BASIC SCIENCE: OBSTETRICS

Amoxicillin pharmacokinetics in pregnant women with preterm premature rupture of the membranes

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OBJECTIVE: This study was undertaken to study the pharmacokinetics of intravenously administered amoxicillin in pregnant women with preterm premature rupture of the membranes (PPROM).

STUDY DESIGN: Healthy women with PPROM were recruited and treated with amoxicillin (2 g initially and 1 g subsequently). Blood samples were obtained from the opposite arm and concentrations determined with the use of high-pressure liquid chromatography. Nonlinear mixed-effects modeling was performed in nonlinear mixed effect (population) modeling.

RESULTS: The pharmacokinetics of 17 patients was described by a 3-compartment model. Clearance and volume of distribution at steady state were 22.8 L/h and 21.4 L/h, respectively, similar to values in nonpregnant individuals. There was little variability between patients. No relationship was observed between values of individual pharmacokinetic parameters and various covariates.

CONCLUSION: The pharmacokinetics of amoxicillin in pregnant patients with PPROM similar to nonpregnant individuals. Given the small interindividual variability in pharmacokinetics, no dose adjustments are required to account for differences between subjects under normal circumstances.

Key words: clearance, interindividual variability, pregnancy, preterm rupture of the membranes, volume of distribution


P reterm premature rupture of the membranes (PPROM) complicates approximately 3% of pregnancies and is responsible for one-third of all preterm births. Subclinical intraamniotic infec-

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Amoxicillin, a penicillin derivative, is an antibiotic frequently used in the management of PPROM. It is active against common pathogens that can cause infection in neonates, in particular Streptococcus agalactiae. The currently recommended amoxicillin dosages in pregnancy are derived from studies that used ampicillin. These dosage regimens essentially do not differ from regimens used in nonpregnant individuals and are based on the assumption that pharmacokinetics in pregnancy and in young men are similar. In nonpregnant individuals, a slow elimination phase has been suggested for penicillin G and amoxicillin. Especially for bacteria with a low minimum inhibitory concentration (MIC), like S agalactiae, a slow elimination phase would be of clinical importance. In women with PPROM, the presence of such elimination phase would be beneficial for efficacy of the prophylaxis by increasing the time the amoxicillin concentration remains above the MIC. However, during pregnancy physiologic changes occur that may modify the pharmacokinetics of drugs, such as increase in plasma volume, increase in fat content, presence of the fetus, and changes in elimination rate or metabolism. These changes can be expected to affect the pharmacokinetics of drugs in various ways. If changes in pharmacokinetics indeed occur, pregnant women and their fetuses are inherently at risk for underdosing or overdosing when they are treated with dosage regimens developed for nonpregnant individuals. A clear example is the drastic decrease in concentration of the antiepileptic drug lamotrigine during pregnancy.

Despite the widespread use of amoxicillin in pregnant women, the pharmacokinetics in patients with PPROM has not been adequately studied. The objective of this study is to describe the phar-
Drug administration and blood sampling
Before the administration of amoxicillin, an intravenous catheter was placed in each arm. Amoxicillin was administered according to local guidelines. The treatment started with an intravenous infusion of 2-g amoxicillin (50 mg/mL) administered over 30 minutes, followed by a second infusion after 4 hours of 1-g amoxicillin over 15 minutes. Blood samples of 2 mL were collected from the second catheter in the contralateral arm at timed intervals beginning at 1 minute after the start of the infusion and, at 7 and 15 minutes (1-g infusion) or 15 and 30 minutes (2-g infusion) during the first 2 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. The exact sampling times were recorded.

Blood samples were placed immediately on ice, allowed to clot, and processed within 1 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.

Pharmacokinetic analysis
Pharmacokinetic parameters were estimated by means of nonlinear mixed effect (population) modeling (NONMEM). The model was implemented in the NONMEM ADVAN5 subroutine and the analysis was performed by using the FOCE method. All fitting procedures were performed with the use of the Compaq Visual Fortran standard edition 6.6 (Compaq Computer Corporation, Houston, TX) and NONMEM software package (v V, release 1.1; GloboMax, Hanover, MD).

To determine the basic structural pharmacokinetic parameters various 1-, 2-, and 3-compartment models were tested. Model selection and identification of variability was based on the likelihood ratio test, pharmacokinetic parameter point estimates, and their respective confidence intervals, and goodness-of-fit plots. For the likelihood ratio test on differences between 2 models, the objective function value (OFV) with a prespecified level of significance of $P < .001$ was used. 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 vs individual or population predictions should be randomly distributed around the line of identity. The weighted residuals vs 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 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 interindividual variability. Possible correlation between interindividual 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 likelihood ratio test and inspection of the goodness-of-fit plots. The residual variability between the observed concentrations and those predicted by the model was described by using a proportional error model. The residual error term contains all the error terms that 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 body weight, body mass index, duration of amenorrhea, blood pressure, pulse, oral temperature, and the amount of edema to determine whether this influenced the pharmacokinetics. The effects of covariates were tested for statistical significance by using the likelihood ratio test and the residual intraindividual and interindividual variability were visually evaluated. The $V$ at steady state ($V_{ss}$) and terminal half-life ($T_{1/2}$) were calculated according to standard procedures. The accuracy of the final population model was established with the use of 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 with the estimates from the original data.

**RESULTS**

In total, 17 patients were included. The population consisted of 15 singleton and 2 twin pregnancies. The gestational age at the time of PPROM ranged from 29.4-36.9 weeks of pregnancy. The patients were born in 8 different countries, illustrating the heterogeneity of the hospital population and the study population. The characteristics of the study patients are presented in Table 1.

A total of 416 blood samples were collected, which was close to the predefined sampling schedule. The 2-g and 1-g infusions resulted in mean peak concentrations of 96.7 mg/L (range, 73.5-136.6 mg/L) and 70.9 mg/L (n = 16; range, 49.1-107.1 mg/L), respectively. A 3-compartment open model best described the data. The estimates of the pharmacokinetic parameters and their respective CVs are summarized in Table 2. The CVs were relatively small with values between 4.5% and 30.8%. Interindividual variability was explained by variation in the parameters CL and $V_2$ (18% for CL and 33% for $V_2$). This means that the variability between subjects was in fact very small. A correlation between the random parameters for interindividuality was found and accounted for in the stochastic model. Values of $T_{1/2}$ and $V_{ss}$ were 1.10 hours and 21.4 L, respectively.

None of the covariates tested (gestational age, body weight, body mass index, blood pressure, pulse, oral temperature, and the amount of edema) could improve the model. Finally, no difference in pharmacokinetics between the 1- and 2-g infusion was observed.

The observed and population-predicted profiles for the final model are shown in Figure 1. The scatter plot of the observed concentrations vs model-predicted concentrations is shown in Figure 2.

The bootstrap validation of the final model was performed with 100 runs. The mean parameter estimates of the runs obtained from the bootstrap analysis did not differ significantly from the predicted values from the NONMEM pharmacokinetic analysis. The standard error obtained from the bootstrap analysis was also comparable to those estimated by the model, except for the intercompartmental clearance between the central and second compartment ($Q_1$). This value differs significantly from the stan-

### Table 1

<table>
<thead>
<tr>
<th>Data</th>
<th>Number of patients</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
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</thead>
<tbody>
<tr>
<td>Maternal age (y)</td>
<td>17</td>
<td>29.42</td>
<td>4.64</td>
<td>19.6-35.1</td>
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<tr>
<td>Gestational age (wk)</td>
<td>17</td>
<td>35.1</td>
<td>1.63</td>
<td>29.4-36.9</td>
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<td>Body mass index (kg/m²)</td>
<td>17</td>
<td>29.1</td>
<td>3.87</td>
<td>21.5-35</td>
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<tr>
<td>Weight (kg)</td>
<td>17</td>
<td>80.9</td>
<td>12.03</td>
<td>56.2-98.9</td>
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<tr>
<td>Edema (no/around the ankle/up to the knee)</td>
<td>16</td>
<td>10/5/1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leucocytes ($\times 10^9$/L)</td>
<td>17</td>
<td>11.8</td>
<td>4.43</td>
<td>6-25.9</td>
</tr>
<tr>
<td>Creatinin ($\mu$mol/L)</td>
<td>17</td>
<td>44.4</td>
<td>10.11</td>
<td>37-74</td>
</tr>
<tr>
<td>Nulliparity</td>
<td>11</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Twin pregnancy</td>
<td>2</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Positive maternal GBS culture</td>
<td>7</td>
<td>—</td>
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</table>

GBS, group B streptococcus (S. agalactiae).

TABLE 2
Population model parameter values and bootstrap estimates

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Final model estimates</th>
<th>Bootstrap estimates(^a)</th>
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<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
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<tr>
<td>Structural model parameters</td>
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<tr>
<td>CL (L/h)</td>
<td>22.8</td>
<td>1.03</td>
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<tr>
<td>(V_1) (L)</td>
<td>5.59</td>
<td>0.826</td>
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<tr>
<td>(V_2) (L)</td>
<td>7.43</td>
<td>1.06</td>
</tr>
<tr>
<td>(V_3) (L)</td>
<td>8.61</td>
<td>0.768</td>
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<tr>
<td>(Q_{1s}) (L/h)</td>
<td>60</td>
<td>18.5</td>
</tr>
<tr>
<td>(Q_{2s}) (L/h)</td>
<td>7.72</td>
<td>1.72</td>
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<tr>
<td>Variance model parameters</td>
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<tr>
<td>Interpatient variability in CL</td>
<td>0.0317</td>
<td>0.0112</td>
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<tr>
<td>Interpatient variability in (V_2)</td>
<td>0.108</td>
<td>0.00440</td>
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<tr>
<td>Residual variability</td>
<td>0.0365</td>
<td>0.00492</td>
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<tr>
<td>Derived pharmacokinetics parameters</td>
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<td></td>
</tr>
<tr>
<td>(T_{1/2}) (h)</td>
<td>1.10</td>
<td>—</td>
</tr>
<tr>
<td>(V_{ss}) (L)</td>
<td>21.4</td>
<td>—</td>
</tr>
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</table>

\(^a\) Mean of 100 bootstrap analyses. The parameter values were compared with the bootstrap estimates by using the unpaired \(t\) test.

**Figure 1**
The observed data and population-predicted profile

The superimposed bold line shows the predicted profile obtained with the final model. The blocks indicate the time at which the infusions of the amoxicillin was started and stopped. Because there was variation in the start-time of the second infusion because of the clinical situation, in this graph the data were adapted assuming that the second infusions started at \(t = 5.05\) hours for all patients.

**Comment**

In this study, a pharmacokinetic model was developed to describe the pharmacokinetics of amoxicillin in pregnant women with PPROM. The pharmacokinetics in our population appear to be only slightly different from nonpregnant individuals with a \(V_{ss}\) of 21.4 L and a \(T_{1/2}\) of 1.10 hours. The variability between the patients was small.

With regard to amoxicillin, values for \(V\), \(C\), and \(T_{1/2}\) were all within the ranges reported in the literature for healthy nonpregnant individuals (Table 3). Only slightly lower peak serum concentrations were observed compared with 7 healthy nonpregnant individuals\(^{17}\) (ie, 96.7 mg/L and 139.3 mg/L, respectively, for the 2-g infusion). The value for \(V_{ss}\) in our study was slightly larger than values found by Dalhoff et al\(^8\) in healthy volun-

**Figure 2**
Individual predicted vs observed concentrations of amoxicillin

Scatter plot of the individual predicted vs observed concentrations of amoxicillin for 17 patients. The correlation coefficient was 0.97. The figure shows the individual data points for the entire population and the line of identity (\(x = y\)).

the use of different methods. The 2 compounds differ very little in pharmacokinetics in healthy volunteers, except with respect to absorption after oral administration of ampicillin (H) and amoxicillin (L). The 2 studies are inconsistent with respect to the range where deviation of linearity occurs. Mastrandrea et al described a difference in clearance in the range from 500-1000 mg, whereas Hill et al found a slight deviation from linearity after a 5-g dose compared with doses of 250-1000 mg. In a study by Sjövall et al, the pharmacokinetics after infusions in doses ranging from 1.9-2.8 g were linear. In our data, covering the range of 1-2 g, there was no evidence for a dose effect on the clear ance. It is unlikely that therapeutic consequences are to be expected.

In general, interindividual variability in pharmacokinetic parameters observed in clinical study populations are caused by biochemical and physiologic differences between subjects. In association with pregnancy, additional physiologic alterations occur, which may further increase the variation in parameters between individuals in pregnant populations. Surprisingly, the interindividual variation in our data was remarkably small. Although this was an unexpected finding, from the clinical perspective this is convenient, because specific adjustments are unnecessary for this patient group.

An important question is whether this dosing regimen is adequate to treat or prevent morbidity in both mother and fetus. The efficacy of the penicillins is determined by the time the concentration exceeds the minimum inhibitory concentration (MIC) and, in general, for 40-50% of the dosing-interval is required for efficacy. The breakpoint MIC value of an antibiotic used is the highest MIC value of different causative microorganisms that results in a high probability of cure, as follows from the target MIC. Because rectovaginal carriage of S. agalactiae has been described in up to 30% of pregnant women, this is an important microorganism after PPROM in the development of neonatal infection. MIC values of amoxicillin for S. agalactiae are scarce, but vary from 0.03-0.12 mg/L.
The peak serum concentrations in our pregnant population were slightly lower than in nonpregnant individuals, but nevertheless well above the MIC. More importantly, maternal serum concentrations remained above the MIC for sufficient percentage of the dosing interval (>95%), even taking into account the protein binding of amoxicillin. The presence of a slow elimination phase, represented by the third compartment, significantly contributes to the high value for $T_{\text{MIC}}$. Because amoxicillin reaches the fetus after transplacental transport, it should be noted that adequate maternal levels are a prerequisite for the prevention of fetal infection, but no guarantee. In treatment of the mother, the added value of a 2-g loading dose above a 1-g dose is doubtful. However, it remains to be confirmed that by using this dosing schedule, adequate fetal and AF levels are established as well.

It is surprising that the pharmacokinetics in pregnant women with PPROM did not differ significantly from nonpregnant individuals. However, it should be noted that this is only valid for pregnant women with PPROM who are otherwise healthy. It has been suggested previously that it is not the state of pregnancy that influences the pharmacokinetics, but being in labor. Because our patients were not in labor, this might explain why our data were similar compared with previously reported data of nonpregnant individuals.

REFERENCES

35. Adam D, Koepe P, Heilmann HD. Pharmacokinetics of amoxicillin and flucloxacillin following the simultaneous intravenous administration of 4 g and 1 g, respectively. Infection 1983;11:150-4.