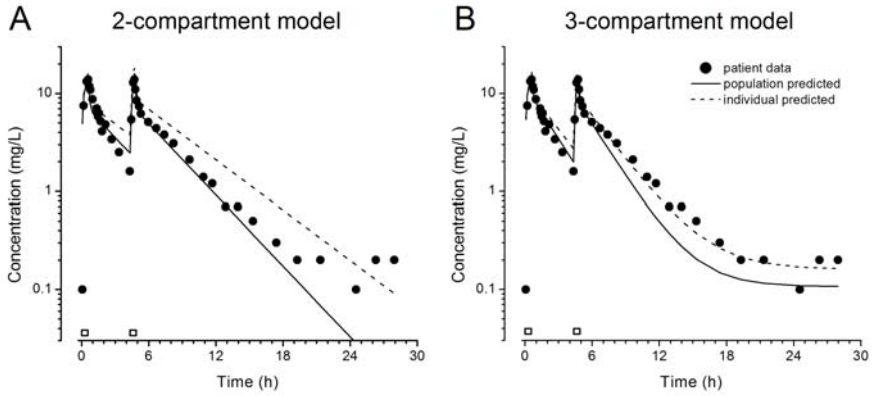

Figure 2*: ID 3 modeled with a 2- compartment model (figure 2A) and a 3-compartment model (figure 2B). The blocks indicate the time at which the infusion of the clindamycin was started and stopped.*

The bootstrap validation of the model of the entire population was performed with 100 runs. The bootstrap validation was successful for 92 runs. From the mean parameter estimates of the runs obtained from the bootstrap analysis only the estimate for V_3 deviated significantly from the predicted values from the NONMEM PK analysis. The estimates for V_3 could not be determined with good accuracy, because in the study population only two patients were included with a prolonged concentration-time profile. Therefore, this indicates that the accuracy of the final model is good.

Parameter	Units	Estimates of all patients		
		Mean	**CV**	**95% confidential interval**
Structural model parameters				
CL	L/h	10.0	41.2	1.92 – 18.1
V_1	L	12.4	11.5	9.62 – 15.2
V_2	L	52.2	6.3	45.8 – 58.6
V_3	L	6260	21.2	3650 – 8870
Q_1	L/h	137	6.27	120 – 154
Q_2	L/h	21.1	10.4	16.8 – 25.4
Variance model parameters				
interindividual variability in CL		0.293	69.3	-0.105 – 0.691
interindividual variability in V3		0.00162	920	-0.0276 – 0.0308
Interindividual variability in error		0.306	50.7	0.22 – 0.61
residual variability		0.0425	48.7	0.00193 – 0.0831

Table 3*: final estimates of the pharmacokinetic parameters and their respective CVs of the 3-compartment model.CL: Clearance, V_1: volume of distribution of the central compartment, V_2: volume of distribution of the first peripheral compartment, V_3: volume of distribution of the second peripheral compartment, Q: intercompartmental clearance between V_1 and V_2, Q_2: intercompartmental clearance between V_1 and V_3,CV: coefficient of variation.*

In the prevention of neonatal GBS disease clindamycin is administered intravenously to the mother. The clinical breakpoint of clindamycin for GBS as determined by the EUCAST is 0.5 mg/L[17]. Using the dosing regimen of 900 mg every 8 hours and 600 mg every 6 hours, the values of $f\text{AUC}_{0\text{-}24h}$/MIC for protein binding ranging from 60% to 95% are shown in table 4. The ratio of $f\text{AUC}_{0\text{-}24h}$/

MIC for the total drug concentration is also shown in table 4. Taking into account the protein binding, the 900mg dosing regimen results in a ratio of at least 32 for a protein binding up to 80%. The limited difference in ratio of fAUC$_{0\text{-}24h}$/MIC for the total drug concentration in maternal serum indicates that the dosing regimen of 900 mg every 8 hours might be more adequate than the dosing regimen of 600 mg every 6 hours.

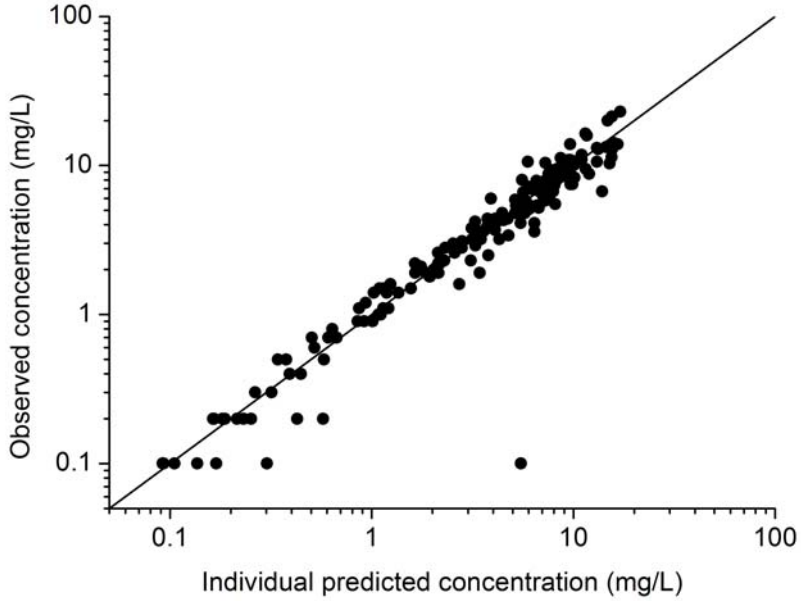

Figure 3: *plot of the observed versus the predicted concentrations using the final 3-compartment model.*

Discussion

In this study a pharmacokinetic model was developed to describe the pharmacokinetics of clindamycin in pregnant women. The pharmacokinetics of clindamycin in pregnant patients is best described using a 3-compartment model. Clearance and gamma-phase half-life were 10.0 L/h and 2.6 h, respectively. For the average pregnant women, the fAUC$_{0\text{-}24h}$/MIC ratio ranges from 64 to 8 for assumed

Percentage of protein binding	600 mg every 6 hours	900 mg every 8 hours	Minimal value for efficacy[16]
Total concentration (free and bound clindamycin)	129	159	147
60%	51.7	63.6	27
70%	38.7	47.8	27
80%	25.8	31.8	27
85%	19.4	23.9	27
90%	12.9	15.9	27
95%	6.46	7.98	27

Table 4: *ratios of $fAUC_{0\text{-}24h}$/0.5 for different percentages of protein binding and two dosing regimens. The MIC value for GBS used (0.5 mg/L) is determined by the EUCAST[17]. Additionally, the minimal value for efficacy as reported in the literature is shown.*

protein binding percentages of 60 to 95% using the 900 mg dosing schedule.

Data on the pharmacokinetics of clindamycin in pregnant women and non-pregnant individuals are scarce. Two previous studies determined the ratio of venous umbilical cord blood and maternal blood[18,19]. Weinstein et al found a ratio of 0.46[18] after intravenous administration during caesarean section. In contrast, Philipson et al[19] found ratios of 0.18 and 0.25 after an oral dose of clindamycin in women with a gestational age of 10 – 22 weeks during a therapeutic abortion. Our values are comparable to the ratio reported by Weinstein et al[18]. The low values reported by Philipson et al might be explained by the low gestational age and the difference in route of administration (orally in the study by Philipson and intravenously in our study). The pharmacokinetic parameter estimate in the mother for clearance using our 3-compartment model, results in a lower value as compared to values reported in the literature (10.3 L/h for our study versus 19.8 – 26.4 L/h in the literature[18,20-23]).

In the final 3-compartment model all structural parameters were estimated with an adequate precision (i.e. CV <51%). When the data were analyzed with a 2-compartment model, the estimates for the volumes of distribution could not be determined with adequate precision. As could be explained by the presence of the third compartment, the estimated value for the clearance using a 2-compartment model was larger than using the 3-compartment model (respectively 28.3 L/h and 10.0 L/h). This supports the inclusion of a third compartment in the pharmacokinetic model.

Clindamycin in mainly bound to alpha1-acid glycoprotein, an acute phase protein. The protein binding is dependent on the serum concentration of both alpha1-acid glycoprotein and clindamycin[8]. High concentrations of alpha1-acid glycoprotein result in a high protein binding, whereas, due to non-linearity in the protein binding an increase in the clindamycin concentration leads to a decrease in protein binding[8]. In our patients the percentage of protein binding is unknown, but compared to non-pregnant healthy volunteers it is likely to be reduced due to the state of pregnancy, but possibly increased due to being in labor, stress or the presence of infection[24]. Since only the free unbound fraction of drugs is active and the plasma protein binding of clindamycin is relatively high, a minor increase in protein binding might influence its efficacy.

Clindamycin has a time-dependent action in vitro, but clinical efficacy is more closely related to $fAUC_{0\text{-}24h}$/MIC[16,25]. The therapeutic goal to achieve a static effect is a ratio of at least 27, taking into account the clindamycin protein binding. However efficacy might be increased with higher ratios. Not for all percentages of protein binding reported in the literature, the current dosing regimen reaches adequate ratios. Furthermore, these concentration time profiles are only applicable for the average pregnant women. When one would take into account the inter-individual variability in pharmacokinetics this regimen is likely to be inadequate for some pregnant women.

Furthermore, to prevent neonatal GBS disease, both concentrations in maternal and in fetal serum have to be adequate. The concentration of alpha1-acid glycoprotein in the neonate increases with gestational age[24]. As has been shown for alprenolol, the affinity of $alpha_1$-acid glycoprotein for clindamycin might be decreased in the first 7 days of life, partly due to displacement by bilirubin[24]. Peak concentrations in the fetus are likely to be lower compared to the maternal peak concentrations, as has been shown for amoxicillin previously[26]. Arterial umbilical blood directly originates from the fetus and therefore these concentrations represent concentrations in the fetus. The number of measured clindamycin concentrations in the arterial umbilical cord samples in our study is relatively low. When the protein binding and the inter-individual variability are taken into account, it is doubtful whether adequate concentration-time profiles are reached in the fetus.

In conclusion, these data indicate that for the average pregnant women the current dosing regimen reach adequate concentrations assuming that the protein binding will not exceed 80% of the total concentration. To prevent the fetus from infection, concentrations in fetal blood have also to be adequate. Unfortunately, these data suggest that the concentration-time profiles in the fetus might be inadequate, at least for a substantial part of the population. More pharmacokinetic studies including data both of the mother and of the neonate are needed to investigate whether the currently advised dosing regimen is adequate to use as preventive measure against neonatal GBS disease.

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