Cerebrospinal Fluid Compartmental Pharmacokinetics of Amikacin in Neonates

K. Allegaert,1* I. Scheers,2 E. Adams,2 G. Brajanoski,1 V. Cossey,1 and B. J. Anderson3

Neonatal Intensive Care Unit, University Hospitals, Leuven, Belgium; Laboratorium voor Farmaceutische Chemie en Analyse van Geneesmiddelen, Faculteit, Pharmaceutische Wetenschappen, Leuven, Belgium; and Department of Anaesthesiology, University of Auckland, Auckland, New Zealand

Received 21 August 2007/Returned for modification 20 November 2007/Accepted 22 March 2008

To describe and investigate the covariate effects of cerebrospinal fluid (CSF) amikacin pharmacokinetics in neonates, CSF samples were prospectively collected from neonates in whom amikacin had been initiated before a diagnostic lumbar puncture was performed. CSF analysis (amikacin concentration, white blood count [WBC], glucose content, and protein concentration) and amikacin therapeutic drug monitoring results (peak and trough concentrations) in serum were recorded. Correlations (Spearman rank) between the CSF amikacin concentration and the CSF WBC and glucose and protein concentration were investigated. There were 44 CSF amikacin concentrations and 83 serum samples available from 43 neonates (mean postmenstrual age, 36 weeks [range, 26 to 41 weeks]; mean weight, 2.43 kg [range, 0.87 to 3.86 kg]). The median time interval between initiation of amikacin administration and CSF sampling was 25 h (range, 2.5 to 93.7 h). The median amikacin concentration in the CSF was 1.08 mg/liter (range, 0.34 to 2.65 mg/liter), and the mean trough and peak amikacin concentrations in serum were 3.8 ± 2.5 mg/liter and 35.7 ± 5.9 mg/liter, respectively. A correlation between CSF amikacin and CSF protein contents (P < 0.01, r = 0.41, 95% confidence interval = 0.13 to 0.63) but not between CSF WBC and CSF glucose was documented. A two-compartment (central and CSF) linear disposition model was used to estimate population pharmacokinetics. The half time for equilibration (Teq) between serum and CSF compartments was used as a measure of blood-brain barrier permeability. The Teq was 7.58 h (coefficient of variation [CV] = 49.1%) with a partition coefficient of 0.103 (CV = 26.4%). There was no relationship between the Teq and CSF WBC, CSF glucose content, or CSF protein content.

The incidence of early-onset bacterial infection in the neonate varies from 0.5 to 1% of the total number of deliveries but is significantly higher in preterm neonates, where infection is causally linked with premature delivery in ca. 50% of cases (14). Late-onset, nosocomial bacterial infections occur in a significant number of preterm and term neonates during their stay in the neonatal intensive care unit. Bacteremia, pneumonitis, necrotizing enterocolitis, and meningitis are the most frequent loci for infection in neonates. Empirical treatment for meningitis, necrotizing enterocolitis, and meningitis are the most frequent loci for infection in neonates during their stay in the neonatal intensive care unit. Bacteremia, pneumonitis, necrotizing enterocolitis, and meningitis are the most frequent loci for infection in neonates. Empirical treatment for meningitis is significantly higher in preterm neonates, where infection is causally linked with premature delivery in ca. 50% of cases (17). Late-onset, nosocomial bacterial infections occur in a significant number of preterm and term neonates during their stay in the neonatal intensive care unit. Bacteremia, pneumonitis, necrotizing enterocolitis, and meningitis are the most frequent loci for infection in neonates. Empirical treatment for meningitis is significantly higher in preterm neonates, where infection is causally linked with premature delivery in ca. 50% of cases (17).

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TABLE 1. Clinical characteristics and CSF observations in 43 neonates from whom 44 CSF samples were collected

<table>
<thead>
<tr>
<th>Clinical characteristics and CSF analysis results</th>
<th>Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical characteristics</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>2.430 (0.865–3.860)</td>
</tr>
<tr>
<td>Gestational age (wk)</td>
<td>35 (25–41)</td>
</tr>
<tr>
<td>Postmenstrual age (wk)</td>
<td>36 (26–41)</td>
</tr>
<tr>
<td>Postnatal age (days)</td>
<td>3 (1–29)</td>
</tr>
<tr>
<td>Creatinemia (mg/liter)</td>
<td>0.86 (0.48–1.26)</td>
</tr>
<tr>
<td>CSF analysis</td>
<td></td>
</tr>
<tr>
<td>Amikacin concn (mg/liter)</td>
<td>1.08 (0.34–2.65)</td>
</tr>
<tr>
<td>WBC (no./µl)</td>
<td>13 (0–4, 177)</td>
</tr>
<tr>
<td>Glucose concn (mg/dl)</td>
<td>50 (10–90)</td>
</tr>
<tr>
<td>Protein concn (mg/liter)</td>
<td>1,116 (10–4, 970)</td>
</tr>
</tbody>
</table>

* Data are reported as the median and range or incidence.

MATERIALS AND METHODS

Clinical characteristics and data reporting. From June 2005 to May 2007, CSF samples were prospectively collected from neonates in whom amikacin treatment had been initiated before a diagnostic lumbar puncture was performed. Although this approach is debatable, lumbar puncture is often not performed as part of the initial neonatal sepsis evaluation (14). A CSF sample for an amikacin concentration assay was taken when a lumbar puncture was performed for clinical indications, i.e., to exclude or document meningoitis as judged by the attending neonatologist. Informed consent of the parents for the additional amikacin concentration assay of the CSF sample and for the study-related registration of clinical data was obtained.

The exact time between the start of the administration of amikacin and CSF sampling was recorded. Clinical characteristics (weight, PMA, postnatal age, creatinemia, iphuprofen administration, blood culture, and hearing assessment), CSF analysis (white blood count [WBC], glucose content, protein concentration, and culture), and amikacin therapeutic drug monitoring (TDM) results in serum were collected. Macroscopic blood during CSF sampling was an exclusion criterion. The data were reported by median and range or incidence. Correlations (Spearman rank) between the amikacin CSF concentration and the CSF-WBC, CSF-glucose, and CSF-protein concentrations, respectively, were investigated.

Amikacin: drug administration and TDM. A PMA-based dosing chart was implemented in 2002 based on the suggestions of Langhendries et al. (16) as follows: PMA of <28 weeks, 20 mg/kg/42 h; PMA of 28 to 30 weeks, 20 mg/kg/36 h; PMA of 31 to 33 weeks, 18.5 mg/kg/30 h; PMA of 34 to 37 weeks, 17 mg/kg/24 h; and PMA of >37 weeks, 15.5 mg/kg/24 h; with an additional dosing interval increase of 6 h if iphuprofen was coadministered or if neonates had suffered asphyxia or hypoxia (1). Amikacin (Amukin, 50-mg/ml pediatric vial; Bristol Myers Squibb Belgium) was given as an intravenous infusion over 20 min via syringe driver (SIMS; Graseby, Watford, United Kingdom). Blood samples for TDM were collected by arterial line or venous puncture just before (“trough”) and 1 h after the initiation of administration (“peak”) of the second dose of amikacin, approximately 40 min after the 20-min intravenous infusion (1, 2).

Amikacin assay in serum and CSF. Amikacin serum concentration measurements were performed by using a fluorescence polarization immunosassay (TDx; Abbott) in the hours after sample collection and were reported in milligrams per liter. Drug recovery from extraction was 100% (standard deviation [SD] = 2.6%) over the tested concentration range of 3 to 35 mg/liter. The precision was assessed at 5, 15, and 30 mg/liter. These concentrations yielded a within-run coefficient of variation (CV) of 3.7% to 2.09%, a between-day CV of 0 to 1.74%, and a total CV of 2.6 to 3.2%. The minimal quantifiable concentration was 0.8 mg/liter as defined by a CV of <20% (Abbott). The CV was typically <5% based on an internal quality assessment covering a concentration range of up to 50 mg/liter (1, 2).

The amikacin concentration in CSF was determined by high-performance liquid chromatography (HPLC) with pulsed electrochemical detection, based on adaptations from methods described elsewhere (29). The lower limit of quantification for amikacin was 0.06 mg/liter, and good linearity was obtained with a correlation coefficient of >0.99 in a concentration range from 0.06 to 4 mg/liter. The recoveries of amikacin reference solutions (0.2 and 2 mg/liter) were 96.6 and 99.8%, respectively.

Population pharmacokinetics. A two-compartment (central and CSF) linear model was used to fit the pharmacokinetic data. Population parameter estimates were obtained by using nonlinear mixed effects modeling (NONMEM) (21). This model accounts for population parameter variability (between and within subjects) and residual variability (random effects), as well as for parameter differences predicted by covariates (fixed effects). The population parameter variability in model parameters was modeled by a proportional variance model. A proportional term was used to characterize the residual unknown variability. The population mean parameters, between-subject variance, and residual variance were estimated by using the first-order conditional interaction estimate method differential equations of ADVAN6 TOL5 of NONMEM V. The convergence criterion was three significant digits. A Compaq Digital Fortran Version 6.6A compiler with an Intel Celeron 333 MHz CPU (Intel Corp., Santa Clara, CA) operating under MS Windows XP (Microsoft Corp., Seattle, WA) was used to compile NONMEM.

Differential equations were used to determine parameter estimates: \(\frac{dC_{\text{serum}}}{dt} = \frac{\text{ratein}}{\text{Cl}} - \frac{\text{rateout}}{\text{CSF}} \), where \(\text{ratein} \) is the rate of amikacin administration (mg/h), \(\text{Cl} \) is the total body clearance (liters/h), \(V \) is the volume of distribution (liters), and \(C_{\text{CSF}} \) is the concentration in serum. The second compartment was used to model the CSF concentration (\(C_{\text{CSF}} \)). These two compartments were linked by using an equilibration rate constant (\(K_{\text{eq}} \)). A partition coefficient (PC) was used to describe the ratio between the CSF and the serum concentration at a steady-state concentration: \(\text{ratein}/\text{rateout} = \frac{C_{\text{serum}}}{C_{\text{CSF}}} \).

The rate constant \(K_{\text{eq}} \) can be described by using an equilibration half-time \(T_{\frac{1}{2}} \) as follows: \(T_{\frac{1}{2}} = \ln(2)/K_{\text{eq}} \).

The population parameter variability is modeled in terms of random-effect (\(\eta \)) variables. Each of these variables is assumed to have mean 0 and a variance denoted by \(\sigma^2 \), which is estimated. We report the estimate of \(\sigma^2 \) for each variability component. The covariance between two elements of \(\eta \) (e.g., \(\eta \)) is a measure of statistical association between these two variables. Their covariance is related to their correlation, i.e., \(R = \frac{\text{covariance}}{\sigma_{\eta C_{\text{serum}}} \cdot \sigma_{C_{\text{serum}}} \cdot \sigma_{C_{\text{CSF}}} \cdot \sigma_{C_{\text{CSF}}} \cdot \sigma_{C_{\text{CSF}}} \)}

The relationships between \(T_{\frac{1}{2}} \) and CSF WBC, CSF glucose, and CSF protein concentration were explored by using both linear and exponential functions: \(F_0 = \text{intercept} + \text{slope} \cdot X \).

![FIG. 1. Correlation (Spearman rank) between amikacin CSF concentration and the CSF protein concentration based on 44 CSF samples collected in neonates \((P < 0.01, r = 0.41, 95\% CI = 0.13 to 0.63)\).](http://aac.asm.org/)
TABLE 3. Correlation of between subject variability for CL, \( V \), \( T_{eq} \) and PC

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Correlation for:</th>
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<tbody>
<tr>
<td></td>
<td>CL</td>
</tr>
<tr>
<td>CL</td>
<td>1</td>
</tr>
<tr>
<td>( V )</td>
<td>0.843</td>
</tr>
<tr>
<td>( T_{eq} )</td>
<td>-0.327</td>
</tr>
<tr>
<td>PC</td>
<td>-0.145</td>
</tr>
</tbody>
</table>

slope_\( x \) \((\text{Obs} - \text{Obs}_{\text{average}})\) and \( F_\text{e} = \text{EXP}(\text{slope}_\text{e} \cdot \text{Obs}_\text{e})\), where \( F_\text{e} \) is the function applied to \( T_{eq} \) for the covariates CSF WBC, CSF glucose content, and CSF protein content (\( \text{x} \)), slope_\( \text{e} \) describes the gradient of this relationship, \text{Obs}_\text{e} is the observation for that covariate, and \text{Obs}_{\text{average}} is the average observation.

The quality of fit of the pharmacokinetic model to the data was judged by NONMEM's objective function and by visual examination of plots of observed versus predicted concentrations. Models were nested, and an improvement in the objective function was subjected to the chi-squared distribution to assess the significance, e.g., an objective function change of 3.84 is significant at \( \alpha = 0.05 \) with one additional parameter in the model.

RESULTS

There were 44 CSF and 83 TDM serum observations available from 43 neonates. Demographic data are presented in Table 1. The median time interval between the initiation of amikacin administration and CSF sampling was 25 h (range, 2.5 to 93.7 h). The median amikacin concentration in the CSF was 1.08 mg/liter (0.34 to 2.65 mg/liter), the mean trough and peak amikacin concentrations in serum were 3.8 mg/liter (SD = 2.5 mg/liter) and 35.7 mg/liter (SD = 5.9 mg/liter), respectively. A significant correlation was determined between the amikacin CSF concentration and the CSF protein content \( (P < 0.01, r = 0.41, 95\% \text{ confidence interval} [\text{CI}] = 0.13 \text{ to } 0.63, \text{Fig. 1}) \), but not with CSF glucose content \( (P = 0.57, r = -0.08, 95\% \text{ CI} = 0.37 \text{ to } 0.22) \) or CSF WBC \( (P = 0.37, r = 0.14, 95\% \text{ CI} = 0.17 \text{ to } 0.41) \).

Blood culture-proven infections were documented in 26 of 44 cases \( (\text{Escherichia coli} [n = 10], \text{Streptococcus agalactiae} [n = 7], \text{Staphylococcus epidermidis} [n = 5], \text{Streptococcus viridans} [n = 1], \text{alpha hemolytic} \text{Streptococcus} \text{sp.} [n = 1], \text{Micrococcus} \text{sp.} [n = 1], \text{and} \text{Sphingomonas paucimobilis} [n = 1]) \). CSF culture-proven infections were documented 6 of 44 cases (enteroviral infections \( [n = 2] \), \text{S. agalactiae} \( [n = 3] \), and \text{E. coli} \( [n = 1] \)).

Hearing evaluation, based on brainstem-evoked response audiometry, was performed before discharge in 39 of 55 neonates, 4 additional neonates were evaluated with an automated auditory brainstem response (ALGO test) at home, and one baby died \( (\text{E. coli} \text{ infection}) \) before a hearing evaluation was performed. Of the 54 neonates tested, 1 had a unilateral hearing loss of 40 to 50 dB.

Population parameter estimates and their variabilities are shown in Table 2. The correlation of between-subject variability for CL, \( V \), \( T_{eq} \) and PC is shown in Table 3. Figures 2 and 3 demonstrate the quality of fit of the pharmacokinetic data. Individual concentration predictions are based on values of maximum a posteriori Bayesian estimates of the parameters, while predicted population concentrations are based on population parameters and covariate information. The population estimate for \( T_{eq} \) was 7.58 h \( (\text{CV} = 49.1\%, \text{standard error} = 29.9\%) \). There was no relationship between \( T_{eq} \) and WBC, glucose content, or protein concentration. CSF markers and their relationship to \( T_{eq} \) are shown figuratively for CSF WBC \( (\text{Fig. 4}) \), CSF glucose content \( (\text{Fig. 5}) \), and CSF protein content \( (\text{Fig. 6}) \).

DISCUSSION

A parameter describing the amikacin half-time between serum and CSF compartments \( (T_{eq}) \) in neonates was estimated \( (7.58 \text{ h}, \text{CV} = 49.1\%) \) and subsequently used to investigate the impact of covariates on CSF permeability. We hypothesized to find two distinct \( T_{eq} \) groups: a short \( T_{eq} \) implying rapid movement of drug across the BBB, and a long \( T_{eq} \) suggesting limited permeability, dependent on the meningeal inflammation. This hypothesis was not confirmed since we were unable to link this \( T_{eq} \) parameter to any biochemical indicator of meningeal inflammation or meningitis.

A single intramuscular administration of amikacin \( (75 \text{ mg}) \) resulted in CSF concentrations of \(<0.5 \text{ mg/liter} \) for up to 8.5 h in healthy adults \( (7) \), while Yow et al. documented that ami-

FIG. 2. Amikacin serum data. (A) Individual Bayesian concentration predictions based on values of the parameters for the specific individual are compared to observed values. (B) Population predictions are compared to observed values. The line \( x = y \) is the line of identity.
Amikacin concentrations were between 0.2 and 2.7 mg/liter in 10 neonates after intravenous administration of amikacin at 10 mg/kg (28). In an attempt to reduce the number of inconclusive assay results below the lower limit of quantification, a specific HPLC method with a lower limit of quantification for amikacin of 0.06 mg/liter was used in the present study (29). Such an approach allowed investigation of amikacin disposition at the anticipated low concentrations. Based on this HPLC method, a median amikacin CSF concentration of 1.08 mg/liter (range, 0.34 to 2.65 mg/liter) was documented in neonates, but the absence of any correlation between markers of CSF inflammation and the $T_{eq}$ are in contrast to other reports in children and adults where CSF amikacin concentrations appear to be increased in the presence of meningitis (7, 8, 13, 24, 26, 28).

We did note a correlation between the amikacin CSF concentration and the CSF protein content (Fig. 1), but we are uncertain about the significance of this finding since the CSF protein concentration also displays age-dependent differences. The mean CSF protein concentration is 900 mg/liter in term neonates and 1,150 mg/liter in healthy preterm neonates, with a PMA-dependent decrease in mean CSF protein concentration in former preterm neonates from 1,770 mg/liter at 26 to 28 weeks PMA to 1,170 mg/liter at 38 to 40 weeks PMA (25), whereas amikacin clearance increases with increasing PMA (1, 2, 9). In the present cohort of preterm and term neonates, we also were able to document a significant inverse correlation between CSF protein and PMA ($r = -0.36, 95\% CI = -0.59$ to $-0.07, P < 0.05$). Consequently, we might expect higher concentrations in younger infants because more amikacin is available in serum to cross the BBB independent of maturational or disease related aspects of this barrier.

CSF WBC higher than 21 cells per cubic millimeter had a sensitivity of 79% and a specificity of 81% for the diagnosis of neonatal meningitis in a recently published cohort of 9,111 neonates (14). Using this WBC threshold (21 cells/mm$^3$) as a dichotomous variable, we were unable to show any significant difference in amikacin CSF concentration in this cohort of neonates (1.16 versus 1.15, $P = 0.95$). Gaillard et al. documented amikacin CSF concentrations in a cohort of 16 children (range, 4 months to 8 years old) with community-acquired bacterial meningitis given intravenous amikacin (7.5-mg/kg twice daily) (13). An inverse correlation with CSF glucose level, but not with the CSF protein concentration or leukocyte count was demonstrated. These findings are similar to observations described by Trujillo et al. in pediatric patients with meningitis (24).

We can only speculate why the amikacin $T_{eq}$ correlates poorly with CSF WBC, glucose content, or protein concentration in neonates in contrast to observations in children (5, 14), but the absence of robust covariates for $T_{eq}$ in neonates in is line with the cohort of 9,111 neonates in whom culture-proven meningitis was neither diagnosed nor reflected accurately by the CSF glucose or CSF protein level (14). Blood and CSF glucose levels are relatively lower in neonates, and there is a
more blunted distress-related surge in glycemia and, as mentioned earlier, the protein content of CSF in neonates is higher than in children in the absence of inflammation (25). The additional meningitis-related increase in protein content of the CSF may be less robust in neonates than in children (5, 14).

In the present study, CSF samples were taken when neonates were already treated with antibiotics. This was a specific prerequisite for inclusion in this pharmacokinetic study. It is to be anticipated that such an approach results in a more limited number of positive bacterial CSF cultures, but the clinical practice not to routinely perform lumbar puncture before the initiation of antibiotics when the a priori risk for a meningitis is perceived to be low or when the clinical condition of the patient necessitates an additional delay before CSF sampling has been reported in the literature (14).

The number of CSF observations \(n = 44\) is the largest cohort of data reported in neonates. Consequently, we were able to investigate the PC. Studies in rabbits with meningitis after the intracisternal injection of \(E.\ coli\) suggest a PC of 0.1 to 0.23 at 8 h, but this ratio increased with time over the study period (22). These rabbit data are consistent with our own finding of a PC of 0.103. The \(T_{eq}\) of 7.58 h suggests the mean peak CSF concentration will rise from 1.5 mg/liter after the first dose (Fig. 7) to a mean peak concentration of 2.5 mg/liter in the CSF after three doses of systemic amikacin. A higher mean amikacin serum/CSF ratio of 3:1 has been reported from one CSF sample taken up to 7 h postdose in children with bacterial meningitis (24). This ratio, however, may change at different stages of the illness.

The present observations on amikacin CSF disposition in neonates are also of relevance for assessing potential risk factors for ototoxicity related to the CSF disposition of this drug (17, 19). Ototoxicity relates to the average concentration that contributes to the saturation of cochlear cell binding sites (4); the antibiotic penetration of the middle ear with inflammation is increased, and this increased permeability may also contribute to hearing loss after meningitis (20). Epidemiologic studies in cohorts of neonates in whom aminoglycosides were administered for a variety of infectious diseases could not document an independent impact of this drug when an extended time interval approach was used (4, 6, 10, 17). In contrast, meningitis is a well-known risk factor for ototoxicity (6, 11).

The present observations with the absence of any link between the amikacin \(T_{eq}\) and indicators of meningitis in neo-

nates suggest that the ototoxicity after meningitis relates more to the disease process itself than to an enhanced amikacin permeability of the BBB. The longer \(T_{eq}\) estimates result in a progressive increase in amikacin CSF concentration during repeated administration in (pre)term neonates, while the age-dependent higher concentrations in both amikacin and CSF protein (Fig. 1) suggest that aminoglycoside-dependent ototoxicity is more likely to occur in preterm neonates. Based on these pharmacokinetic observations, it is anticipated that preterm neonates after repeated dose administration are most likely to develop aminoglycoside-mediated ototoxicity.

ACKNOWLEDGMENTS

The clinical research of K.A. is supported by the Clinical Research Fund of the University Hospitals, Leuven, Belgium. E.A. is a postdoctoral fellow of the Fund for Scientific Research, Flanders, Belgium.

REFERENCES