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

Muller, A.E.

Chapter 6

Clavulanic acid does not influence amoxicillin pharmacokinetics in pregnant women during labor.

Anouk E. Muller, Rob A. Voskuyl, Johan W. Mouton, Paul M. Oostvogel, Joost DeJongh, Eric A.P. Steegers, Meindert Danhof, P. Joep Dörr

Abstract

We studied the pharmacokinetics of amoxicillin when co-administered with clavulanic acid in pregnant women during labor. Co-administration of amoxicillin with clavulanic acid did not result in changes in amoxicillin pharmacokinetics. The mean values (+/- SD) of the estimates for the CL of amoxicillin in patients treated with amoxicillin and amoxicillin in combination with clavulanic acid were, respectively, 22.8 +/- 2.4 L/h and 21.3 +/- 6.9 L/h. For V_1 the mean values (+/- SD) of the estimates were 11.0 +/- 2.6 L and 13.4 +/- 7.0 L for patients treated with, amoxicillin with and without clavulanic acid respectively. Based on these pharmacokinetic findings dose adjustments for amoxicillin during labor are not necessary when the drug is co-administered with clavulanic acid.

Introduction

Augmentin® or co-amoxiclav, an antibiotic formulation of amoxicillin and clavulanic acid, is widely used in pregnant women as treatment of choice for intra-amniotic infection (IAI). IAI during delivery is a serious complication and affects both mother and neonate. This can occur in up to 10.5 percent of all deliveries[1-5]. The frequency of IAI is highest in preterm deliveries[6].

When amoxicillin and clavulanic acid are administered simultaneously, the activity spectrum of amoxicillin against both gram-positive and gram-negative bacteria is enhanced. Clavulanic acid irreversibly inhibits a wide range of β-lactamases produced by micro-organisms, thereby protecting the β-lactam antibiotics from enzymatic inactivation[7]. The intrinsic antibacterial activity of clavulanic acid alone is negligible[7].

Previously, several other studies have reported that the pharmacokinetic behavior of amoxicillin in healthy volunteers and children is not modified by the co-administration of clavulanic acid[8-11]. On the other hand, one study administering varying doses of oral amoxicillin together with 125 mg clavulanic acid, showed that at the highest amoxicillin dose (875 mg) clavulanic acid absorption was reduced while amoxicillin absorption was unaffected[12]. Dose-dependency in the pharmacokinetics of amoxicillin has also been described in other studies[13,14].

Thus, it cannot be excluded that under certain circumstances co-administration of clavulanic acid might influence the concentration-time profile of amoxicillin. Potential interactions between simultaneously administered drugs might be caused by inhibition of metabolic enzymes, competition for protein binding in plasma or changes in renal transporter-systems. Consideration of the elimination routes of the simultaneously administered drugs is therefore important to assess the probability of drug interactions. Both amoxicillin and clavulanic acid are eliminated by hepatic metabolization and renal excretion. Amoxicillin is in part metabolized to the inactive compound penicilloic acid and is eliminated by the kidneys. Clavulanic acid is also partly metabolized and in animal studies it has been shown that the metabolites are excreted by feces, urine and lung secretions[15,16]. Physiological changes taking place during pregnancy and labor, such as an increase in cardiac output and glomerular filtration rate[17-19], may also influence the pharmacokinetics of (co-administered) drugs. These adaptations may include changes in protein binding and the function of and/or blood flow to the metabolizing and eliminating organs. The aim of this study was to investigate whether co-administration of clavulanic acid influences the pharmacokinetics of amoxicillin in pregnant women during labor.

Methods

Patients

In the period between February 7, 2005 and February 28, 2007, all women who needed antibiotic treatment with amoxicillin or co-amoxiclav during labor and were admitted to the hospital, were eligible for inclusion in this study. Following local guidelines, women were treated with amoxicillin in the prevention of GBS, in case of a proven or unknown *Streptococcus agalactiae* (group B streptococcus, GBS) carriage, in the presence of generally recognized risk factors for neonatal GBS disease but without signs of infection[20]. In case of suspected intra-amniotic infection, women were treated with co-amoxiclav. The study was approved by the Medical Ethics Committee of the Medical Center Haaglanden, the Hague, the Netherlands. Written informed consent was obtained from all patients. Women were excluded from the study if they i) had been treated with oral or intramuscular antibiotics within 2 days before starting therapy, ii) were unwilling to comply with the requirements of the study, iii) were known to be allergic to amoxicillin or other penicillins, or iv) were receiving co-medication that exhibits interaction with amoxicillin. At the start of the study all patients were at least 18 years of age and in labor.

Drug administration and blood sampling

Before the administration of amoxicillin or co-amoxiclav an intravenous catheter was placed in each arm. The choice of the antibiotic and the dosing schedule was in accordance to the guidelines of the local hospital. Treatment with amoxicillin started with an intravenous infusion of 2 gram amoxicillin (50 mg/mL) administered over 30 minutes, followed by a second infusion after 4 hours of 1 gram amoxicillin (50 mg/mL) over 15 minutes. Treatment with co-amoxiclav (consisting of 1 gram amoxicillin (50 mg/mL) with 200 mg clavulanic acid) consisted of an infusion for 15 minutes every 8 hours. Blood samples of 2 mL were collected from the second catheter in the contra-lateral arm at timed intervals beginning at 1 min after the start of the infusion and, at 7 and 15 min (1 gram infusion) or 15 and 30 min (2 gram infusion) during the first two amoxicillin administrations. After the infusion sampling was scheduled at 3, 6, 10, 16 and 36 minutes, and afterwards every 30 minutes until the next antibiotic dosage. Exact sampling times were recorded.

All 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 minutes. The supernatants were transferred into plastic storage tubes and frozen at –70° C until analysis.

Patient information

All patients received a standard work-up at the onset of the study which included a medical history, biochemical and hematological examination. Furthermore blood pressure, pulse, oral temperature, and body weight were recorded. The amount of edema was scored semi-quantitatively from 0 (no edema), 1 (around the ankle), 2 (up to the knee) to 3 (above the knee).

High-performance liquid chromatography

Amoxicillin concentrations were determined by an isocratic high-pressure liquid chromatography (HPLC) (Shimadzu, Den Bosch, The Netherlands (NL)) method, using an ODS Gemini column (Bester, Amstelveen, NL) with 0.066 M KH2PO4 solution containing 10 % methanol as a mobile phase. A perchloric acid solution of 0.1 ml was added to the sample in an equal volume and after vortexing, added to 0.56 ml 0.028 M citric acid containing cefadroxil (Sigma, Zwijndrecht,NL) as an internal standard. The assay was linear over the concentration range measured. Controls were included in every run. The lower limit of detection was 0.2 mg/L and the between run CV < 4%.

Pharmacokinetic analysis

Pharmacokinetic parameters were estimated by means of Non-Linear Mixed Effect (population) Modeling (NONMEM). The model was implemented in the NONMEM ADVAN5 subroutine and the analysis was performed using the FOCE method. 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, USA).

The data of all patients were analyzed simultaneously to determine the basic structural pharmacokinetic parameters. Various 2- and 3- compartment models were tested. The previously described pharmacokinetic population model in pregnant women with preterm premature rupture of the membranes (PPROM) consisting of 3 compartments, was used to start the analysis[21]. Model selection and identification of variability was based on the objective function value (OFV), pharmacokinetic parameter point estimates, and their respective confidence intervals, and goodness-of-fit plots. To estimate the structural parameters, a significance level of 0.001 was selected (corresponding to differences 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. The 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 visually be randomly distributed around zero. Population values were

estimated for the parameters clearance (CL), the volumes of distribution (V) and the 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 OFV, their respective confidence intervals and inspection of the goodness-of-fit plots. A additive error model, proportional error model and a combined additive and proportional error model were evaluated. 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 were plotted independently against the covariates bodyweight, body mass index, gestational age, creatinin, leucocytes, oral temperature, and the amount of edema to determine whether this influenced the pharmacokinetics. The effects of covariates were tested for statistical significance using the OFV and the residual intra- and inter-individual variability were visually evaluated. A significance level of 0.05 was selected, corresponding to differences in OFV of 3.84 points. A further criterion for acceptance of the covariate effects is that the estimated 95% confidence interval of the covariate effect did not overlap with it's null value.

The effect of co-administration of clavulanic acid on structural amoxicillin PK parameters was investigated using the entire population as one group. The difference between treatment with amoxicillin alone of co-amoxiclav was implemented in the model as covariate on the structural parameters with significant inter-individual variability. The effect of co-administration of clavulanic acid was evaluated using the OFV with a significance level of 0.05, the 95% confidence interval and the goodness-of-fit plots. The residual intra- and inter-individual variability were visually evaluated.

The accuracy of the final population model for the entire population was established using a bootstrap method 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.

Results

Eighteen patients were included in the study. Nine patients were treated with co-amoxiclav and 9 with amoxicillin alone. In total 218 serum samples were collected

in the study, 94 samples from patients treated with co-amoxiclav and 124 samples from patients treated with amoxicillin. All samples were used as a part of other study groups[22, 23]. The gestational age of patients treated with amoxicillin was lower (35.3 weeks) compared to patients treated with co-amoxiclav (39.8 weeks). Of the patients treated with co-amoxiclav 5 patients had a temperature above 37.8ºC, compared to 2 patients treated with amoxicillin. 9 patients were nulliparae. Of the patients receiving co-amoxiclav 2 delivered after a secondary caesarean section. All patients treated with amoxicillin delivered vaginally. The characteristics of the study patients are presented in table I.

		Patients treated with amoxicillin			**Patients treated with co-amoxiclav**		
Data	**units**	**mean**	**SD**	**Range**	**Mean**	**SD**	**Range**
Maternal age	year	30.0	6.9	21.2-37.9	31.6	4.2	24.9-38.5
Gestational age	week	35.3*	2.6	31.0-39.1	39.8*	1.9	36-42.4
Weight	kg	73.4	10.8	53.0-87.3	81.3	16.2	56-108
Body Mass Index	kg/m^2	26.0$^{\nabla}$	4.3	18.3-32.0	31.7$^{\nabla}$	6.0	24.2-41.6
Leucocytes	$x10^9/L$	16.4	6.4	9.4-28.2	14.3	4.8	8.4-23.1
Creatinin	µmol/L	45.8	7.6	35-56	56.3	17.8	34-89
Temperature	ºC	36.9	1.1	35.4-39.1	37.7	0.6	36.7-38.6
Edema**		1.22	0.4	1-2	1.78	0.7	1-3

SD: standard deviation; The unpaired t-test was used to detect significant differences between patients treated with amoxicillin and patients treated with co-amoxiclav. * significant difference ($p<0.001$); significant difference ($p<0.05$) ** The amount of edema was score as: no (0) /around the ankle (1) /up to the knee (2) /above the knee (3).

Table 1*: characteristics of the study patients.*

A 3-compartment model best described the data. The inter-individual variability was mainly due to differences in CL and V_1. A correlation between the random parameters for inter-individual variability was not found. An increase of V_1 of 2.1% per kg body weight gain was found and incorporated into the model. Implementation of the other covariates did not improve the model-fit. The residual error was found to be proportional to the blood concentrations. The randomly distributed observed concentrations versus model-predicted concentrations as shown in figure 1, illustrate the unbiased model fit. For some observed concentrations the predicted concentrations are overestimated. These blood samples were taken during the

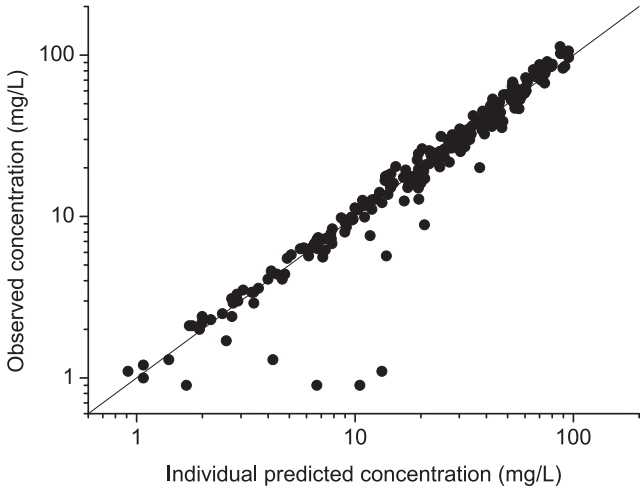

***Figure 1**: observed versus individual predicted concentrations.*

Parameter	Units	Mean	SE
Structural model parameters			
CL	L/h	21.3	1.58
V_1	L	11.8	1.3
V_2	L	5.72	1.01
V_3	L	8.01	1.52
Q_1	L/h	28.2	7.89
Q_2	L/h	5.4	1.37
Variance model parameters			
Interindividual variability in CL		0.080	0.051
Interindividual variability in V_1		0.055	0.017
Residual variability		0.040	0.0092

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_1 intercompartmental clearance between V_1 and V_2; Q_2 intercompartmental clearance between V_1 and V_3.

***Table 2:** Parameter estimates of the final model.*

antibiotic infusion. The antibiotic concentration increases very fast during the infusion. We recorded sampling times with a precision of 0.5-1 minute. Therefore, the model will predict concentrations during the infusion less accurately compared to concentrations obtained after the infusion. Table 2 shows the estimated values for the pharmacokinetic parameters of the final model. The parameter estimates obtained with the bootstrap analysis did not differ significantly from the parameters estimated from the final model (99 of 100 runs successful, data not shown).

Co-administration of clavulanic acid to the amoxicillin did not influence the pharmacokinetics of amoxicillin during labor after intravenous drug administration. The OFV decreased with 1.01 and 0.15 points after implementation of the covariate for the difference between the drugs on CL and V_1, respectively. This is supported by the individual estimates of CL and V_1, as generated by NONMEM. The mean values (+/- SD) of the estimates for the CL of amoxicillin in patients treated with

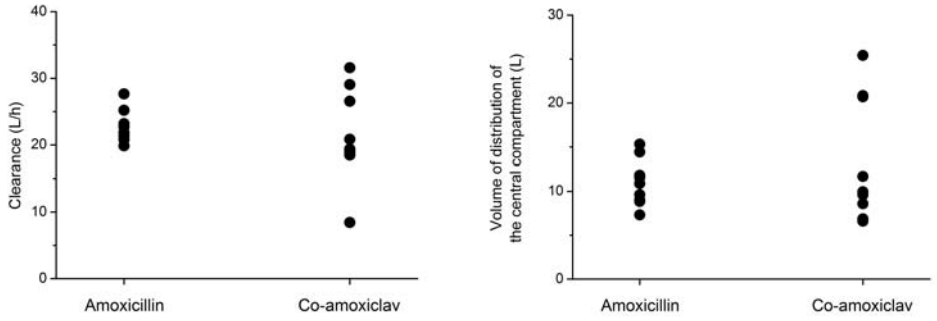

Figure 2:
Distribution of clearance (figure 2A) and central volume of distrubution (figure 2B) for patients receiving amoxicillin and co-amoxiclav.

amoxicillin and co-amoxiclav were, respectively, 22.8 +/- 2.4 L/h and 21.3 +/- 6.9 L/h. For V_1 the mean values (+/- SD) of the estimates were 11.0 +/- 2.6 L and 13.4 +/- 7.0 L for patients treated with amoxicillin and co-amoxiclav, respectively. Figure 2A and 2B show the distribution of the structural parameters CL and V_1 for patients treated with amoxicillin and patients with co-amoxiclav.

Discussion

Pharmacokinetics of amoxicillin in pregnancy has been described previously and it has been shown that adherence to the guidelines leads to adequate concentration-time profiles in the mother in the prevention of GBS[21]. However, as co-administration of other drugs might influence the concentration-time profile of an individual drug, we investigated in this study the frequently used alternative, the combination of amoxicillin and clavulanic acid. In this study, we show that the pharmacokinetics of intravenously administered amoxicillin during labor is not significantly influenced by co-administration of clavulanic acid.

A number of potential interactions had been anticipated. Both drugs bind to serum proteins and are eliminated via the same routes. Distribution might be influenced by competition of the drugs for the same serum proteins. This might in principle increase the unbound fraction of the drug, resulting in a different tissue penetration and a higher rate of biotransformation and excretion. However, since both amoxicillin and clavulanic acid have low plasma protein binding of respectively 20% and 22%[24], the probability that this would lead to a significant change after the administration of co-amoxiclav is small. Inhibition or induction of enzymes involved in metabolism might also have caused changes in pharmacokinetics of the drugs. Both amoxicillin and clavulanic acid are in part metabolized, but the absence of pharmacokinetic drug interaction in this study indicates that the individual drugs do not influence the enzyme activity involved in each others metabolism. Both changes in passive and active excretion of drugs could have caused drug interaction. Passive re-absorption may change due to a change in the pH of the urine and on the other hand, transporters involved in the active excretion of drug may be influenced. Our results indicate that administration of co-administration of clavulanic acid with amoxicillin did not significantly influence the distribution, metabolization and excretion of amoxicillin.

Data on the pharmacokinetics of drugs in pregnant patients are difficult to obtain both for ethical and practical reasons. Therefore, comparison of different patient groups and discovering sources of variability is nearly impossible by using conventional statistical methods. Particularly in this type of studies the use of population modeling is an advantage, because covariate analysis applied to the whole group of patients maximizes the utilization of all information that is available for each patient. The finding that body weight has a slight influence on clearance illustrates this point. Furthermore, if data of more patients would became available, this can be simply added to the dataset, without the need to carry out a study with new groups of patients.

In the analysis of the pharmacokinetics of amoxicillin in this patient group the volume of distribution of the central compartment increased with body weight. In an earlier study the volume of distribution of the central compartment increased

with an advancing gestational age[23]. Because gestational age is correlated with body weight ($p<0.01$), this is not a discrepancy between these analysis. The use of amoxicillin is indicated in patients during a preterm delivery. Consequently, the gestational age in patients treated with amoxicillin is lower than in patients treated with co-amoxiclav. The increasing body mass index increases with advancing gestational age, will also result in a significant difference in body mass index between the two groups. The effect of covariates on the PK is investigated in the entire patient group and these differences will therefore not influence the results.

A proper dosing regimen of amoxicillin is essential for the treatment of IAI in pregnant women. Co-administration of clavulanic acid to amoxicillin increases the antibacterial spectrum of the treatment. Different dose ratios of amoxicillin/clavulanic acid had been used. But since the higher doses of clavulanic acid was associated with a higher rate of adverse effects, such as diarrhea, nausea and vomiting, the amount of clavulanic acid was decreased[16]. Data on the pharmacokinetics of clavulanic acid during labor are not available. Unfortunately, the concentrations of clavulanic acid could not be determined in this study due to stability issues. As there are no reports that the efficacy of the these dose ratio's are different, the amount of clavulanic acid may not be that critical[12].

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