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

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ABSTRACT

Changes in pharmacokinetic parameters of critically 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 haemodialysis to reduce the exposure to high dose amikacin and prevent nephrotoxicity. Amikacin 50 mg/kg was administered intravenously to six adult sheep once-daily for four days. The sheep were divided into two groups according to the implementation (group 1) or not (group 2) of haemodialysis. In group 1, haemodialysis was performed for 4 h, initiated 2 h 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 haemodialysis amikacin clearance was 2.14 L/h (35.6 mL/min), 14% of the mean total body clearance for 24 h. Haemodialysis reduced C_{min} (group 1: 0.3 μg/mL [0.3–1.1]; group 2: 1.4 μg/mL [1.1–3.9]; $P = 0.0003$). A trend towards reduced AUC with haemodialysis was observed (group 1: 1450 μg/mL·h [1311–1716]; group 2: 3126 μg/mL·h [2581–3171]; $P = 0.10$). In conclusion, haemodialysis seems interesting in reducing AUC and C_{min} after the injection of high-dose of amikacin, parameters known to be involved in its induced nephrotoxicity, in an experimental ovine model.

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 involving Gram-negative pathogens [3–8]. Among the aminoglycosides, amikacin is a concentration-dependent antibiotic commonly prescribed in ICU patients. 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 clinical breakpoint of *Enterobacteriaceae* and *Pseudomonas* spp. are 8 μg/mL for sensitive strains and 16 μg/mL for intermediate strains [10], indicating that to improve the antibacterial activity, C_{max} should reach plasma concentrations ≥64 μg/mL or ≥128 μg/mL.

In critically ill patients, pharmacokinetic parameters are impaired, 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 ≤30 mg/kg, a C_{max} of ≥64 μg/mL is reached in less than 77% of patients [4,5,8,11]. Doses higher than

Abbreviations: AKI: acute kidney injury; AUC: area under the curve; CL_{cr}: creatinine clearance; CL_d: haemodialysis 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.

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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. The area under the time–concentration curve (AUC) and trough concentration (C_{min}) are pharmacokinetic parameters quantifying this exposure. Increasing the amikacin dose will increase these pharmacokinetic parameters, implying an increased risk of renal toxicity [12]. With a dose of 25 mg/kg, a C_{min} of >5 μg/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 amikacin [5]. Survivors had a C_{min} significantly lower than that of non-survivors [5].

The use of renal replacement therapy (RRT) 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 favourable clinical response in 8 of 15 patients with MDR-induced sepsis [3].

Despite medical and economical concerns, only few data are 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 dialysed and non-dialysed sheep. We hypothesized that intermittent haemodialysis may reduce the risk of amikacin nephrotoxicity.

2. Materials 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 63–81 kg were included in this study. A 14-day 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 anaesthetized with intramuscular injection of xylazine (0.1 mg/kg) and midazolam (0.2 mg/kg), and an 11.5Fr 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 (of the actual body weight) dose of amikacin was then administered intravenously over a 30-min period through the jugular vein every day from day 2 to day 5. The sheep were divided into two groups: with haemodialysis (group 1, n = 3) and without haemodialysis (group 2, n = 3). In group 1, haemodialysis was initiated 2 h after the beginning of the infusion of amikacin from day 2 to day 5 and was performed for 4 h with a Prismaflex® dialyser unit (Gambro Hospal, Meyzieu, France) equipped with a ST100® set constitute with an artificial kidney with a AN69ST® membrane (Gambro 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® (Gambro 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, haemodialysis was not performed.

2.4. Sampling and analytical 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;

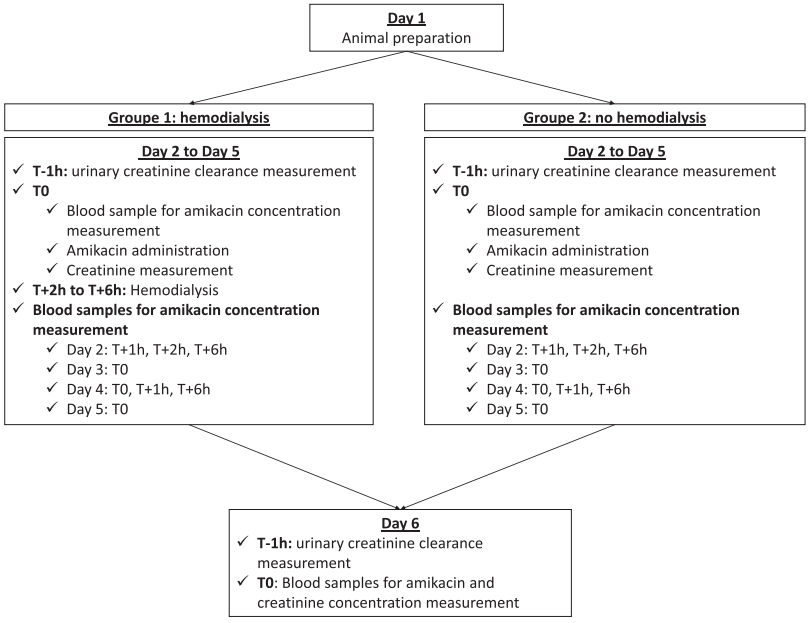

Fig. 1. Study protocol.

at 0, 1 and 6 h after the beginning of the infusion during day 4 and at 0 h after the beginning of the infusion during days 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 millilitres 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 µg/mL.

2.5. Nephrotoxicity and AKI

Comparisons of AUC and C_{min} between the two groups were used to evaluate the impact of haemodialysis on nephrotoxicity. As toxicity is associated with amikacin exposure, the time spent with a concentration greater than 2.5 µg/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 µg/mL [16]. The AKI was defined by an increase in serum creatinine concentration of ≥50% and/or a decrease in the glomerular filtration rate (GFR, evaluated by the urinary creatinine clearance) of ≥25% between baseline and last day values [17].

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 [18]. This model was described by a system of ordinary differential equations as follows:

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

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

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 the elimination rate constant.

A linear relationship was introduced between the V_d and the actual body weight. Elimination is described by renal elimination linked to creatinine clearance, elimination by haemodialysis in sheep of group 1 and non-renal elimination. The analysis was performed using the non-parametric modelling software Pmetrics® (LAPKB, Hollywood, CA) [19].

Individual pharmacokinetic parameters were determined by Bayesian estimation for each sheep. The individual Bayesian posteriors were calculated using the population joint density obtained with Pmetrics as a prior. 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) [20].

The residual error was modelled as a polynomial function (describing the assay error) multiplied by a parameter (γ) taking into account uncertainties of the clinical environment. Error = $\gamma \times (3.62 + 0.000975Y + 0.0.003454^2)$, where Y is the 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 mean ± standard deviation (SD) or median (interquartile range). The value of 0.3 µg/mL was used for amikacin concentration lower than 0.6 µg/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.

3.2. Predictive performance

The model had good predictive performance: bias of –0.55 mg/L, imprecision of 3.94 mg^2/L^2 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 mg^2/L^2, 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).

3.3. C_{max}

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

3.4. Impact of haemodialysis on pharmacokinetic parameters

The median clearance of amikacin by haemodialysis was 2.14 L/h (35.6 mL/min), 14% of the total body clearance for 24 h. The C_{min} was significantly lower in group 1 compared with group 2 (respectively 0.3 µg/mL [0.3–1.1] and 1.4 µg/mL [1.1–3.9]; $P = 0.0003$). Fig. 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. Unlike in group 2, no case of C_{min} greater than the toxic level of 2.5 µg/mL was observed in group 1.

The median AUC tended to be lower in group 1 compared with group 2 (respectively, 1450 µg/mL·h [1311–1716] versus 3126 µg/mL·h [2581–3171]; $P = 0.10$), although this difference did not reach statistical significance. The time with serum amikacin concentration exceeding 2.5 µg/mL was significantly lower in group 1 compared with group 2 (99.7% [99.7–99.8] versus 99.9% [99.8–99.9]; $P = 0.049$).

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

Table 1
Pharmacokinetic parameters.

	Median	Standard deviation	CV
CL_r	0.04	0.01	22.6
CL_{cr}	2.05	0.58	31.9
CL_d	2.14	0.48	20.2
V_d	0.19	0.03	17.3

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, haemodialysis clearance of amikacin (L/h); V_d, volume of distribution (L/kg); CV, coefficient of variation (%).

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

	Group 1 (haemodialysis)			Group 2 (no haemodialysis)			*P*
	Median	25th percentile	75th percentile	Median	25th percentile	75th percentile	
C_{max} (μg/mL)	214.1	208.6	272.1	208.3	165.8	214.2	0.09
C_{min} (μg/mL)	0.3	0.3	1.1	1.4	1.1	3.9	0.0003
AUC (μg/mL·h)	1450	1311	1716	3126	2581	3171	0.10

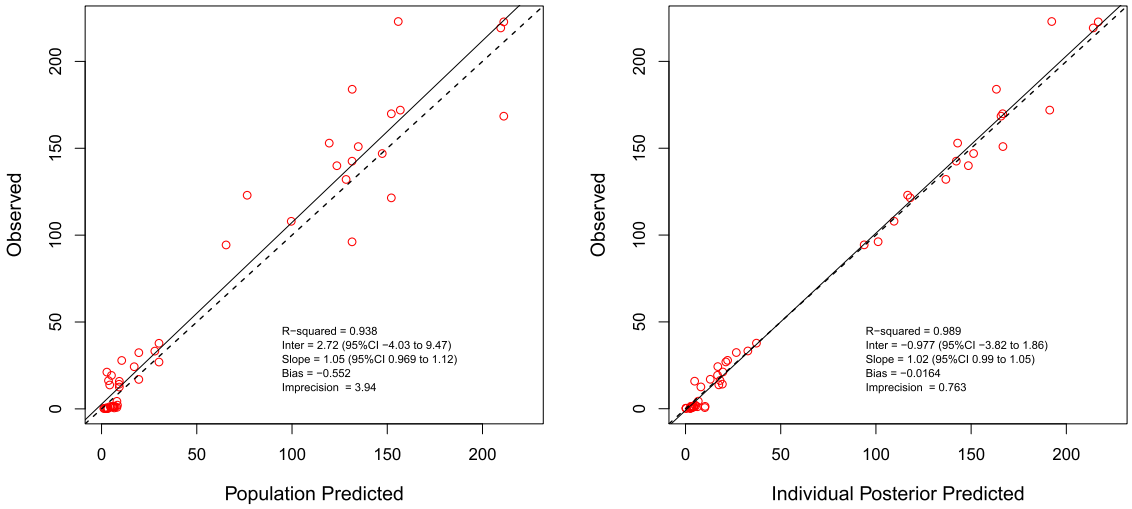

Fig. 2. Representation of observed versus population predicted concentrations (A), and observed versus individual predicted concentrations (B).

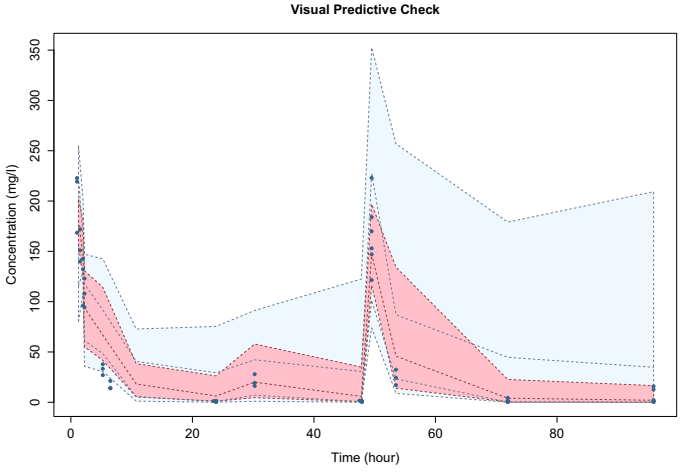

Fig. 3. Visual predictive check for the 5th (red) and the 95th percentiles (blue).

creatinine clearance were observed during the study. Based on the previously defined criteria, no sheep developed AKI.

4. Discussion

In the current study, haemodialysis increased the elimination of a high dose of amikacin in an ovine model. This effect is illustrated by the significant reduction in the C_{min} and time of exposure to a concentration exceeding 2.5 μg/mL. A trend towards reducing AUC with haemodialysis was also observed. Since these parameters are considered as predictive of toxicity [12], haemodialysis seems an interesting technique in the prevention of nephrotoxicity induced by a high-dose aminoglycoside regimen.

Table 3
Individual creatinine serum concentrations and urinary creatinine clearance measured during the study (D: day of the study, UV: usual values, IQR: interquartile). Group 1: sheep with haemodialysis; group 2: sheep without haemodialysis.

	Serum creatinine concentration (mg/dL, UV [21]: 8–20)		1-h urinary creatinine clearance (mL/kg/min, UV [15]: 1.12–1.52)	
	Group 1	Group 2	Group 1	Group 2
	Sheep A	***Sheep D***	***Sheep A***	***Sheep D***
D2	1.41	0.80	0.7	2.1
D3	1.31	0.84	0.8	1.8
D4	1.29	0.79	1.4	1.0
D5	1.43	0.76	0.6	2.2
D6	1.38	0.80	1.5	1.7
	Sheep B	***Sheep E***	***Sheep B***	***Sheep E***
D2	1.03	0.81	2.0	2.2
D3	0.90	0.80	2.2	2.2
D4	0.93	0.73	2.0	1.1
D5	0.97	0.75	1.2	2.1
D6	0.94	0.74	2.5	2.2
	Sheep C	***Sheep F***	***Sheep C***	***Sheep F***
D2	1.20	0.82	1.5	2.6
D3	1.26	0.78	1.7	2.3
D4	0.94	0.70	2.1	2.5
D5	0.99	0.72	2.2	2.7
D6	1.13	0.82	1.5	2.4
Median (IQR)	1.13 (0.94–1.31)	0.79 (0.74–0.81)	1.5 (1.2–2.1)	2.2 (1.8–2.4)

Infections by strains of *Enterobacteriaceae* and *Pseudomonas* spp. with intermediate resistance to amikacin require high serum concentration of antibiotics [10]. The pharmacokinetic parameters of hydrophilic antibiotics are strongly altered in patients with sepsis because of a major increase in V_d in these patients. This increase seems to be correlated with the severity of sepsis [8]. So, higher loading doses may be required to obtain clinically relevant peak

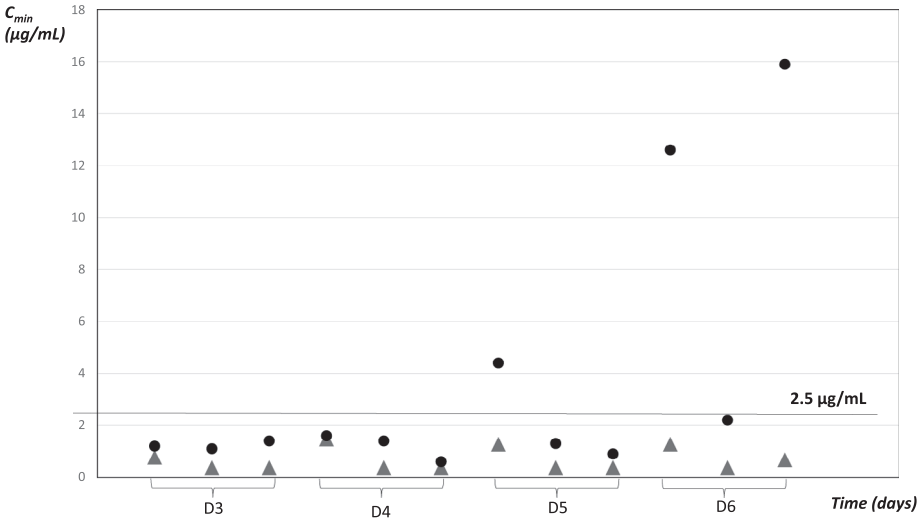

Fig. 4. Minimal concentrations (abscissa: time, D: day; ordinate: C_{min} (µg/mL); triangles: group 1 (with haemodialysis), circles: group 2 (without haemodialysis)).

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. Serum concentration of amikacin ≥64 µg/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 µg/mL. Nevertheless, the V_d of amikacin in sheep was 0.2 L/kg, comparable to that described in the literature [22,23], but half of the mean V_d of a human patient in ICU [4,5,7,11]. For a V_d comparable to that of a human patient in ICU, the dose of 50 mg/kg would provide a median C_{max} of about 100 µg/mL, higher than 64 µg/mL and relatively close to the second therapeutic goal.

All renal replacement techniques are efficient for the elimination of amikacin from the blood. Continuous haemodiafiltration gave an amikacin clearance of 40 mL/min or around 89% of the mean total body clearance [24]; intermittent haemodialysis gave a clearance of 37.5 mL/min amikacin, approximately 21% of the total clearance (for haemodialysis sessions of 3 to 4 hours) [25]. In the current study, the clearance of amikacin related to haemodialysis was very close to the values found in the literature: clearance of 35.6 mL/min or 14% of the total clearance for sessions of 4 h. The hydrophilic nature of amikacin and its low protein binding fraction make removal with RRT possible by diffusion, convection and adsorption on the membranes of the artificial kidney [24–26]. 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 haemodiafiltration over haemodialysis for the treatment of renal failure [27]. However, the comparison between studies is complex, considering the differences between RRT parameters in the studies, as the elimination of amikacin seems to be correlated with these parameters [28]. Our choice of haemodialysis is based on literature and on the technical expertise of our team: although some studies have shown a reduction in side effects with haemofiltration or haemodiafiltration, this benefit remains to be confirmed [29–31]. In addition, there is no consensus on the best RRT method, and all are still currently used [3,24,28]. The choice of intermittent sessions versus continuous RRT was made because continuous RRT may be technically complex in animals. Besides, continuous techniques have not shown their superiority over intermittent sessions [32,33].

The accumulation of aminoglycosides in renal tubular cells is responsible for their nephrotoxicity and limits their use, especially in critically ill patients [34]. The toxicity is correlated with 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 µg/mL were significantly lower in the dialysed sheep. The AUC also tended to be lower in this group. These results suggest a positive effect of haemodialysis in the prevention of amikacin 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 µg/mL [6]. So theoretically, there is no limitation to increase the administrated dose of amikacin for a patient if elimination is increased, which may be obtained with RRT. As simulations show that to obtain a satisfactory clinical response against bacteria with MIC = 16 µg/mL, the administered doses of amikacin caused AKI in 100% of the patients [34], combination with RRT, may be a good clinical choice.

No sheep developed biological 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 haemodynamic alterations in healthy sheep. The occurrence of AKI induced by aminoglycosides correlates with the duration of treatment in humans [35]. Acute kidney injury may appear after more than five to seven days of treatment [36]. In an experimental model of AKI induced by the administration of high doses of gentamicin in healthy dogs, sixteen days of treatment were needed to observe the occurrence of AKI diagnosed with an increase of ≥50% in serum creatinine concentration [37]. In that study, the measurement of serum creatinine concentration did not seem to be the optimal early biomarker to identify AKI induced by aminoglycosides [32]. Indeed, neutrophil gelatinase-associated urinary lipocalin (NGAL) was able to diagnose AKI over a week before the increase in serum creatinine [37]. Post-mortem examination of the kidney could have help to diagnose renal injury.

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 dialysed 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 anaesthetize them for the haemodialysis sessions. This is an advantage because anaesthesia is known to induce changes in renal perfusion, which could make the interpretation of the results more difficult [38,39]. Second, our team has much clinical experience with haemodialysis in sheep. However, this model has some limitations. There is no information in the literature about the nephrotoxicity of amikacin in sheep. We observed variations in urinary creatinine clearance.

These variations limited the diagnostic of AKI. They were most likely related to urine losses that passed by the urinary catheters. Post-mortem examination of the kidney could have help to diagnose renal injury, although this was not performed in the current study. In addition, sheep have rapid elimination [22,23] compared with critically ill patients [40], 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

Haemodialysis reduces C_{min}, exposure time to amikacin and AUC after injection of high dose of amikacin (50 mg/kg) in an ovine model, 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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Competing interests: None.

Ethical approval: 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 with the agreement 1548-V2.

Appendix

Pharmacokinetic parameters after injection of 7.5 mg/kg intravenously of amikacin in 5 sheep [22].

Parameter	Mean	Standard deviation
C_{max} (μg/mL)	146.6	45.2
Total body clearance (mL/min/kg)	0.7	0.06
V_d (L/kg)	0.2	0.03
AUC (μg/mL·min)	11,018.6	2,321

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