Original Article

Relationship between amikacin blood concentration and ototoxicity in low birth weight infants☆

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ABSTRACT

Amikacin (AMK) is used as empiric therapy for severe infections such as sepsis in low birth weight (LBW) infants. AMK administered once daily (OD) in adults is reported to be therapeutically effective and prevent side effects, however, evidence on AMK administration in LBW infants is limited, with no clear indications of effectiveness. We performed therapeutic drug monitoring analysis of 20 infants treated with AMK OD for severe infections such as bacteremia. Treatment effectiveness was admitted by the patients' medical records, and side effects of renal dysfunction and ototoxicity were investigated. The mean gestational age was 30.4 ± 5 weeks and mean body weight (Bw) was 1280.2 ± 809.8 g. The mean AMK dose was 14.1 ± 2.6 mg/kg and mean administration period was 10.1 ± 4.1 days. Blood concentration was measured 6.3 ± 2.3 days after AMK administration; mean peak and trough concentrations were 29.1 ± 7.5 μg/mL and 7.6 ± 6.9 μg/mL, respectively. Additionally, therapeutic effect was observed in all patients, and no significant change in serum creatinine (CRE) concentration (a marker of renal dysfunction) was observed, suggesting no renal dysfunction. Ototoxicity was observed in 4 patients, 3 of whom had trough concentrations ≥ 10 μg/mL. When we categorized patients into two groups using a trough cut-off value of 10 μg/mL, no difference in AMK dose was observed. However, there were significant differences in peak concentration, Bw, volume of distribution and CRE. Our findings suggest AMK trough concentration ≥ 10 μg/mL significantly affects ototoxicity in neonates.

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1. Introduction

Empiric antibiotic treatment is prescribed for severe bacterial infections in newborns with immature immune function. In such cases, aminoglycosides (AGs), which have activity against Gram-negative bacteria (GNB) such as *Escherichia coli (E. coli)* are often used [1]. In the neonatal intensive care unit (NICU) of our hospital, amikacin (AMK) has shown better sensitivity against *E. coli* isolates with past sensitivity to gentamicin and AMK.

AMK is an AGs that is mainly used to treat GNB such as *Pseudomonas aeruginosa* and *Serratia*, and its therapeutic effect is considered to be high based on its peak concentration from pharmacokinetic-pharmacodynamic studies [2]. Other reports have demonstrated that the risk of impaired renal and auditory function is dependent on blood concentration and total dose [3–5]. Multiple daily dosing (MDD) of AGs had been recommended for adults. Similarly, in our hospital, neonates had received AGs therapy by MDD according to postnatal age and weight. However, in recent years, pharmacokinetic-pharmacodynamic studies have suggested that treatment once daily (OD) is more effective in adults compared to MDD, and the onset of side effects is reduced. Therefore, at present, OD dosing is routinely used for AGs [6]. Furthermore, by conducting therapeutic drug monitoring (TDM), it is possible to maintain a stable AGs blood concentration in the treatment area, which contributes to a reliable clinical effect and suppression of side effects [7]. However, few reports have assessed the relationship between renal dysfunction, ototoxicity, and AMK concentration, and the toxic blood concentration range is unclear.

Furthermore, in neonates, especially low birth weight (LBW) infants, is difficult to adjust the drug dose because of fluctuation in the volume of distribution (V_D) and delayed elimination due to undeveloped kidneys. For this reason, treatment with TDM is indispensable when using AGs treatment in neonates [8].

While there are reports of AGs use including AMK therapy in premature infants [9], few reports have evaluated AMK therapy in LBW infants, especially extremely low birth weight (ELBW) and very low birth weight (VLBW) infants. Therefore, in this study, we evaluated AMK therapy using TDM in preterm infants, including ELBW and VLBW, with a focus on therapeutic effects and side effects by OD.

2. Materials and methods

2.1. Study design

We enrolled 20 premature infants hospitalized in the NICU during November 2013 to January 2015 and who underwent TDM during AMK therapy. All subjects enrolled in this study were treated with AMK for severe infections of sepsis, respiratory infections, and intraperitoneal infections. We excluded patients in whom AMK blood concentrations were not measured. We evaluated gestational age (GA), sex, body weight (Bw), AMK dose, AMK peak and trough concentrations, calculated V_D, effect of indomethacin (IND) administration (which impairs renal function), fluctuation of serum creatinine (CRE) level at each blood sampling time point, and ototoxicity.

2.2. AMK administration

AMK was intravenously administered at 15 mg/kg OD for 0.5—1 h.

2.3. AMK blood sampling

We measured AMK blood concentration during steady state 3 days after the start of treatment. The peak concentration was measured 1—1.5 h after completion of administration, and the trough concentration was measured within 1 h before administration. We thought that AMK remained in the catheter at the time of blood collection for about 30 min after administration, and that the dose would not reflect uniform distribution in the body. Therefore, the peak blood sampling time was delayed in order to avoid the risk. For sampling, approximately 0.3—0.5 mL of whole blood were collected per patient, and the centrifuged serum was used. Blood concentration measurements were performed by kinetic interaction of microparticles in a solution (SRL Inc., Tokyo, Japan). The measured values were analyzed using PEDA VB (Jiho Inc., Tokyo, Japan), and the dosing schedule was calculated. V_D was calculated using the following formula: $V_D = Dose/C\ peak$.

2.4. Evaluation of therapeutic effect and side effects

Effectiveness of AMK treatment was obtained from the patients' medical records which were evaluated by a doctor.

We focused on two side effects: renal dysfunction and ototoxicity. Renal dysfunction was defined as a 1.5-fold increase in CRE value from the baseline value according to the RIFLE classification [10]. Blood sampling of CRE value was performed at four time points: before AMK administration, at the time of AMK blood concentration measurement, after AMK administration, and before discharge, and we confirmed the transition of influence to renal function by AMK administration. In addition, we investigated the effect of IND administration as a factor that affects fluctuations in CRE value.

Regarding ototoxicity, infants underwent automated auditory brainstem response testing. Infants with abnormal results underwent subsequent auditory brainstem response testing. Furthermore, if newborns were suspected to have an abnormality with ototoxicity, an otolaryngologist was consulted, who then determined ototoxicity.

2.5. Statistical analysis

Differences in GA, sex, Bw, AMK dose and peak concentration, CRE value at each blood sampling time point, IND administration, and ototoxicity were each evaluated using a correlated the Wilcoxon signed-rank test, t-test and Fisher's exact probability test.

2.6. Ethical consideration

The study obtained ethical clearance from clinical and genome research ethics review committee of our institution (register number 29—27). In order to secure confidentiality unique identifiers like names were not recorded. Data were kept in the computer using password protection by the principal investigator.

3. Results

3.1. Patient background

In this study in 20 neonates, GA was mean 30 ± 5.1 weeks and Bw was mean 1280 ± 810 g. Antimicrobials were used against early onset and late onset in sepsis, respiratory infections, intraperitoneal infections etc. The mean dose was 14.1 ± 2.6 mg/kg, which was almost the same as the target dose of 15 mg/kg based on a previous report [9] that recommended by the Japanese Society of Chemotherapy guideline; the mean administration period was 10.1 ± 4.1 days; and the mean measurement day of AMK blood concentration was 6.3 ± 2.3 days (Table 1).

3.2. AMK concentration transition

Peak and trough concentrations obtained by blood sampling are shown in Fig. 1. The mean peak concentration was 29.1 μg/mL; the highest peak concentration was 42.5 μg/mL and the lowest peak concentration was 19.4 μg/mL. When comparing them between the group with ototoxicity and without ototoxicity, there were no differences (Fig. 1a). The mean trough concentration was 7.86 μg/mL; the highest trough concentration was 28.4 μg/mL and the lowest trough concentration was 1.8 μg/mL. When comparing them between the group with ototoxicity and without ototoxicity, trough concentration tended to increase in the group with ototoxicity (Fig. 1b).

3.3. Therapeutic effect

Therapeutic effect of AMK in all patients suggested from the patients' medical records which was evaluated by a doctor.

Table 1
Mean dose, administration period, and blood sampling date for amikacin (AMK).

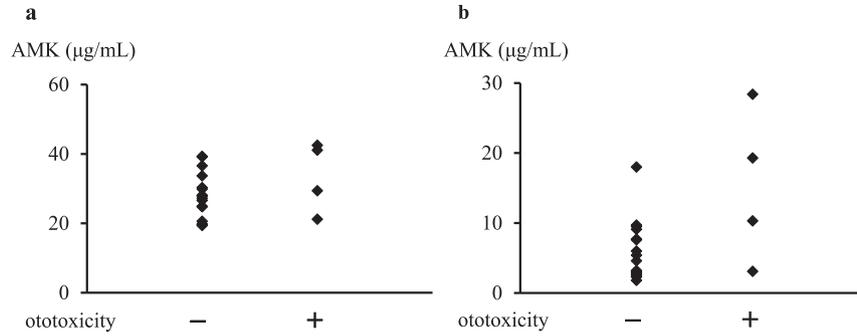| AMK	Total (n = 20)
Dose (mg/kg)	14.1 ± 2.6
Administration period (day)	10.1 ± 4.1
Serum blood sampling (day)	6.3 ± 2.3

Fig. 1. AMK peak and trough concentrations. (a) Blood concentration transition of the peak concentration. (b) Blood concentration transition of the trough concentration.

3.4. Side effects

Overall, no significant fluctuation in the CRE value was observed (before treatment: 0.72 ± 0.54; after treatment completion: 0.76 ± 0.7). In addition, the CRE value improved to 0.25 ± 0.07 before discharge, and no renal dysfunction was observed in all patients (Fig. 2).

Ototoxicity was confirmed in 4 of 20 patients (Table 2a). When we categorized ototoxicity using a trough cut-off value of 10 μg/mL (based on a previous report [11] that ototoxicity frequency increases with trough concentrations $\geq$10 μg/mL), 3 patients had trough concentrations $\geq$10 μg/mL and 1 patient had a concentration <10 μg/mL (Table 2b). Using this trough cut-off value, we then divided the patients into two groups ($\geq$10 μg/mL and <10 μg/mL) and evaluated differences in patient characteristics (Table 3). Of the 20 patients, 4 had trough concentrations $\geq$10 μg/mL and 16 had trough concentrations <10 μg/mL. In 16 patients of trough concentrations $\geq$10 μg/mL, 4 was Bw $\geq$ 1500 g. There was no significant difference between the two groups according to sex. In addition, there was no significant difference according to GA, although trough concentration tended to increase with younger GA (Table 3, Fig. 3a). However, a significant difference in Bw was observed, with lower Bw noted in the $\geq$10 μg/mL group ($\geq$10 μg/mL group: 742 g, <10 μg/mL group: 1414 g; $p < 0.05$). The AMK dose did not differ between the two groups, however, the peak concentration was significantly higher in the $\geq$10 μg/mL group ($\geq$10 μg/mL group: 38.1 μg/mL, <10 μg/mL group: 26.9 μg/mL; $p < 0.05$). One factor that affects the blood concentration of AMK is the fluctuation of V_D, therefore we analyzed the V_D. As a result, V_D calculated by measured peak concentrations and dosage was significantly different between the two groups ($\geq$10 μg/mL group: 0.37 L/kg, <10 μg/mL group: 0.55 L/kg; $p < 0.05$). In addition,

IND was administered to 7 of 20 infants, 3 in the $\geq$10 μg/mL group and 4 in the <10 μg/mL group; the IND administration rate in the $\geq$10 μg/mL group was 75%. The CRE value at the time of AMK blood sampling was significantly higher in the $\geq$10 μg/mL group than in the <10 μg/mL group ($p < 0.05$), although the CRE value before discharge improved to within the normal range in both groups (Table 3). The proportion of patients with ototoxicity was significantly higher in the $\geq$10 μg/mL group (75.0%) compared to the <10 μg/mL group (6.25%) ($p < 0.05$).

4. Discussion

In TDM of AGs, the peak concentration can be used to evaluate the therapeutic effect and the trough concentration is thought to correlate with side effects. Based on this concept, a comparison study on OD and MDD of AGs reported OD was superior to MDD in terms of therapeutic effect, nephrotoxicity, and ototoxicity [6]; thus, OD is currently recommended for adults. The recommended AMK dose to achieve a peak blood concentration ranging 40–60 μg/mL and trough concentration <4 μg/mL is 15 mg/kg/day [12]. However, there are few reports on AMK blood concentrations in newborns, and there are no clear indications on therapeutic effects and occurrence of side effects, especially in LBW infants.

In this study, we investigated the therapeutic effect and side effects of AMK treatment given OD in preterm infants, especially ELBW and VLBW infants. Based on thought that AMK remained in the catheter at the time of peak blood collection for short term, the peak concentration was measured 1–1.5 h after completion of administration. Therefore, we thought possibility that this peak may be lower than exact peak. Although it is difficult to accurately evaluate the therapeutic effect, no deterioration of the patients' infectious condition was observed in this study. Namely, in all patients, treatment efficacy was clinically obtained even at this low peak in administration of 15 mg/kg OD. Consequently, our findings suggest it may be
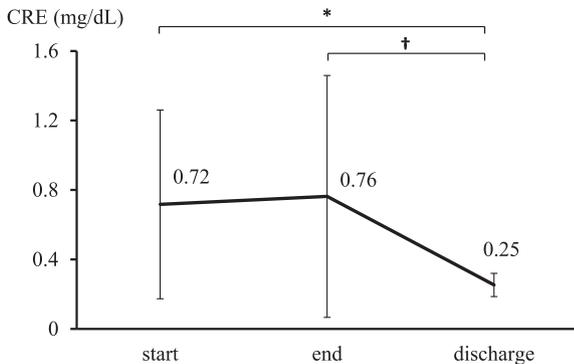

Fig. 2. Serum creatinine (CRE) transition at the three time points; the start and the end of AMK administration and before discharge. *$p < 0.05$ and †$p < 0.05$, Wilcoxon signed-rank test.

Table 2
Number of patients with ototoxicity.
(a) Number of infants with ototoxicity.
(b) Ototoxicity determined by a trough cut-off value of 10 μg/mL.

(a)		Ototoxicity		
		(+)	(−)	
(b)	n = 20	4	16	
			Ototoxicity	
			(+)	(−)
	Trough (μg/mL)	$\geq$10 (n = 4)	3*	1
		<10 (n = 16)	1*	15

*$p < 0.05$, Fisher's exact probability test.

Table 3
Differences in patient characteristics in groups stratified by an AMK trough cut-off value of 10 μg/mL.

	Trough (μg/mL)		*p* value
	≥10	<10	
Number of patients	4	16[*]	
Sex (M/F)	02/02/2018	05/11/2018	0.59[†]
Gestational age (w)	28.1 ± 5.1	31.1 ± 5.4	0.15[‡]
Body weight (g)	742 ± 195.7	1414.8 ± 852.3	< 0.05[‡]
(ELBW, VLBW)	(3, 1)	(6, 6)	
AMK dose (mg/kg)	13.9 ± 2.6	14.2 ± 2.7	0.40[‡]
Peak concentration (μg/mL)	38.1 ± 5.9	26.9 ± 6.1	< 0.05[‡]
V_D (L/kg)	0.37 ± 0.07	0.55 ± 0.16	< 0.05[‡]
IND administration	3/4 (75%)	4/16 (20%)	0.10[†]
CRE at AMK sampling time (mg/dL)	1.88 ± 0.75	0.66 ± 0.29	< 0.05[‡]
CRE before discharge (mg/dL)	0.26 ± 0.07	0.25 ± 0.07	0.44[‡]
Ototoxicity	3 (75%)	1 (6.25%)	< 0.05[†]

[*] 4 patients are Bw ≥ 1,500 g.
[†] Analysis by the Fisher's exact probability test.
[‡] Analysis by the *t*-test.

possible to design AMK administration guidelines while considering the trough concentration that is an index of side effects.

Moreover, when the trough concentration was used to stratify infants into two groups (≥10 μg/mL and <10 μg/mL), Bw and V_D increased in the ≥10 μg/mL group compared to the <10 μg/mL group (Table 3). These results suggest that the AMK trough concentration is increased in ELBW infants. We thought like follows as one of the factor for this phenomenon. Considering that AMK is hydrophilic and transported through extracellular fluid, the peak concentration is likely increased because V_D is small in ELBW infants compared to VLBW infants. Furthermore, AMK elimination delayed due to immature kidneys, and the trough concentration is likely increased. Consequently, we consider that the AMK blood concentration in ELBW infants generally increases. Therefore, both peak and trough concentrations are likely higher in ELBW infants than in VLBW infants. However, when the V_D value was calculated as shown in Table 3, we found a incompatibility result that V_D decreases in a lower body weight infants. The V_D value was calculated from the measured peak value. Therefore the V_D value may not be an accurate, but it significantly decreased in patients with lower body weight. We couldn't lead to conclusion on this result. We considered that further study with increasing the patients is necessary.

In addition, when we examined the effect of IND on renal function, the IND administration rate in the ≥10 μg/mL group was 75%. ELBW infants, who are younger, have a high risk of developing patent ductus arteriosus. In addition, the mean CRE value at the time of AMK blood sampling was 1.88 mg/dL in the ≥10 μg/mL group and 0.66 mg/dL in the <10 μg/mL group, which was significantly different. Based on the report that is typically poor to evaluate renal function using CRE value in neonates [13], we are difficult to judge kidney damage in this study. However, considering that CRE is excreted from the kidney, renal function is predicted to decline in the high-risk group. From these results, we consider that IND administration in LBW infants can lead to renal dysfunction by decreasing renal blood flow (by inhibiting prostaglandin E2 synthesis in the kidney), and AMK excretion is delayed. AMK excretion is more delayed by the addition of water and sodium accumulation. These factors likely contributed to AMK concentration transition of trough concentration ≥10 μg/mL. However, we regard as one of the possibilities due to no elevation of CRE value and AMK trough concentration in all patients with IND administered. In all patients, CRE values significantly improved and recovered to within the normal range at discharge. Therefore, we thought that possibility of renal dysfunction occurrence by AMK defined using the RIFLE classification was low. Additionally, a report on impaired renal function induced by AMK treatment described temporary renal impairment in newborns that subsequently improved [5], which may have also occurred in our patients. Based on the above findings, renal dysfunction may be temporarily observed with IND administration but renal function will likely recover after several days.

Regarding renal function and age, a previous report described that renal function in newborns including preterm infants is determined by the post-conceptual age, and the rate of kidney development does not change before and after birth [14]. Conversely, another study reported that kidney development is completed by 34 weeks of gestation [5]. In the present study, AMK trough concentrations tended to be higher in patients with younger GA, and some infants showed high concentrations before 34 weeks of age (Fig. 3a). However, no significant relationship was observed between GA and AMK trough concentration. Furthermore, no significant relationship was observed between trough concentration and Bw, although the trough concentration tended to be higher in infants with lower Bw (Fig. 3b). Specifically, ELBW infants weighing <1000 g had AMK trough concentrations ≥10 μg/mL and VLBW infants weighing >1000 g had AMK trough concentrations <10 μg/mL.

AMK excretion in newborns has been reported to occur through glomerular filtration [15]. However, there are few markers measuring glomerular filtration in neonates, and it is difficult to predict AMK excretion. Therefore, in the present study, when we examined the relationship between CRE value and AMK trough concentration, AMK trough concentration was positively correlated with CRE value (Fig. 3c). This result suggests that we can predict the trough concentration based on the CRE value and set the appropriate AMK dose. However, because V_D was small and excretion may be delayed in newborns, AMK blood concentration measurement is necessary.

A previous study reported increased ototoxicity with AMK trough concentrations ≥10 μg/mL [11]. Therefore, we compared the presence or absence of ototoxicity using the trough cut-off value of 10 μg/mL and observed significant differences between the two groups (Tables 2b and 3). This result was similarly significant difference when we examined with only Bw < 1500 g in this study (data not shown). There are many risk factors for ototoxicity including long-term NICU stay, medication (AGs, loop
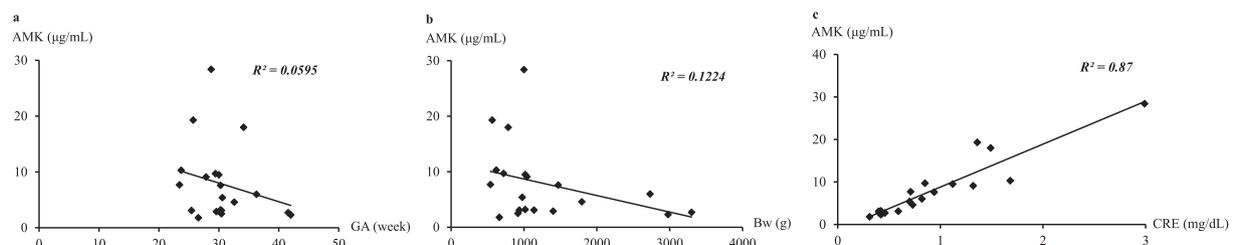
Fig. 3. (a) Relationship between AMK trough concentration and gestational age (GA). (b) Relationship between AMK trough concentration and body weight (Bw). (c) Relationship between AMK trough concentration and CRE.

diuretic), hyperbilirubinemia requiring plasma exchange, *Cytomegalovirus*, herpes, rubella, syphilis, intrauterine infection such as toxoplasmosis, blood culture-positive bacterial infection, extracorporeal membrane oxygenation, ventilator management, head and neck malformation (craniofacial anomalies), and teratogenic syndrome [16]. However, we are limited in the present study that investigates the relationship between ototoxicity and the above factors including genetic factors. When we performed binomial logistic regression analysis, no significant correlation was observed between ototoxicity and trough concentration $\geq$10 μg/mL ($p = 0.107$), although they tended to be related (data not shown). Similarly, when we evaluated the relationship between GA and ototoxicity, no significant correlation was observed ($p = 0.096$), although they tended to be related (data not shown). We speculate that ELBW infants with younger GA have delayed AMK excretion due to both IND administration and immature renal function, which subsequently leads to increased risk of developing ototoxicity at trough concentrations $\geq$10 μg/mL. However, further study with a larger sample size is necessary to confirm our findings.

The affect of concomitant administration of other drugs and IND under administration AMK is unclear. A study reported that the administration interval must be increased when administering gentamicin and IND concomitantly [17], whereas other groups reported both dose adjustment and increased administration interval are needed to maintain the target concentration [3,18]. Therefore, administration intervals (e.g., every other day) should be considered.

Our results suggest an appropriate AMK dose to prevent ototoxicity can be determined by comprehensively judging three factors: GA, Bw, and CRE value. In addition, chronic renal dysfunction is unlikely to occur even at trough concentrations $\geq$10 μg/mL, although the mean AMK administration period in this study (10.1 $\pm$ 4.1 days) was relatively short. However, a previous study on renal dysfunction induced by AMK reported that the total AMK treatment period when given OD ranges 10–14 days [19]. Therefore clinicians and pharmacists must pay attention to renal dysfunction due to AMK accumulation with long-term administration >10 days.

In conclusion, our findings suggested that AMK trough concentration $\geq$10 μg/mL significantly affects ototoxicity in neonates, especially ELBW and VLBW infants. Therefore, when using AGs, it is necessary to examine the dose and administration interval after blood concentration measurement.

Conflicts of interest

The authors declare no conflicts of interest.

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