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Evaluation of hemodialysis as a protective technique for preventing high daily dose amikacin nephrotoxicity: an experimental study in an ovine model

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Abstract (248 words)

Changes in pharmacokinetic parameters of critical ill patients make the treatment of infections challenging, particularly when multidrug-resistant bacteria are involved. The aim of this study was to evaluate the ability of hemodialysis to reduce the exposure to high dose amikacin and prevent nephrotoxicity. Amikacin 50 mg/kg was administered intravenously to 6 adult sheep once daily for 4 days. Sheep were divided into two groups according to the implementation (group 1) or not (group 2) of hemodialysis. In group 1, hemodialysis was performed for 4h, initiated 2h after starting amikacin infusion. Amikacin area under the curve (AUC) and trough concentrations (C_{\min}) were used as markers of amikacin-induced nephrotoxicity. The median hemodialysis amikacin clearance was 2.14 L/h (35.6 mL/min), 14% of the mean total body clearance for 24 h. Hemodialysis reduced C_{\min} (group 1: 0.3 $\mu\text{g/mL}$ [0.3 – 1.1]; group 2: 1.4 $\mu\text{g/mL}$ [1.1 – 3.9]; $P = 0.0003$) and time of exposure to a concentration exceeding 2.5 $\mu\text{g/mL}$ (group 1: 99.7 % [99.7 – 99.8]; group 2: 99.9 % [99.8 – 99.9]; $P = 0.049$). A trend toward reduced AUC with hemodialysis was observed (group 1: 1450 $\mu\text{g/mL}\cdot\text{h}$ [1311 – 1716]; group 2: 3126 $\mu\text{g/mL}\cdot\text{h}$ [2581 – 3171]; $P = 0.10$). No sheep has developed acute kidney injury. In conclusion, hemodialysis seems interesting in reducing AUC and C_{\min} after injection of high-dose of amikacin, parameters known to be involved in its induced nephrotoxicity, in an experimental ovine model.

Keywords

Amikacin-induced nephrotoxicity, pharmacokinetics, hemodialysis, multidrug-resistant bacteria

Abbreviations

AKI: acute kidney injury

AUC: area under the curve

Cl_{cr} : creatinine clearance

Cl_{d} : hemodialysis clearance of amikacin

Cl_{r} : renal clearance of amikacin

C_{max} : maximal concentration

C_{min} : trough concentration

CV: coefficient of variation

ICU: intensive care unit

MDR: multidrug-resistant bacteria
MIC: minimal inhibitory concentration
RRT: renal replacement therapy
 V_d : volume of distribution
VPC: Visual Predictive Check

1. Introduction

Multidrug-resistant (MDR) bacteria are tremendously emerging in the intensive care unit (ICU) environment, increasing mortality and morbidity of critically ill patients [1]. The treatment of these patients is challenging as only few new drugs have been developed in recent years. New strategies need to be promoted in order to optimize the use of available antibiotics [2].

Aminoglycosides are important drugs for the treatment of sepsis and septic shock with involvement of Gram-negative pathogens [3–8]. Among the aminoglycosides, amikacin is a well-used concentration-dependent antibiotic. Optimum antibacterial effect is obtained when the ratio between the maximal concentration (C_{max}) of the drug and its minimal inhibitory concentration (MIC) is more than 8 [2]. This target is also related to a better clinical response [9]. For amikacin, the MIC of *Enterobacteriaceae* and *Pseudomonas spp* are 8 $\mu\text{g/mL}$ for sensitive strains and 16 $\mu\text{g/mL}$ for intermediate strains [10], indicating that to improve the antibacterial activity, C_{max} should reach plasma concentrations $\geq 64 \mu\text{g/mL}$ or $\geq 128 \mu\text{g/mL}$.

Critically ill patients have modifications of their pharmacokinetic parameters, with increased volume of distribution (V_d) due to the large volume of administered fluids and increased vascular permeability resulting in interstitial fluid shifts [2]. Consequently, serum target concentrations of hydrophilic drugs such as amikacin are difficult to obtain. With a dose of amikacin of $\leq 30 \text{ mg/kg}$, a C_{max} of $\geq 64 \mu\text{g/mL}$ is reached in less than 77% of patients [4,5,8,11]. Higher doses than 30 mg/kg may therefore be needed to achieve the clinical breakpoint in critically ill patients.

Amikacin is a nephrotoxic agent with toxicity related to excessive antibiotic exposure, providing increased area under the time-concentration curve (AUC) and increased trough concentration (C_{min}) [12]. Increasing the amikacin dose will increase these pharmacokinetic parameters, implying increased renal toxicity. With a dose of 25 mg/kg , a C_{min} of $> 5 \mu\text{g/mL}$ is observed in more than 50% of the patients [7]. Acute kidney injury (AKI) is reported in

24% of ICU patients with 30 mg/kg of amikacin [5]. In this study, survivors had a C_{\min} significantly lower than non survivors [5].

The use of renal replacement therapy to improve the elimination of the antibiotic and reduce its toxicity after the administration of high dose of amikacin has been reported with success in two cases [6] and was associated with a favorable clinical response in 8 of 15 patients with MDR-induced sepsis [3].

Despite medical and economical concerns, only few data is available on this subject. The aim of the present study was to compare the elimination of a high dose of amikacin (50 mg/kg) in an ovine model between a population of dialyzed and non-dialyzed sheep. We hypothesized that intermittent hemodialysis may reduce the risk of amikacin nephrotoxicity.

2. Material and methods

This study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals and approved by the Ethics Committee of VetAgro Sup (Campus Vétérinaire de Lyon) with the agreement 1548-V2.

2.1. Animals

Six adult female sheep weighing from 63 to 81 kg were included in this study. A 14-days acclimation period was implemented before the study. Animals were fed with hay *ad libitum* and with alfalfa pellets and given free access to water. All sheep were screened by physical examination, complete blood cell count, serum biochemistry, coproscopy and serologic test for *Brucella* and *Coxiella*.

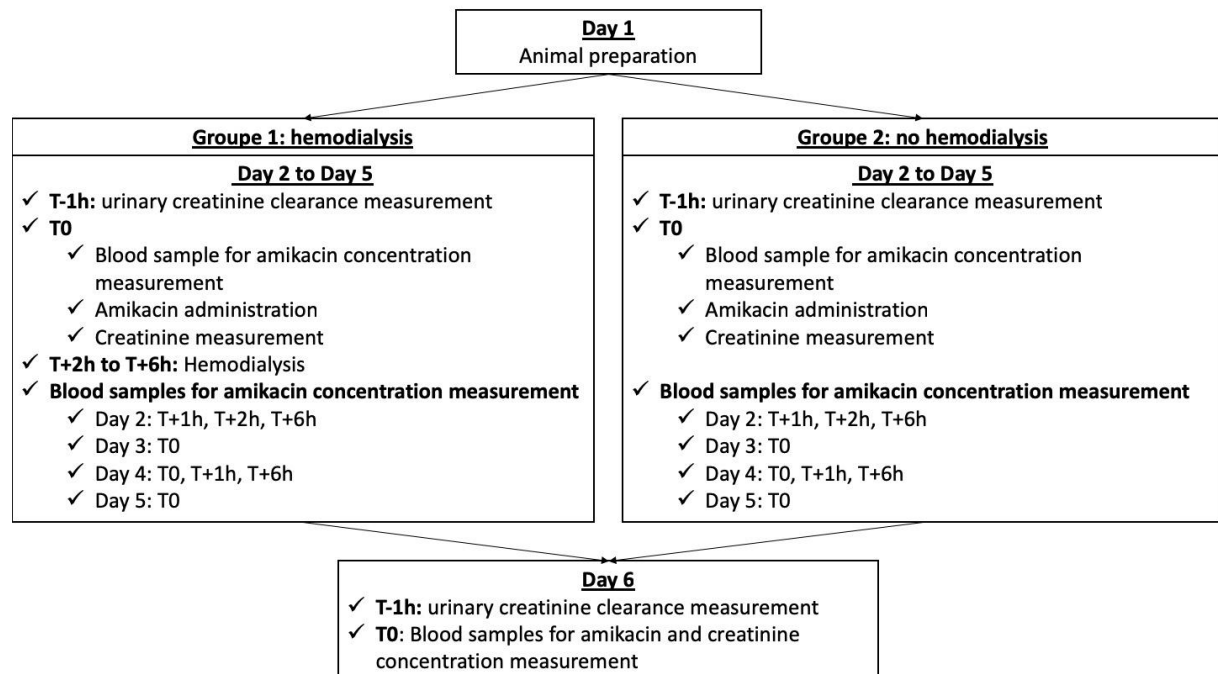
2.2. Animal preparation

Animals were anesthetized with intramuscular injection of xylazine (0.1 mg/kg) and midazolam (0.2 mg/kg), and a 11.5 Fr double lumen catheter (Hemo-cath[®], Medical Components, Harleysville, PA) was placed with the transcutaneous Seldinger technique [13] in the right jugular vein. A 14 CH Foley urinary catheter (Uromedia[®], Euromedis, Neuilly-Sous-Clermont, France) was also placed and a one-day recovery period was then allowed.

2.3. Experimental protocol

The experimental protocol is detailed in Fig. 1. Urine was collected over one hour with the conventional technique [14] before amikacin administration for urinary creatinine clearance calculation every day from day 2 (considered as the reference value) to day 6 [15]. A single 50 mg/kg dose of amikacin was then administered intravenously over a 30-min period through the jugular vein every day from day 2 to day 5. Sheep were divided into two groups: with hemodialysis (group 1, n=3), without hemodialysis (group 2, n=3). In group 1, hemodialysis was initiated 2 h after the beginning of the infusion of amikacin from day 2 to day 5 and was performed during 4 h with a Prismaflex dialyzer unit (Prismaflex[®], Hospal, Meyzieu, France) equipped with a ST100[®] set constitute with an artificial kidney with a AN69ST[®] membrane (Hospal, Meyzieu, France). Blood was pumped at a rate of 160 mL/min. The dialysate fluid flow rate was set at 1200 mL/h. The dialysate solution used was Hemosol B0[®] (Hospal, Meyzieu, France) supplemented with potassium at 4.5 mmol/L. A low ultrafiltration rate was set at 100 mL/h offset by the infusion of a predilution replacement solution. Heparin was used for anticoagulation at 1000 units every hour. In group 2, hemodialysis was not performed.

Figure 1: Study protocol



2.4. Sampling and analytic method

Blood samples were collected from the jugular vein at 1, 2 and 6 h after the beginning of the amikacin infusion during day 2; at 0, 1 and 6 h after the beginning of the infusion during day 4 and at 0 h after beginning the infusion during day 3, 5 and 6. Blood samples were collected in heparinized tubes and were centrifuged. Urine samples were collected every morning for one hour (day 2 to day 6) before amikacin administration and their volume was measured. Five mL of each urine sample were immediately stored at -80°C and protected from light until creatinine analysis. Creatinine was measured with a colorimetric technique realized by Konelab 30[®] (Thermo Fisher Scientific, Waltham, MA). The minimum detectable concentration was 1 mg/L in serum and 20 mg/L in urine. Amikacin was measured by an immunoturbidimetric technique realized by Architect c8000[®] (Abbott Laboratories, Abbott Park, IL). The minimum detectable concentration in serum was 0.6 $\mu\text{g/mL}$.

2.5. Nephrotoxicity and AKI

Comparisons of AUC and C_{min} between the two groups were used to evaluate the impact of hemodialysis on nephrotoxicity. As toxicity is associated with amikacin exposure, the time spent with a concentration greater than 2.5 $\mu\text{g/mL}$ was studied. The French National Agency of Drug Safety recommends not to administer another dose of amikacin if the C_{min} is not below the threshold of 2.5 $\mu\text{g/mL}$ [16]. The AKI was defined by an increase in serum creatinine concentration of $\geq 50\%$ and/or a decrease in the glomerular filtration rate (GFR, evaluated by the urinary creatinine clearance) of $\geq 25\%$ between baseline and last day values.

2.6. Pharmacokinetic analysis

The pharmacokinetic analysis was based on a compartmental approach using a two-compartment model, as described for amikacin in patients with renal replacement therapy [17]. This model was described by a system of ordinary differential equations as follows:

$$dX(1)/dt = -[(K_{12} + K_{\text{el}}) * X(1)] + K_{21} * X(2)$$

$$dX(2)/dt = -K_{21} * X(2) + K_{12} * X(1)$$

Where $X(1)$ is the amount of amikacin in the principal compartment and $X(2)$ is the amount of amikacin in the second compartment. K_{12} and K_{21} are the transfer rate constants and K_{el} is elimination rate constant.

The V_d was linearly linked to the body weight. Elimination is described by renal elimination linked to creatinine clearance, elimination by hemodialysis in sheep of group 1 and non-renal elimination. The analysis was performed using the non-parametric modeling software Pmetrics[®] (LAPKB, Hollywood, CA) [18].

Individual pharmacokinetic parameters were determined by Bayesian estimation for each sheep. Adjusted coefficient of determination, bias (mean weighted prediction error) and imprecision (bias-adjusted mean weighted squared prediction error) of concentration predictions were used to measure predictive performance. The validation of the model was made by Visual Predictive Check (VPC) [19].

The residual error was modeled as a polynomial function (describing the assay error) multiplied by a parameter (gamma) taking into account uncertainties of the clinical environment. Error = $\gamma \times (3.62 + 0.000975Y + 0.003454^2)$, where Y is observed concentration

2.7. Statistical analysis

Statistical analyses were performed using Prism 6[®] software (GraphPad Software, Inc., La Jolla, CA). Continuous variables were expressed as means \pm standard deviation (SD) or median (interquartile range). The value of 0.3 $\mu\text{g/mL}$ was used for amikacin concentration lower than 0.6 $\mu\text{g/mL}$, as non-measurable by the automate. Differences between groups were assessed using the Mann-Whitney U test. A value of $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Pharmacokinetic parameters

The pharmacokinetic parameters are presented in Table 1.

Table 1. Pharmacokinetic parameters

(Cl_r : renal clearance of amikacin (without unit, it must be multiplied by the creatinine clearance), Cl_{cr} : creatinine clearance (mL/kg/min), Cl_d : hemodialysis clearance of amikacin (L/h), V_d : volume of distribution (L/kg), CV: coefficient of variation (%))

	Median	Standard deviation	CV
Cl _r	0.04	0.01	22.55
Cl _{Cr}	2.05	0.58	31.88
Cl _d	2.14	0.48	20.18
V _s	0.19	0.03	17.34

3.2. Predictive performance

The model had good predictive performance: bias of - 0.55 mg/L, imprecision of 3.94 mg²/L² and adjusted coefficient of correlation of 0.94 between predicted and observed amikacin concentrations. These predictive performances were improved after Bayesian estimation of individual pharmacokinetic parameters (bias = - 0.02 mg/L, imprecision = 0.76 mg²/L², adjusted coefficient of correlation = 0.99) (Fig 2).

The model was validated by VPC: only three concentrations were not included in the 95% confidence interval (Fig 3).

Figure 2: Representation of observed versus population predicted concentrations (A), and observed versus individual predicted concentrations (B)

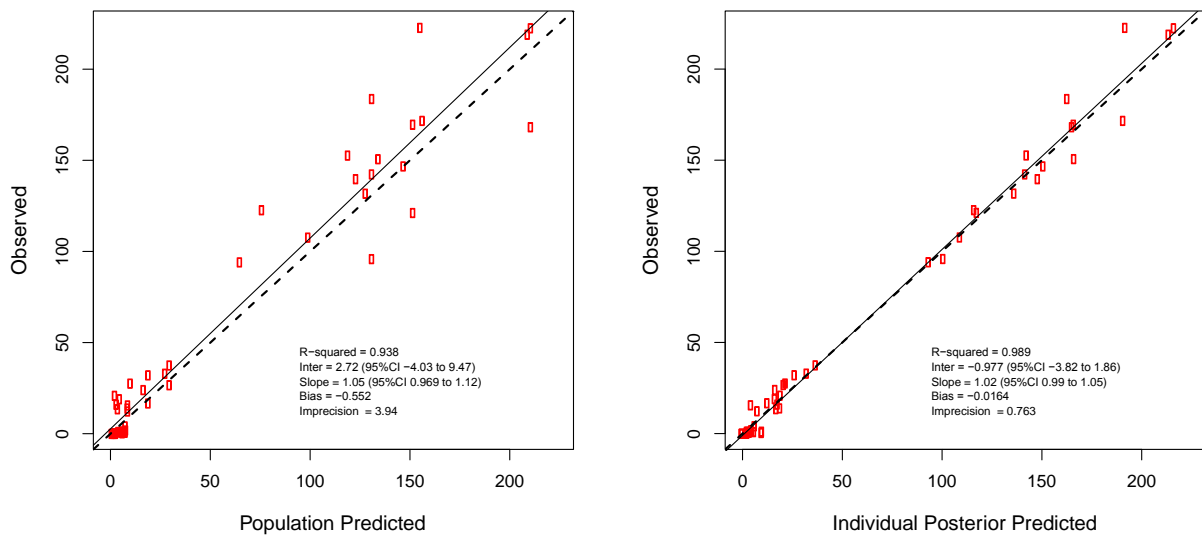
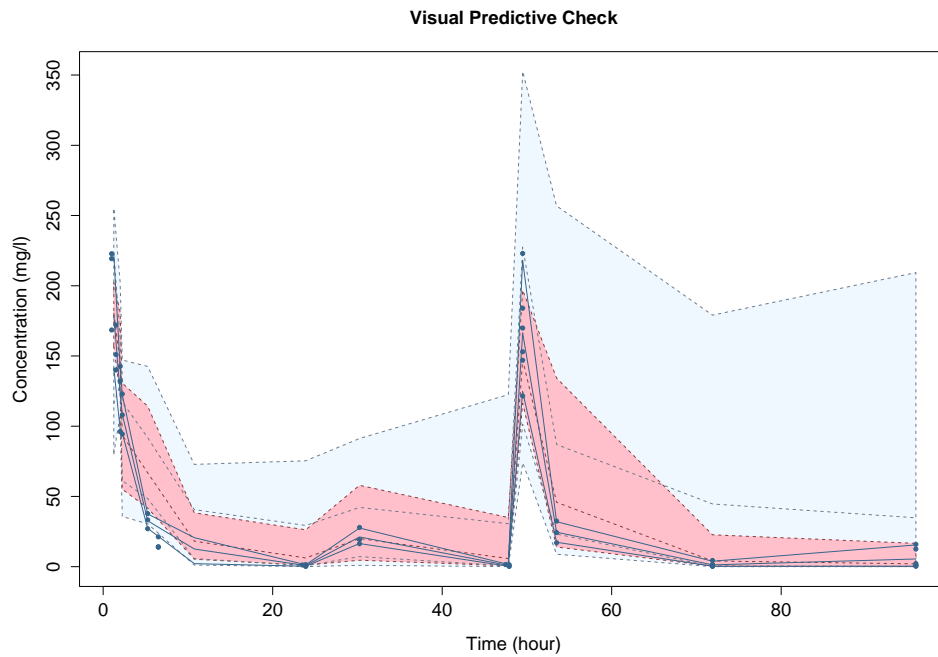


Figure 3: Visual predictive check for the 5th (red) and the 95th percentile (blue)



3.3. C_{max}

The C_{max} predicted by the model were used. The medians were 214.1 $\mu\text{g/mL}$ (208.6 – 272.1) in group 1 and 208.3 $\mu\text{g/mL}$ (165.8 – 214.2) in group 2 ($P = 0.09$) (Table 2).

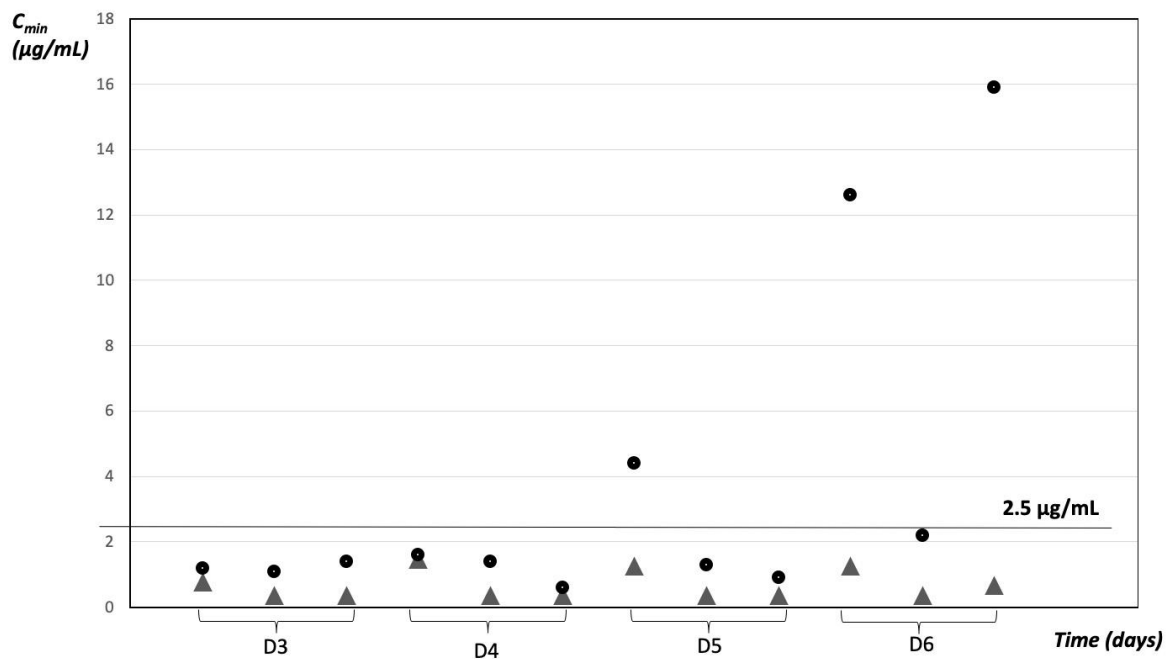
Table 2. Maximal concentrations (C_{max}), minimal concentrations (C_{min}) and area under the time-concentration curve (AUC) of amikacin after the injection of 50 mg/kg.

	Group 1 (hemodialysis)			Group 2 (no hemodialysis)			P
	Median	25 th percentile	75 th percentile	Median	25 th percentile	75 th percentile	
C_{max} ($\mu\text{g/mL}$)	214.1	208.6	272.1	208.3	165.8	214.2	0.09
C_{min} ($\mu\text{g/mL}$)	0.3	0.3	1.1	1.4	1.1	3.9	0.0003
AUC ($\mu\text{g/mL.h}$)	1450	1311	1716	3126	2581	3171	0.10

3.4. Impact of hemodialysis on pharmacokinetic parameters

The median clearance of amikacin by hemodialysis was 2.14 L/h (35.6 mL/min), 16% of the total body clearance for 24 h. The C_{min} was significantly lower in group 1 compared with group 2 (respectively 0.3 $\mu\text{g/mL}$ [0.3 – 1.1] and 1.4 $\mu\text{g/mL}$ [1.1 – 3.9]; $P = 0.0003$). The Figure 4 represents the evolution of C_{min} during the study in both groups: an increase was observed in sheep from group 2 the last two days. The median AUC tended to be lower in group 1 compared with group 2 (respectively, 1450 $\mu\text{g/mL.h}$ [1311 – 1716] versus 3126 $\mu\text{g/mL.h}$ [2581 – 3171]; $P = 0.10$), although this difference did not reach statistical significance. The time with serum amikacin concentration exceeding 2.5 $\mu\text{g/mL}$ was significantly lower in group 1 compared with group 2 (99.7 % [99.7 – 99.8] versus 99.9 % [99.8 – 99.9]; $P = 0.049$).

Figure 4: Minimal concentrations (abscissa: time, D: day; ordinate: C_{min} ($\mu\text{g/mL}$); triangles: group 1 (with hemodialysis), circles: group 2 (without hemodialysis))



3.5. Acute kidney injury

Results of serum creatinine concentration and clearance of urinary creatinine are presented in Table 3. All the serum creatinine concentrations were in the normal range. Some

variations in urinary creatinine clearance were observed during the study. Based on the previously defined criteria, no sheep developed acute kidney injury.

Table 3. Individual creatinine serum concentrations and urinary creatinine clearance measured during the study (D: day of the study, UV: usual values).

Group 1: sheep with hemodialysis, group 2: sheep without hemodialysis.

	Serum creatinine concentration (mg/L, UV [39]: 8 – 20)		1-h urinary creatinine clearance (mL/kg/min, UV [15]: 1.12 – 1.52)	
	Group 1	Group 2	Group 1	Group 2
D2	14.1	8.0	0.7	2.1
D3	13.1	8.4	0.8	1.8
D4	12.9	7.9	1.4	1.0
D5	14.3	7.6	0.6	2.2
D6	13.8	8.0	1.5	1.7
D2	10.3	8.1	2.0	2.2
D3	9.0	8.0	2.2	2.2
D4	9.3	7.3	2.0	1.1
D5	9.7	7.5	1.2	2.1
D6	9.4	7.4	2.5	2.2
D2	12.0	8.2	1.5	2.6
D3	12.6	7.8	1.7	2.3
D4	9.4	7.0	2.1	2.5
D5	9.9	7.2	2.2	2.7
D6	11.3	8.2	1.5	2.4

4. Discussion

This study showed that hemodialysis reduces the C_{min} and time of exposure to a concentration of at least 2.5 $\mu\text{g/mL}$ after the injection of a high dose of amikacin. A trend toward reducing AUC with hemodialysis was also observed. Thus, this technique seems interesting in preventing nephrotoxic risk induced by a high-dose aminoglycoside regimen.

Amikacin has a dose-dependent bactericidal efficacy. The usual therapeutic goal is a C_{max}/MIC ratio ≥ 8 [9], yielding serum concentrations exceeding 64 $\mu\text{g/mL}$ for sensitive strains or greater than 128 $\mu\text{g/mL}$ for strains with intermediate resistance [10]. Pharmacokinetic parameters of hydrophilic antibiotics are strongly altered in patients with sepsis, due to a major increase in the volume of distribution in these patients. This increase seems to be correlated to the severity of sepsis [8]. So higher loading doses may be required to obtain clinically relevant peak concentration values, as is occurring in practice. However, several studies highlight the difficulties encountered in these patients to achieve the therapeutic goals even with doses as high as 15 to 30 mg/kg. In these studies, a serum

concentration of amikacin ≥ 64 $\mu\text{g/mL}$ was obtained in less than 77% of cases [4,5,8,11]. That is why we chose a 50-mg/kg amikacin dose. In the current study, we obtained high peaks, with median C_{max} close to 210 $\mu\text{g/mL}$. Nevertheless, the volume of distribution of amikacin in sheep was 0.2 L/kg, comparable to that described in the literature [20,21], but half that of the mean volume of distribution of a human patient in ICU [4,5,7,11]. For an increased volume of distribution in a critically ill patient, the dose of 50 mg/kg would provide a median C_{max} of about 100 $\mu\text{g/mL}$, higher than 64 $\mu\text{g/mL}$ and relatively close to the second therapeutic goal. It therefore seems appropriate to use that dose in clinical practice for the treatment of infection caused by bacteria with a MIC of 8 $\mu\text{g/mL}$. The administration of higher doses of amikacin, associated with longer sessions of hemodialysis should be considered for intermediate resistance strains.

All renal replacement techniques are efficient for the elimination of amikacin from the blood. Continuous hemodiafiltration gave an amikacin clearance of 40 mL/min or around 89% of the mean total body clearance [22]; intermittent hemodialysis gave a clearance of 37.5 mL/min amikacin, approximately 21% of total clearance (for hemodialysis sessions of 3 to 4 hours) [23]. In the current study, the clearance of amikacin related to hemodialysis was very close to values found in the literature: clearance of 35.6 mL/min or 14% of total clearance for sessions of 4 h. The hydrophilic nature of amikacin and its low protein binding fraction make possible removal with renal replacement therapy by diffusion, convection and adsorption on the membranes of the artificial kidney [22–24]. Few studies exist regarding the removal of amikacin by different renal replacement therapy so it is difficult to determine which technique is the most effective. Some authors emphasize the superiority of hemodiafiltration over hemodialysis for the treatment of renal failure [25]. However, comparison of studies is complex, considering the differences between the renal replacement therapy parameters in the studies, as the elimination of amikacin seems to be correlated with these parameters [26]. The choice of hemodialysis in our study is based on literature and the technical expertise of our team: although some studies show a reduction of side effects with hemofiltration or hemodiafiltration, this benefit remains to be confirmed [27–29]. In addition, there is no consensus on the best choice of renal replacement therapy, and all are still used today [3,22,26]. The choice of intermittent sessions versus continuous renal replacement therapy was a technical choice: continuous renal replacement therapy could be technically complex in animals. Besides, continuous techniques have not shown their superiority over intermittent sessions [30,31].

The accumulation of aminoglycosides in renal tubular cells is responsible for their nephrotoxicity and limits their use, especially in critically ill patients [32]. The toxicity is correlated to the exposure, expressed as C_{\min} or AUC [12]. In the current study, even with this small sample, the C_{\min} and time spent above a concentration $>2.5 \mu\text{g/mL}$ were significantly lower in the dialyzed sheep. The AUC also tended to be lower in this group. These results demonstrate the effect of hemodialysis in the prevention of nephrotoxicity. Similar findings were described in a retrospective study [3] and a case report [6]. Despite the administration of high doses of aminoglycosides, C_{\min} remained low. There was no direct link between C_{\max} and toxicity: indeed, renal accumulation was saturated when the serum concentration of amikacin was exceeding $15 \mu\text{g/mL}$ [6]. So theoretically, there is no limitation to increase the administered dose of amikacin for a patient if elimination is increased, which is possible with renal replacement therapy. As simulations show that to obtain a satisfactory clinical response against bacteria with $\text{MIC} = 16 \mu\text{g/mL}$, the administered doses of amikacin caused AKI in 100% of the patients [32], combination with renal replacement therapy seems to be a good clinical choice.

No sheep developed AKI in our study. This observation could be explained by the short period of experimentation over which the study was conducted and by the absence of hemodynamic alterations in healthy sheep. The occurrence of AKI induced by aminoglycosides correlates with the duration of treatment in humans [33]. Acute kidney injury may appear after more than 5 to 7 days of treatment [34]. In an experimental model of AKI induced by the administration of high doses of gentamicin in healthy dogs, 16 days of treatment were needed to observe the occurrence of AKI diagnosed with an increase of $\geq 50\%$ in serum creatinine concentration [35]. In that study, the measurement of serum creatinine concentration did not seem to be the optimal early biomarker to identify AKI induced by aminoglycosides [31]. Indeed, neutrophil gelatinase-associated urinary lipocalin (NGAL) was able to diagnose AKI over a week before the increase in serum creatinine [35].

The current study has several limitations. First, the small number of animals limited the conclusion of this study. In particular, although the median AUC was clearly lower in dialyzed sheep, this difference did not reach statistical significance probably because of lack of power. Experimentation with large animals is difficult and costly, limiting the use of more animals. However, using a mathematical model allowed increasing the amount of

data without increasing the number of blood samples, which is an important ethical concern. The choice of ovine model can also be discussed. It has been chosen for several reasons. First, sheep are very calm animals. So unlike other animal models, it is not necessary to anesthetize them for the hemodialysis sessions. This is an advantage because anesthesia is known to induce changes in renal perfusion, which could make the interpretation of the results more difficult [36,37]. Second, our team has much clinical experience with hemodialysis in sheep. However, this model has some limitations. There is no information in the literature about the nephrotoxicity of amikacin in sheep. In addition, sheep have rapid elimination [20,21] compared with critically ill patients [38], leading to low accumulation of amikacin in blood after several days of treatment. In this regard, the healthy sheep model is not fully representative of the pharmacokinetics of amikacin observed in ICU patients.

5. Conclusion

The current study shows, in a sheep model, that hemodialysis reduces C_{min} , exposure time to amikacin and AUC after injection of high dose of amikacin (50 mg/kg), responses that are known to be involved in the risk of nephrotoxicity of amikacin. Renal replacement therapy sessions may thus be useful in preventing kidney failure when treating infections with multidrug-resistant Gram-negative bacteria, with intermediate sensitivity to amikacin, requiring the administration of high doses of amikacin. Further study is required to evaluate this technique in ICU patients.

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