Introduction

Amikacin is commonly used with \( \beta \)-lactam antibiotics for the treatment of presumed or proven sepsis in neonates. Concentration-dependent pharmacokinetics, post-antibiotic effect, and adaptive resistance provide a starting point for once-daily (extended) dosing regimen (OD) where higher doses are given less frequently (1, 2). OD is as effective and safe as multiple-daily (traditional) dosing regimen (3). Nevertheless, when aminoglycoside antibiotics are administered OD, better pharmacokinetic profile with target drug levels is achieved (3, 4). However, there is still uncertainty, and no consensus on the target amikacin concentration range. According to British National Formulary for Children, target amikacin peak levels \( (C_{\text{peak}}) \) for twice daily (TD) should not be \( > 30 \mu g/ml \), and trough levels \( (C_{\text{trough}}) \) should be \( < 10 \mu g/ml \), while for OD, \( C_{\text{trough}} \) should be \( < 5 \mu g/ml \) (5).

Amikacin pharmacokinetics depends on physiological characteristics of neonates. Due to hydrophilic properties, amikacin distributes in the extracellular space. As neonates have rather high percentage of extracellular fluid compared to total body weight, volume of distribution (Vd) expressed in liters per body weight is larger in neonates than infants, children, adolescents, and adults (6, 7). Reported Vd values in neonatal patients range from 0.3 to 0.77 l/kg (8 – 13). Amikacin is almost completely eliminated by the kidneys. Hence, glomerular filtration rate (GFR) determines the clearance (CL) of amikacin. Anatomical and functional renal development

Pharmacokinetic Variability of Amikacin After Once-Daily and Twice-Daily Dosing Regimen in Full-Term Neonates

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Abstract. The purpose of the study was to compare peak (\( C_{\text{peak}} \)) and trough (\( C_{\text{trough}} \)) amikacin levels after twice-daily (TD) or once-daily dosing (OD) in full-term neonates. Additionally, the study aimed to address amikacin pharmacokinetics and its variability. Data included 31 patients born on term. Amikacin daily dose was 15 or 20 mg/kg depending on the neonate’s age. Patients randomly received amikacin every 12 or 24 h. In all patients corresponding \( C_{\text{peak}} \) and \( C_{\text{trough}} \) were taken. Volume of distribution (Vd), clearance (CL) and half-life (\( t_{1/2} \)) were calculated. Mean \( C_{\text{peak}} \) of 21.79 \( \mu g/ml \) in the TD group was statistically different from \( C_{\text{peak}} \) of 36.39 \( \mu g/ml \) in the OD group. Average \( C_{\text{trough}} \) in TD (5.67 \( \mu g/ml \)) was statistically different from the corresponding 3.99 \( \mu g/ml \) in the OD group. Mean amikacin Vd, CL, and \( t_{1/2} \) were 0.78 ± 0.38 l/kg, 86.99 ± 48.22 ml/h∙kg, and 6.81 ± 2.51 h, respectively. High interindividual pharmacokinetic variability was observed. Further analysis showed that neonatal age contributed to the pharmacokinetic parameters’ values. Statistically significant difference in CL and \( t_{1/2} \) was observed between patients age \( \leq 2 \) and \( > 2 \) days on therapy initiation. As expected, amikacin given OD achieved higher \( C_{\text{peak}} \) and lower \( C_{\text{trough}} \) than TD. Based on the results, observed variability in amikacin pharmacokinetics was possibly due to the renal maturation process.

Keywords: amikacin, pharmacokinetics, full-term neonate, dosing regimen
Amikacin Pharmacokinetics in Neonates

The daily dose of amikacin was 15 mg/kg for patients (TD or OD) according to the amikacin dosing regimen.

Materials and Methods

Data for this prospective study were collected in the period from 1st September 2009 until 31st January 2010, in the Neonatal Intensive Care Unit (NICU), Institute for Mother and Child Health Care of Serbia “Dr. Vukan Ćupić”, Belgrade, Serbia. The protocol of the study was reviewed and approved by the Ethics Committee of the Institute. Written informed consent was obtained from the patients’ parents before the enrollment in the study. The study was performed in accordance with the Declaration of Helsinki and its amendments and in compliance with the Guidelines of Good Clinical Practice. Inclusion criteria for the enrollment in the study were neonates (aged 0 – 28 days) born on term (≥ 37 gestation weeks) with suspected or confirmed sepsis. Exclusion criteria were neonates born before 37 weeks of gestation, renal insufficiency, patients already treated with other antimicrobial drugs, and patients with congenital anomalies requiring surgical intervention. Before the initiation of therapy the diagnosis of sepsis (proven, probable, possible, nosocomial) was made according to the recommendations of the International Sepsis Definitions Conference.

The neonates were randomly allocated into two groups (TD or OD) according to the amikacin dosing regimen. The daily dose of amikacin was 15 mg/kg for patients of neonatal age ≤ 7 days or 20 mg/kg for age > 7 days.

In all patients two blood samples (volume of 0.5 – 1 ml) were obtained immediately after the discontinuation of the infusion and just before the next dose. These samples corresponded to C_peak and C_trough. In patients treated with the TD dosing regimen, samples were taken after the 5th dose and in patients with the OD dosing, after the 3rd dose from the initiation of the therapy. In patients with existing central line, venous/arterial sampling was performed. Otherwise a venepuncture procedure was performed. An appropriately trained and experienced medical nurse was responsible for taking blood samples.

Relevant demographic characteristics were collected, including the following: gestation age (weeks), body weight (kg) and body length (cm) at the birth, gender, neonatal age (days) and body weight (kg) at the moment of admission to the hospital, and Apgar score at 1 and 5 min. Moreover, data on maternal age, illness during pregnancy, maternal status during labor as well as therapy experienced medical nurse was responsible for taking blood samples.

Additional C-reactive–protein level was determined after the 3rd dose from the initiation of the therapy. During the first 3 days of hospitalization, β2-microglobulin was assessed and between the 7th and 10th day if the patient was still on the ward. Additional C-reactive–protein level was determined after 12 – 24 h following the initial measurement. Auditory function was also assessed with the transient evoked otoacoustic emission (TEOAE) method: during the first 3 days and between the 7th and 10th day of the treatment.

The bioanalytical method used for measuring amikacin concentrations was turbidimetric immunoassay (Thermo Scientific, UniCel® DxC Synchron® Systems, Beckman Coulter Inc., USA).

Amikacin concentrations were used for calculating individual pharmacokinetic parameters applying the one-compartment linear model based on the Sawchuk and Zaske method (24). Based on the following equation,
the elimination rate constant ($\beta$) was calculated:
\[
C_{\text{rough}} = C_{\text{peak}} \cdot e^{-\beta (t - t_i)}
\]
where $t$ is the dosing interval, and $t_i$ is the infusion time.
Vd was calculated from the following equation:
\[
C_{\text{peak}} = R_0 \cdot \frac{(1 - e^{-\beta t_i})}{\beta \cdot Vd \cdot (1 - e^{-\beta t})}
\]
where $R_0$ is the rate of drug infusion (mg/kg·h), while CL was calculated from estimated values of Vd and $\beta$.

Descriptive and statistical analysis was performed using PASW Statistics® (ver.18.0; SPSS Inc., Chicago, IL, USA). Test of normality and equal variances were applied in order to assess if parametric tests may be used. Parameters with normal distribution were presented as the mean value, whereas data with non-normal distribution were given as the median. For comparing the differences in mean/median values between two groups, the unpaired Student’s $t$-test or the Mann-Whitney U test was employed. Level of statistical significance was set at $P < 0.05$.

**Results**

Based on the inclusion/exclusion criteria, 31 neonates were included in the study: 16 patients on TD and 15 patients on OD amikacin dosing regimen. There were no statistical differences in demographic characteristics of patients between groups (Table 1). In 5 patients, sepsis was confirmed by the isolation of *Acinetobacter* spp, *Escherichia coli* (2 patients), *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.

Amikacin levels after the TD and OD dosing regimen are presented on Fig. 1. Mean amikacin $C_{\text{peak}}$ in the TD group was statistically different ($P < 0.001$) from the corresponding level in the OD group, as given in Table 2. In 2 (12.5%) patients of the TD group, $C_{\text{peak}}$ was below 15 μg/ml and 2 (12.5%) patients reached concentrations above 30 μg/ml. In 2 patients to whom amikacin was administered OD, $C_{\text{peak}}$ was below 30 μg/ml. Mean $C_{\text{trough}}$ in the TD group was statistically different ($P < 0.001$) from the mean $C_{\text{trough}}$ in the OD group (Table 2). In all patients in both groups, $C_{\text{trough}}$ values were below 10 μg/ml. However, in the TD group 5 (31.25%) patients had $C_{\text{trough}}$ less than 5 μg/ml, whereas in the OD group, in all except 1 patient, $C_{\text{trough}}$ values were about or less than 5 μg/ml. No statistical differences in the mean pharmacokinetic parameters’ values were observed between groups. According to the results of this study, mean Vd, CL, and $t_{1/2}$ of amikacin in full-term neonates were $0.78 \pm 0.38$ l/kg, $86.99 \pm 48.22$ ml/h·kg and $6.81 \pm 2.51$ h, respectively. High interindividual pharmacokinetic variability was observed. Hence, further analysis revealed that neonatal age contributes to the changes in pharmacokinetic parameters of elimination. Statistically significant difference was observed in CL and $t_{1/2}$ between patients whose neonatal age was ≤ 2 days and those with neonatal age of > 2 days on the day of initiating amikacin therapy, as presented in Table 3. The data on efficacy and safety are not presented, as this study was focused on pharmacokinetic aspects. However,
no difference was observed in the monitored parameters of efficacy and toxicity between groups on different dosing regimens at the beginning and at the end of the therapy. In this study, Scr levels and TEOAE did not show marked change by the end of the amikacin therapy between groups. The results of our study did not show any relationship between amikacin C<sub>trough</sub> and ototoxicity. All patients were cured by the end of the treatment, and there was no follow up on amikacin ototoxicity upon discharge of the patients from the hospital. Therefore, these data did not allow us to determine correlations of the amikacin concentrations with efficacy and toxicity parameters.

**Discussion**

The results of the present study confirmed that significantly higher C<sub>peak</sub> and lower C<sub>trough</sub> were achieved with OD dosing regimen of amikacin in comparison to TD dosing. Lower C<sub>peak</sub> levels might be risky for the efficacy, since they may not attain the desired ratio of C<sub>peak</sub> and minimal inhibitory concentration (MIC) (25). According to the results of our study, this was more probable when amikacin was administered TD, and it might be due to the large values of amikacin Vd. Additionally, as previously described, this might delay the start of the amikacin effect for 1 – 2 days from the initiation of therapy to when the therapeutic steady-state C<sub>peak</sub> occurs. On the contrary, when amikacin was administered OD, it was expected to reach levels above the usual ones for TD dosing. C<sub>peak</sub> after extended dosing might be up to 60 µg/ml (26), and they usually relate to enhanced therapeutic outcomes. Measured C<sub>peak</sub> in patients in this study were consistent with previously reported maximum concentrations in pre-term and term neonates (17.1 – 43.3 µg/ml) (8, 12).

Evidence from in vitro and animal studies indicated that aminoglycoside antibiotics exhibit a post-antibiotic sub-MIC effect that suppressed bacterial growth when a low concentration (≤ 0.3 × MIC) was added to bacteria previously exposed to a supra-inhibitory concentration (27). Due to prolonged dosing interval in OD compared to TD, a lower C<sub>trough</sub> was observed in our study (Table 2 and Fig. 1). Since it is not required for the drug to accumulate, target amikacin C<sub>trough</sub> after OD was lower than that proposed for TD dosing. Therefore, C<sub>trough</sub> may approximate zero at the end of the extended dosing interval, as it was shown for other aminoglycoside antibiotics (2, 3). Additionally, the developed predictive pharmacokinetic–pharmacodynamic model for gentamicin dosing schedules in neonates supported equivalent efficacy when the dosing interval was 24, 36, or 48 h for the same total dose (28). This result may be extrapolated to amikacin as well, where C<sub>trough</sub> after OD can be targeted at 1.5 to 3 µg/ml (18, 29). In our study, 53.33% of patients in the OD group had C<sub>trough</sub> > 3 µg/ml. This indicates the need to extend the dosing interval from 24 to 36 h or even to 48 h in order to achieve the defined concentration. The results from our study are in compliance with previously reported data on amikacin C<sub>trough</sub> in term neonates, which were in the range from 0.5 to 6.6 µg/ml (8). Furthermore, target concentrations for the individual patient will depend on the patient’s clinical condition, site of the infection, and particularly on MIC of the suspected/isolated bacterial pathogen (26).

Amikacin pharmacokinetic parameters in our study are comparable to previously published values of Vd, CL, and t<sub>1/2</sub> (8, 21). No statistical differences in the mean parameters values were observed between groups, as amikacin shows linear pharmacokinetics. However, relatively high coefficients of variation for aforementioned parameters demonstrate significant interindividual pharmacokinetic variability of amikacin in full-term neonates. The findings of other authors confirm that variability in amikacin elimination was caused by gestation age or birth weight which correlates to gestation age. However, in these studies, pre- and full-term neonates were included (10, 18, 30). There were as well conflicting data if postmenstrual (sum of gestational and neonatal) age was a good predictor of aminoglycoside CL (12, 17, 23, 31). Considering the fact that the major changes in blood flow occur during the transition from the intrauterine to the extraterine environment, it seems rational to observe gestational and neonatal age as independent factors since their influences on amikacin CL are different due to distinct causes (32, 33). For that reason, we must emphasize that our study included neonates ≥ 37 weeks, and it would not be expected to

### Table 3. Median values of amikacin pharmacokinetic parameters in neonates

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Neonatal age 1 and 2 days (n = 18)</th>
<th>Neonatal age ≥ 2 days (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume of distribution, Vd (l/kg)</td>
<td>0.68</td>
<td>0.62</td>
</tr>
<tr>
<td>Clearance, CL (ml/h/kg)</td>
<td>65.11</td>
<td>79.98*</td>
</tr>
<tr>
<td>Half-life, t&lt;sub&gt;1/2&lt;/sub&gt; (h)</td>
<td>6.94</td>
<td>5.64*</td>
</tr>
</tbody>
</table>

*P < 0.05.
observe variability due to gestation age. However, in our study patients born on 37–38 weeks of gestation showed the tendency for lower CL (without significant difference) in comparison to newborns born on 41–42 week of gestation, as previously observed (11). Due to the limited number of patients in our study, it was not possible to assess the age influence as continuous covariate. Therefore, the result in the study may seem overemphasized, but we tried to explain the variability observed in the pharmacokinetic parameters. On the other hand, nephrogenesis is completed by 36 weeks of gestation (14), so it may not be expected to detect significant differences in pharmacokinetics between neonates born on 37 and 41 weeks of gestation. Therefore, our results indicate that the change in CL and amikacin t_{1/2} was possibly due to kidney maturation. Findings of this study indicate that amikacin CL was lower in patients in whom therapy was initiated in the first two days of life in contrast to the older full-term neonates (Table 3), and it perhaps reflects the changes in GFR with neonatal age. Data on measured Scr were not presented in this report as no difference in Scr was observed between the initiation of treatment and the end of the treatment. However, it was noticed that Scr levels decreased with neonatal age with the steepest slope in the youngest neonates. Observed changes were physiologically altered, due to maturation of kidneys’ function that was extensive in the first days of life (33, 34). Hence, our data show that amikacin t_{1/2} was prolonged immediately after birth in comparison to older neonates. As mentioned, the limitation of this study was that the majority of patients were neonatal age 1–2 days, while other age categories (up to 1 month) included only 1–2 patients. Consequently, it was not possible to observe the tendency of the changes in CL and t_{1/2} during the neonatal period in the present study, but it has been shown by population pharmacokinetic models (18). However, based on the presented results it may be reasonable to explain the variability in CL with neonatal age and kidney maturation.

Large interindividual pharmacokinetic variability of amikacin in full-term neonates emphasizes the need for further research where factors that contribute to pharmacokinetic variability and hence dosing regimen will be quantitatively determined.

The results of this study highlighted the differences between amikacin C_{peak} and C_{trough} in full-term neonates on twice-daily and once-daily dosing regimen, and our results contribute to defining the optimal therapeutic range of amikacin. Additionally, calculated pharmacokinetic parameters revealed high interindividual variability that may be partially explained by neonatal age.

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**Conflicts of Interest**

The authors indicated no potential conflicts of interest.

**References**

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