Pharmacokinetics of penicillin G in infants with a gestational age of less than 32 weeks.

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Abstract

The pharmacokinetics of penicillin G was studied in 20 preterm neonates with a gestational age of less than 32 weeks on day 3 of life using a population approach performed with the nonlinear mixed effects modeling program NONMEM. The derived population estimates and the correlation matrix of these estimates were used to perform Monte Carlo Simulations (MCSs) and obtain the probability of target attainment (PTA). Penicillin G pharmacokinetics was best described by a two-compartment pharmacokinetic model. The population estimates of the central volume of distribution, peripheral volume of distribution, intercompartmental clearance and the total body clearance were $0.359 \pm 0.06$ L, $0.152 \pm 0.03$ L, $0.774 \pm 0.28$ L/h and $0.103 \pm 0.01$ L/h (mean $\pm$ SE), respectively. The terminal $t_{1/2}$ was 3.9 h. Clearance increased significantly with increasing birth weight. Assuming the percentage of time that the concentration of unbound drug remained above the MIC (%T>MIC) of 50% for preterm neonates, the susceptibility breakpoint based on a 100% PTA was $\leq 4$ mg/L simulating the current dosing regimen of 50,000 U/kg every 12 h. This regimen is therefore adequate for the treatment of common neonatal infections on the third day of life.
Introduction

Penicillins are important antimicrobial agents in treatment of bacterial infections in newborn infants, with a high clinical efficacy in eradicating common pathogens in combination with a high degree of safety. Because of lack of evidence from randomized clinical trials in favor of any particular antibiotic or antibiotic regimen\(^1\), penicillin G remains important among the antibiotics most commonly given to neonates in the treatment of presumed early neonatal sepsis.

Infectious complications during the immediate postnatal period are not uncommon and require prompt antibiotic treatment\(^2-5\). Differences in body composition and organ function can significantly affect the pharmacokinetics in neonates. In very premature neonates (i.e. gestational age of less than 32 weeks) the disposition of antibiotics may differ from full term neonates as a result of differences in absorption, distribution, biotransformation and excretion\(^6-8\). As a result, dose estimation on the basis of body size or allometric scaling may be inadequate. Specifically, in very premature neonates maturation processes of the organs may influence the relevant pharmacokinetic parameters.

The efficacy of the penicillins is primarily correlated to the percentages of time that concentrations of unbound drug remained above the MIC (%\(T>MIC\))\(^9-11\). In general, the therapeutic goal to cure infections caused by Gram-positives is a %\(T>MIC\) of at least 40% of the antimicrobial, which corresponds to an in vivo static effect in animal studies\(^12\). Studies showing a clear relationship between exposure and efficacy in premature neonates are not available. Because prematures have to be regarded as immunocompromised, to our opinion it is reasonable that in this patient group the exposure should correspond to exposures that correlate to a 1 to 2 log drop of CFU in various models, and thus be bactericidal rather than bacteriostatic. Thus, for penicillins, this percentage should be at least 50%\(^13\). Since the goal of treatment is to attain this target for every individual in the population, the dosing regimen in this age group should be defined taking the inter-individual pharmacokinetics variability into account. Monte Carlo Simulations (MCS) is a technique that is commonly used to determine the probability of achieving therapeutic concentrations on the basis of population pharmacokinetic parameter estimates and their measures of dispersion\(^14-19\). We investigated the pharmacokinetics of penicillin G and the adequacy of the dosing regimen in very premature neonates on the third day of life to allow us to construct a population pharmacokinetic model and to use the parameter estimates to perform MCS in this specific age group. We then used that information to define optimal dosing regimens.
Materials and methods

Patients and treatment
Preterm neonates with suspected or documented septicemia or invasive infection were eligible for this study. The neonates were hemodynamically stable (diuresis > 1 mL/kg/h; systolic and diastolic blood pressure above the third percentile adjusted for gestational age), had a normal liver function, had not received inotropic or nephrotoxic drugs, did not have an intracranial hemorrhage beyond grade II, and had an indwelling arterial catheter for clinical purposes. The partial pressure of oxygen in arterial blood was kept at greater than 50 mmHg or oxygen saturation between 87% and 92%, and hematocrit values were maintained above 0.32 by packed erythrocyte transfusions. Neonates were excluded from the study if they had life-threatening illnesses or became hemodynamically unstable (systolic and diastolic blood pressure below the third percentile adjusted for gestational age; diuresis < 1 mL/kg/h). Also excluded were infants with severe asphyxia, defined as having: profound umbilical artery acidemia (pH < 7.00), persistence of an Apgar score of 0 to 3 longer than 5 minutes, neonatal neurological sequelae (e.g., seizures, coma, hypotonia), and multiorgan system dysfunction (e.g., cardiovascular, gastrointestinal, hematologic, pulmonary or renal). Subjects were enrolled after parental informed consent. During that period, empirical treatment consisted of penicillin G or amoxicillin in combination with tobramycin or cefotaxime. Patients treated with penicillin G were eligible for this study.

Penicillin G was administered as an intravenous bolus injection of 50,000 U/kg every 12 hours. From each subject leucocytes-count, platelets-count, blood and superficial cultures were performed as part of their routine work-up.

Pharmacokinetics study
The pharmacokinetics of penicillin G was studied on day three of life. Blood samples (200 μL) were taken from an indwelling arterial line just before the administration of an intravenous bolus dose and at 0.03, 0.5, 1, 2.5, 4, 8, 12 h after administration. A 24 h sample was taken from those patients that did not receive a subsequent dose. Samples were immediately centrifuged in a microcentrifuge (Merck-type Eppendorf 5414: 3.000 x g) for 1 minute and serum was stored at -70° C.

Penicillin G HPLC assay
Chromatographic analysis was performed with a glass-prepacked column (100 by 3 mm) containing ODS-2 Chromospher Spherisorb beads (5-μm-diameter particle size; Chrompack, Middelburg, The Netherlands) combined with a guard column. A Bio LC pump (model 410, Perkin-Elmer, Norwalk, Conn.) was used to deliver the eluent consisting of 16% (vol/vol) acetonitrile and 50 mM sodium phosphate buffer (pH 6.9) at a flow rate of 0.8 ml/min. The separations were carried out at room
Penicillin G PK in premature infants

temperature. The eluate was monitored with a Perkin-Elmer LC-95 UV/visible spectrophotometer detector at a wavelength of 215 nm. As an internal standard 25 μg/ml methicillin in 100 % methanol (vol/vol) was used. Briefly, hundred μl of the internal standard was added to a 100 μl aliquot of the serum sample. This mixture was immediately vortexed for 30 seconds. Subsequently, the sample was kept for 10 min at -20 °C, again vortexed for 30 seconds and finally centrifuged at 1,500 g for 10 min at room temperature. The supernatant was filtered (millipore) and 10 μl was injected onto the column.

HPLC-grade acetonitrile was purchased from Rathburn (Walkerurb, Scotland). The other chemicals were purchased from Aldrich-Chemie (Steinheim, Germany). All chemicals applied were of the highest grade commercially available.

The lower limit of detection of penicillin was 0.5 μg/ml. The coefficients of inter-assay variation determined at concentrations of 100 and 20 μg/ml were 2.6% and 2.3%, respectively. The intra-assay values were 0.75% and 1.05%, respectively.

Pharmacokinetics analysis

Pharmacokinetic parameters were estimated by means of Non-Linear Mixed Effect (population) Modeling (NONMEM). This approach estimates the structural PK parameters considering both inter-individual variability within the population and the intra-individual (i.e. residual) variability. The model was implemented in the NONMEM ADVAN5 subroutine and the analysis was performed using the first-order conditional estimation (FOCE) method with interaction option. 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 version V (NONMEM project group, University of California, San Francisco, USA).

To determine the basic structural pharmacokinetic parameters several models were evaluated. One, two, and three-compartment models were tested and evaluated for goodness-of-fit. 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 two models, the objective function value (OFV) with a pre-specified level of significance of P<0.001 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 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.

The stochastic part of the model was selected to describe inter-individual
variability in the pharmacokinetic parameters and assumed a log normal distribution of all model parameters over the population. Therefore an exponential distribution model was used to account for inter-individual variability:

\[ P_i = \theta \times \exp(\eta_i) , \]

in which \( P_i \) is the individual value of the model parameter \( P \), \( \theta \) is the population estimate for parameter \( P \) and \( \eta_i \) is the normally distributed inter-individual random variable with mean zero and variance \( \omega^2 \). Selection of an appropriate residual error model was based on the likelihood ratio test and inspection of the goodness-of-fit plots. The model was modified to objectively account for unexplained inconsistency in the data. To reduce the influence of neonates with large unexplained inconsistency in the concentration-time profile on the population estimates, these neonates were objectively determined by means of the residual error and weighted less in the estimates of the population.

To refine the stochastic model covariate analysis was also performed. The estimated pharmacokinetic parameters were plotted independently against the covariates gestational age, birth weight, gender and the presence of a dosing history to determine whether this influenced the pharmacokinetics. The effects of covariates were tested for statistical significance using the likelihood ratio test and the residual intra- and inter-individual variability were visually evaluated. A covariate was retained in the model if it produced a decrease in objective function of > 10.8 (p<0.001). In addition, we investigated whether there were significant differences in the pharmacokinetics between neonates with a birth weight of less than 1000 gram and neonates with birth weight of more than 1000 gram. \( V_{ss} \) and \( t_{1/2} \) were calculated following standard procedures.

**Estimation of \%T>MIC and Monte Carlo Simulations**

The estimates of the pharmacokinetic parameters and measures of dispersion were used to simulate various dosing regimens and obtain \%T>MIC as a function of MIC. Protein binding was estimated at 40% ± 2.5%\(^2\). The protein binding of penicillin G in premature neonates is unknown. However, protein binding in neonates is generally lower compared to adults. It is therefore likely that the estimated protein binding of 40% overestimates the not-active protein bound fraction of penicillin G in these neonates. The use of 40% is therefore a conservative estimate. MCS was performed using the MICLAB version 2.36 program (Medimatics, Maastricht, the Netherlands) simulating 10.000 subjects for each regimen. The program allows inclusion of the covariance matrix (or correlation matrix) of the parameter estimates used in the simulations. The output consisted of a probability distribution, a cumulative probability distribution, and specific confidence intervals over user defined MIC and \%T>MIC ranges.
Results

Demographic data.
Twenty neonates with a gestational age under 32 weeks were included in the study. Demographic, laboratory and clinical parameters are shown in table 1. The average weight was 1195 g (range 650-2030 g). Half of the subjects were born from mothers with preeclampsia or HELLP-syndrome. Other reasons for premature birth were: suspected intra-amniotic infection, preterm contractions with meconium stained amniotic fluid and PPROM.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (wk)</td>
<td>29 5/7</td>
<td>1 5/7</td>
<td>26 3/7- 32 0/7</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>12/8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (g)</td>
<td>1195</td>
<td>387</td>
<td>650-2030</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>46</td>
<td>7</td>
<td>33-63</td>
</tr>
<tr>
<td>Leucocytes (10^3/mm^3)</td>
<td>15</td>
<td>13</td>
<td>5-54</td>
</tr>
<tr>
<td>Platelets (10^3/mm^3)</td>
<td>203</td>
<td>103</td>
<td>74-497</td>
</tr>
<tr>
<td>Creatinine</td>
<td>46</td>
<td>17</td>
<td>10-82</td>
</tr>
<tr>
<td>APGAR 1 minute</td>
<td>6</td>
<td>3</td>
<td>1-10</td>
</tr>
<tr>
<td>APGAR 5 minutes</td>
<td>8</td>
<td>1</td>
<td>6-10</td>
</tr>
<tr>
<td>Artificial ventilation (Yes/No)</td>
<td>9/11</td>
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<td></td>
</tr>
<tr>
<td>No. of positive bloodculture</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of positive superficial culture</td>
<td>1</td>
<td></td>
<td>(Streptococcus agalactiae)</td>
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</tbody>
</table>

*Table 1: Demographic, laboratory and clinical parameters of 20 patients studied on day three after birth.*
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Population pharmacokinetics

167 samples were included in the pharmacokinetic analysis. In 11 neonates samples were obtained only in the first 12 hours after the i.v. bolus and in 9 neonates a sample could be obtained after 24 hours. A bi-phasic rate of decline in the penicillin G concentration versus time (i.e. a two-compartment model) best described the data using a combined error model with an additive and proportional error. Figure one shows the observed concentrations in the individual patients as well as the predicted concentration time curves as obtained from the final model, while figure 2 shows the observed concentrations versus the individual predicted concentrations for the whole population. The distribution around the reference line of perfect prediction was symmetric with a correlation coefficient of 0.80, but deviates significantly from one. The pharmacokinetic parameter estimates are shown in table 2. The percent coefficient of variation reflects both the inter-individual or intra-individual

Figure 1: Individual plots of the 20 very preterm neonates. The black dots correspond with the individual datum points. The line represents the individual estimate and the dotted line the population estimate. Neonate 1, 3 and 6 were weighted less in the population estimation.
Figure 2: Plot of individual predicted versus observed concentrations of penicillin G for 20 patients. The correlation coefficient was 0.801. The individual datum points for the entire population and the x=y line is also shown.

Figure 3: Observed relation between body weight and clearance.
Table 2: Pharmacokinetic parameters of penicillin G in 20 preterm neonates.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean value</th>
<th>Standard error</th>
</tr>
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<tbody>
<tr>
<td><strong>Structural model parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL (L/h)</td>
<td>0.103</td>
<td>0.0104</td>
</tr>
<tr>
<td>V₁ (L)</td>
<td>0.359</td>
<td>0.0558</td>
</tr>
<tr>
<td>V₂ (L)</td>
<td>0.152</td>
<td>0.0312</td>
</tr>
<tr>
<td>Q (L/h)</td>
<td>0.774</td>
<td>0.277</td>
</tr>
<tr>
<td><strong>Variance model parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interindividual variability in CL</td>
<td>0.164</td>
<td>0.0865</td>
</tr>
<tr>
<td>Interindividual variability in V₁</td>
<td>0.39</td>
<td>0.126</td>
</tr>
<tr>
<td>Residual variability (proportional component)</td>
<td>0.104</td>
<td>0.0316</td>
</tr>
<tr>
<td>Residual variability (additive component)</td>
<td>1.12</td>
<td>0.891</td>
</tr>
<tr>
<td><strong>Derived pharmacokinetics parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vₚₜ (L)</td>
<td>0.540</td>
<td>-</td>
</tr>
<tr>
<td>t₁/₂β (h)</td>
<td>3.9</td>
<td>-</td>
</tr>
</tbody>
</table>

variability of the pharmacokinetic parameters. Estimation of inter-individual variability was possible for the parameters CL corrected for body weight, V₂ and the infusion rate (34.5 % for CL corrected for body weight, 17.1% for V₂ and 89.9% for the infusion rate). The residuals were generally small, but the additive error of neonate 1, 3 and 6 differs 2.5 to 3.5 times from the median of these neonates.

We examined the relationship between CL and gestational age, gender, body weight and the presence of a dosing history (i.e. whether the neonate had received a previous dose of penicillin or not). There were no significant correlations between CL and gender or the presence of a dosing history. CL increased significantly with an increasing body weight (Figure 3, p<0.01). Incorporation of body weight on
clearance in the model improved the model fit significantly. There was a significant correlation between gestational age and body weight (p<0.01). Incorporation of the gestational age did not further improve the model fit. No significant differences in pharmacokinetic parameter estimates could be demonstrated between neonates with a birth weight of more and less than 1000 gram.

To determine the probability of target attainment (PTA) for the dosing regimen used, MCS was performed. Assuming a %fT>MIC of 50% for preterm neonates, a PTA of 100% was reached with the currently recommended dosing regimen of 50.000 U/kg every 12h for pathogens with MICs of ≤4 mg/L. Figure 4 shows the %fT>MIC for the dose of 50.000 U/kg and 3 different dosing intervals based on mean population parameter estimates and the correlation matrix of these estimates.

Figure 4: Percent of time the unbound fraction of penicillin G remained above the MIC (%fT>MIC), based on the pharmacokinetic estimates and the correlation matrix of the parameter estimates, as a function of the MIC for 3 regimens.
Discussion

Pharmacokinetics of penicillin G in neonates with a gestational age of less than 32 weeks was best described by a two-compartment model, yielding estimates of the terminal $t_{1/2}$ of 3.9 h, $V_{ss}$ of 0.54 L and CL of 0.103 L/h. The dosing regimen of 50,000 U/kg every 12 h is adequate for the treatment of neonatal infections caused by common microorganisms on day 3 of life.

Most studies on the pharmacokinetics of penicillin G in neonates have been performed after intramuscular administration. However, the intramuscular route should be avoided, because this may result in erratic absorption in the sick, infected newborn with restricted blood supply to the extremities. Mulhall et al. performed a study on the pharmacokinetics of penicillin G after intravenous administration in 4 neonates with a gestational age ranging from 27 to 40 weeks and found a CL of 0.12 +/- 0.07 (mean +/- SD) L/h/kg, $V$ of 0.61 +/- 0.28 L/kg (mean +/- SD) and $t_{1/2}$ of 3.8 h. These results are similar to our data.

Other penicillins have larger $V$ and longer terminal $t_{1/2}$ in prematures, especially when compared with adults. $V$ varied from 0.3 L/kg for ampicillin to 0.41-0.68 L/kg for amoxicillin in neonates, compared to 0.45 L/kg found for penicillin G in our study. The terminal $t_{1/2}$ of penicillin G in neonates with a gestational age of less than 32 weeks was longer compared with the value of 0.5 h as reported for healthy adults, but is in the same range as the values of between 2 to 9.5 h that have been reported for other penicillins in neonates.

Within the limited number of samples available from patients in this age group, there is some unexplained inconsistency in the data. This might be caused by subcutaneous administration of penicillin G, erratic sampling times, or accidental exchange of samples. Three neonates had large unexplained inconsistency in the data based objectively on their residual error and were therefore weighted less in the population estimates. Using this method none of the neonates were excluded from the study and all data were used in the analysis. Consequently, there is a deviation between the line of identity and the regression line of the observed versus predicted concentrations, indicating that the description of the pharmacokinetics in this age group can be improved. To this end more data are needed.

The presence of a third elimination phase ($t_{1/2,\gamma}$) for penicillin G has been described previously, both in animal models as well as in adult humans. Our data could not be described by a three-compartment model. But the terminal elimination phase ($t_{1/2,\beta}$) found in our study, was comparable to the $t_{1/2,\gamma}$ of 3.1 h in human adults. Both the limited number of samples taken in the initial distribution phase and the unique body composition of the neonate, comprising approximately 75% water, complicate the distinction between the initial distribution and second elimination phase. Possibly, the initial phase in our study represents both the initial and second phase as found in the study of Ebert et al. Thus, the slow elimination
we found may represent the third elimination phase for penicillin G as determined in that study.

Inter-individual variability was partly explained by variation in CL, V₂ and the infusion rate. The variability in infusion rate represents not only the variation in rate of the manually administered intravenous bolus injection, but also variation in the sampling times between the first samples. Especially for the first samples the exact sampling times are crucial.

Growth and development are major aspects in infants, therefore both size and gestational age may have an impact on the prediction of CL\(^3\). Maturation of CL begins before birth, suggesting that gestational age would be a physiologically appropriate covariate to explain the time course of changes in CL\(^3\). In our data changes in CL were best explained by differences in body weight. Probably because both gestational age and other factors influencing the development of renal function are represented by an adequate increase of body weight in time. Furthermore, the range in gestational age as included in our study was relatively small. While CL slightly increased as a function of birth weight for the entire group, differences between subgroups with a birth weight of more and less than 1000 gram could not be demonstrated. The analysis of correlations between covariates and pharmacokinetic parameters estimates is more sensitive when all neonates are included in the study.

The enhanced inter-individual pharmacokinetics variability in prematures complicates the calculation of the therapeutic dosing regimen. The dosing regimen should be adequate for the entire population. We concluded that the 100% PTA obtained with simulation of the recommended regimen was adequate to treat neonatal infection on the third day of life. In case of meningeal involvement effective concentrations are required in the cerebrospinal fluid (CSF). Little is known on the pharmacokinetics of penicillin G in the CSF of premature neonates. We do not know what percentage of penicillin G penetrates the CSF in premature neonates with meningitis. Given the low MIC of GBS to penicillin (up to 0.12 mg/L), with use of the currently recommended dosing regimen it is sufficient when the penetration of penicillin into the CSF is at least 3%.

Shortening the dosing interval to 8 h, will not have additional value in treatment of infections in neonates with a gestational age of less than 32 weeks. When these infections are caused by microorganisms with low MICs, like *Streptococcus agalactiae*, a regimen with a prolonged dosing interval of 24 h, is also likely to have clinical success. However, for empirical therapy this regimen is suboptimal.
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References


