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Ampicillin pharmacokinetics in azotemic and healthy dogs

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Abstract

Background: Little is known about effects of factors such as kidney disease, affecting ampicillin pharmacokinetics in dogs.

Objectives: Determine the pharmacokinetics of ampicillin after a single intravenous dose in healthy and azotemic dogs.

Animals: Nine dogs presenting with acute kidney injury and 10 healthy dogs.

Methods: This was a prospective study. An ampicillin dose of 22.2 mg/kg (mean dose) was administered once intravenously. Blood samples were obtained at timed intervals (just before administration, 1, 2, 4, 12, and 24 hours), analyzed using high-pressure liquid chromatography followed by pharmacokinetic analysis of the plasma drug concentrations.

Results: Peak ampicillin concentration (mcg/mL; 97.07 (36.1) vs 21.3 (50.26)), P<.001 (geometric mean (coefficient of variation, CV%)), half-life (hours; 5.86 (56.55) vs 0.97 (115.3)), P<.001) and AUC (h × mcg/mL; 731.04 (83.75) vs 33.57 (53.68)), P<.001) were greater in azotemic dogs than in healthy dogs. Azotemic dogs also had significantly lower clearance (30.06 (84.19) vs 655.03 (53.67); mL/kg h, P < .001) and volume of distribution (253.95 (30.14) vs 916.93 (135.24); mL/kg, P <.001) compared to healthy dogs.

Conclusion and Clinical Importance: Increased drug concentrations and slower clearance of ampicillin in azotemic dogs could have clinical importance in contributing to antibiotic associated morbidity requiring indicating the need to adjust ampicillin dosing in dogs with decreased kidney function.

KEYWORDS
acute kidney injury, drug concentration, half-life, plasma clearance, volume of distribution

1 | INTRODUCTION

Ampicillin, a semisynthetic aminopenicillin, is commonly used in acutely azotemic dogs for the empirical treatment of the bacteremic phase of leptospirosis or for pyelonephritis.

Aminopenicillins are excreted from the body by the kidneys, primarily via renal tubular secretion, and partially via glomerular filtration. Aminopenicillins have low protein binding and when administered PO

Abbreviations: λ_z, terminal slope; AUC, area under the curve; CL, clearance; CLSI, Clinical and Laboratory Standards Institute; Cn, last measured concentration point; GFR, glomerular filtration rate; VD, volume of distribution; LOQ, limit of quantification.

produce extracellular tissue fluid concentrations that are similar to unbound concentrations in plasma, but concentrations in urine are much higher, exceeding 300 mcg/mL, in healthy dogs.[1-3]

Penicillins have a high index of safety in animals and are associated with few adverse effects in dogs. Adverse events reported in humans with administration of penicillins include central nervous system signs (seizures and encephalopathy) as well as acute interstitial nephritis.[4,5]

Kidney disease can have wide-ranging effects on drug disposition, including changes in drug clearance, protein binding, and drug metabolism.[6] Drugs that are excreted by renal clearance accumulate in patients with kidney disease and dysfunction if the dosage is not modified.[7-9] More than 80% of a single dose of ampicillin is excreted by the kidneys.[3] Kidney disease and acute kidney injury in dogs is detected using serum creatinine concentration, among other criteria. Although increases in serum creatinine concentration are associated with low glomerular filtration rate (GFR) in dogs,[10] it is an imperfect clinical surrogate to predict drug clearance, and decreased GFR might not predict clearance for drugs that undergo tubular secretion.[11,12] However, serum creatinine concentration is the most often surrogate measure used to stage kidney disease in dogs. Information for animals with a decrease in GFR on drug elimination in dogs is sparse.[7]

Ampicillin was chosen for our study because it has a wide therapeutic range, a low incidence of toxicity, is available for intravenous administration, and is a common choice in acutely azotemic dogs for the treatment of leptospirosis and often administered to dogs, either alone or in combination with sulbactam (Unasyn) as empirical treatment for dogs with infections suspected to be caused by bacteria susceptible to ampicillin.

The objective of our study was to determine the plasma clearance of ampicillin after a single IV dose in healthy dogs, compared to dogs with documented renal azotemia. We selected a dose of 22 mg/kg because this is the FDA-approved dose for dogs, and is the dose used by the Clinical and Laboratory Standards Institute (CLSI)[13] to determine susceptibility testing clinical breakpoints for dogs. We hypothesized that this dose administered IV to the azotemic dogs would produce high plasma ampicillin concentrations and prolonged increased concentrations compared to that in healthy dogs.

2 | MATERIALS AND METHODS

2.1 | Patients

Nine client-owned dogs with documented kidney disease were prospectively enrolled from October 2010 to April 2011. Ten healthy dogs were also enrolled over that same period. Healthy dogs were selected from employee or student owned pets on a volunteer basis. Informed consent was obtained for all participants. This study was approved by the Institutional Animal Care and Use Committee.

The inclusion criteria for healthy dogs was a normal physical examination, no known history of kidney disease or nephrotoxicosis, and a normal kidney biochemical profile with a urine specific gravity ≥1.030 at the time of enrollment. Dogs presenting to the Hospital for Small Animals at Cummings School of Veterinary Medicine with evidence of acute or acute on chronic kidney injury were eligible for enrollment into the study group. We included dogs with known chronic kidney disease if they presented for an acute exacerbation of their azotemia. Renal azotemia was defined as a serum creatinine concentration of ≥4.0 mg/dL and a urine specific gravity of >1.008 and <1.020. In dogs with known chronic kidney disease, an increase in serum creatinine concentrations by >25% as compared with previous baseline values within the past 3 months as well as acute progression of clinical signs was considered eligible for enrollment. Dogs with a packed cell volume of less than 30% at presentation and those weighing less than 4.5 kg were not included due to concern regarding volume of blood required for pharmacokinetic sampling. Dogs with a serum albumin of less than 1.5 g/dL were also excluded. Other exclusion criteria included dogs that were administered fluids parenterally before documentation of urine specific gravity, as well as dogs receiving diuretics, dopamine, fenoldopam, or corticosteroids within 48 hours before enrollment. Anuric dogs were excluded.

Azotemic dogs were medically managed by the primary clinician assigned to the case, not necessarily the study investigators. Fluids were administered intravenously based on the individual needs of the dog as judged by the attending clinician. Other treatments consistent with the management of acute kidney injury (AKI) in our hospital included administration of antiemetics, antacids, phosphate binders, antihypertensive drugs, antithrombotic agents, and prokinetic medication.

2.2 | Treatment design and sample analysis

Each dog was weighed at the time of enrollment and a dose of 22 mg/kg of ampicillin was administered intravenously on day 1 of presentation to the hospital for treatment after diagnosis of kidney disease in the diseased dogs or at the time of enrollment in the healthy dogs. Ampicillin sodium was reconstituted according to the manufacturer's label directions and the total volume was infused slowly through an IV catheter over 15 to 20 minutes. Two milliliters of blood were collected from direct venipuncture or through an intravenous sampling catheter that was not used to administer the ampicillin just before administration of drug and at 1, 2, 4, 12, and 24 hours after administration of drug. Centrifuged plasma samples were separated and frozen at −80°C for submission to the North Carolina State University laboratory for measurement of ampicillin concentrations using a validated high-pressure liquid chromatography assay from the laboratory.[14]

2.3 | Pharmacokinetic analysis

Concentrations for ampicillin after the IV dose were analyzed using noncompartmental analysis and a pharmacokinetic program (Phoenix WinNonlin, Version 6.4.0.768. 2 Certara, St. Louis, Missouri). The area under the plasma concentration vs time curve (AUC) from time 0

TABLE 1 Values of healthy and azotemic dogs

	Healthy group (n = 10)	Azotemic group (n = 9)	*P* value <.05
Age (y)	3.2 (1-11.3)	8.25 (1.5-11.6)	.1
Weight (kg)	15 (7-46.5)	24.4 (8-47.2)	.2
Blood urea nitrogen (mg/dL)	16 (11-35)	105 (66-217)	**<.001**
Creatinine (mg/dL)	0.95 (0.7-1.4)	7.2 (4.2-9.3)	**<.001**
Phosphorus (mg/dL)	4.05 (3-4.4)	10.9 (5.4-24.7)	**<.001**
Albumin (g/dL)	3.6 (3.1-3.9)	2.8 (1.6-3.6)	**.001**

Note: Significance is $P < .05$. Healthy dogs N = 10, azotemic dogs N = 9. Median and ranges. Bold terms are highlighting p values that met significance (<0.05).

to the last measured concentration (defined by the limit of quantification, LOQ) was calculated using the log-linear trapezoidal method. The AUC from time 0 to infinity was calculated by adding the terminal portion of the curve to the $AUC_{0\text{-}cn}$, estimated from the relationship Cn/λ_Z, where λ_Z is the terminal slope of the curve, and Cn is the last measured concentration point. Mean residence time, systemic clearance (CL), and apparent volume of distribution (VD) were calculated using statistical moment theory according to methods described by Gilbaldi and Perrier.[15]

2.4 | Statistical analysis

The pharmacokinetic data were presented as the geometric mean and coefficient of variation (CV%). Statistical differences in pharmacokinetic parameters between groups were assessed using the Mann-Whitney *U* test. Assessment for correlation between clearance and patient serum creatinine was performed using Spearman's rank correlation. Significance was set at $P < .05$.

Statistical analysis was performed with Microsoft Excel (version 1908) and the statistical program SAS Statistical Software (version 9.4: SAS Institute, Cary, North Carolina).

3 | RESULTS

Ten healthy dogs were recruited for the control group including 3 intact males, 1 castrated male, and 6 spayed females. The median age of this group was 3.2 years (1-11.3). The study group consisted of 9 dogs of which 4 were castrated males and 5 were spayed females. The median age of the study group was 8.25 years (1.5-11.6). There was no significant difference in age between the groups ($P = .1$). The median weight of the control group was 15 kg (7-46.5) and of the study group was 24.4 kg (8-47.2). There was no significant difference in weight between the groups ($P = .2$).

Six dogs in the azotemic group were diagnosed with protein losing nephropathy, 2 were suspected of having leptospirosis, and 1 with a history of chronic kidney disease of unknown etiology presented with an acute exacerbation (an increase in creatinine and clinical signs attributed to azotemia). Median serum creatinine in the control group was 0.95 mg/dL (0.7-1.4) vs 7.2 mg/dL (4.2-9.3) in the study group; these were significantly different ($P \leq .001$) (Table 1).

3.1 | Pharmacokinetics

The mean ampicillin concentration at each collection interval was significantly lower for the healthy dogs vs the azotemic dogs ($P = .006$) (Figure 1). The peak ampicillin concentration for the azotemic dogs was 4.55 times greater than the healthy dogs, CV% 36.10 ($P \leq .001$). Plasma half-life was significantly longer by (6× higher) in the azotemic dogs compared to the healthy control dogs ($P \leq .001$). The apparent volume of distribution and clearance of ampicillin were also significantly lower in the azotemic dogs ($P \leq .001$ for each). Ampicillin clearance was significantly lower (21.79 times) in the azotemic dogs compared to the healthy dogs ($P \leq .001$). The AUC (0 to infinity) was significantly greater (21.77 times) in azotemic dogs compared to healthy dogs ($P \leq .001$) (Table 2). LOESS graphs, a nonparametric technique using local weighted regression to fit a smooth curve through points in a scatter plot, are presented for creatinine versus ampicillin half-life for healthy and azotemic dogs in Figure 2 and for creatinine vs ampicillin clearance for both groups of dogs in Figure 3.

4 | DISCUSSION

Our study evaluated the pharmacokinetics of ampicillin after intravenous administration of 22 mg/kg to a group of dogs with severe azotemia (creatinine >4 mg/dL) as compared to healthy dogs. There was a significant difference in peak concentration, half-life, clearance, volume of distribution, and AUC between these groups. All dogs in the azotemic group achieved higher maximum plasma concentrations than the healthy dogs and they maintained measurable plasma concentrations of ampicillin for the full 24-hour period. The azotemic dogs all had VDs that were significantly less than the healthy dogs, which likely reflects dehydration had not been corrected during the initial treatment, even though fluid treatment administered intravenously was ongoing. Lower VD as well as lower systemic clearance during the first hour after drug administration likely caused the large difference in peak plasma ampicillin concentration.

The relationship between GFR and serum creatinine concentration is more difficult to predict when serum creatinine levels are greater than 4 mg/dL, which is only a modest rise in creatinine for many dogs.[10] The relationship between serum creatinine concentration and GFR in dogs is not linear, and at serum creatinine

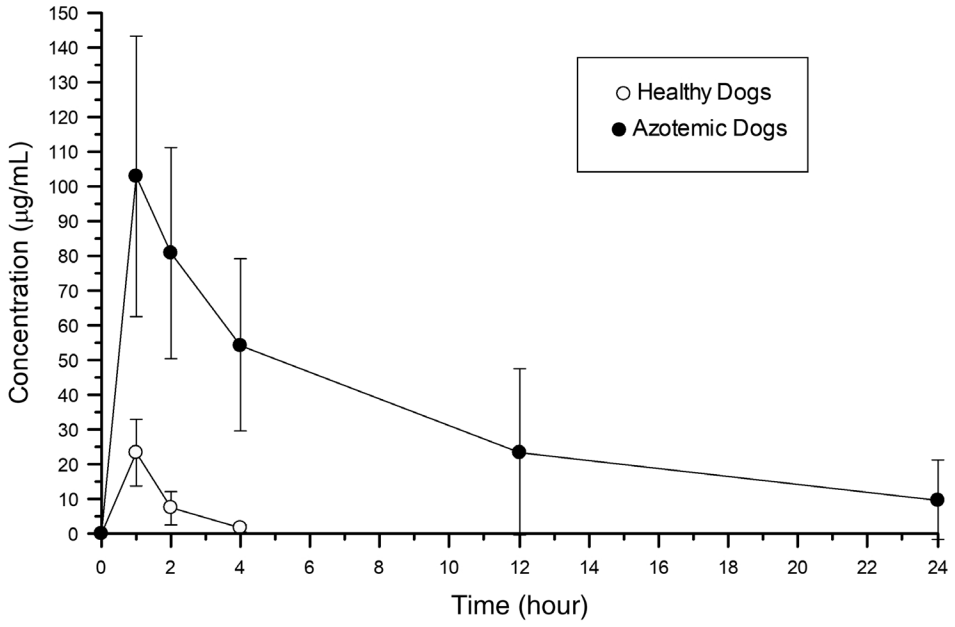

FIGURE 1 Plasma ampicillin concentrations in dogs. Each point represents the mean with ±SD bars. Healthy dogs N = 10. Azotemic Dogs N = 9, ampicillin dose 22 mg/kg (mean dose)

TABLE 2 Pharmacokinetic data of healthy vs azotemic dogs

	Healthy group (n = 10) Geo mean CV% Geo mean	Azotemic group (n = 9) Geo mean CV% Geo mean	*P* value
Peak concentration (mcg/mL)	21.30 50.26	97.07 36.10	**<.001**
Half-life (h)	0.97 115.30	5.86 56.55	**<.001**
Clearance (mL/[kg h])	655.03 53.67	30.06 84.19	**<.001**
Volume of distribution area (mL/kg)	916.93 135.24	253.95 30.14	**<.001**
Area under the curve 0 to infinity (h × mcg/mL)	33.57 53.68	731.04 83.75	**<.001**

Note: GEO mean, CV% Geo mean normal dogs N = 10, azotemic dogs N = 9, $P < .05$.
Abbreviation: GEO mean, geometric mean.
Bold terms are highlighting p values that met significance (<0.05).

concentrations above 4 mg/dL, the GFR drops off to less than 0.5 mg/(kg min).[12]

In human uremic patients, a dose adjustment of ampicillin and cephalexin based on creatinine clearance alone still produced drug accumulation. Therefore, such findings suggest that these methods may be inappropriate for drugs for which clearance depends on active tubular secretion.[16] Prediction of renal drug clearance in people using GFR for drugs secreted by the proximal tubule might not be suitable for dogs because the relationship between serum creatinine and GFR in not linear.[10,17] Thus, relying on serum creatinine to distinguish between normal and abnormal GFR can be "misleading" because of the substantial variation in the relationship between serum creatinine concentrations and GFR among individuals.[9]

Most healthy dogs had undetectable ampicillin concentrations at the 12-hour measurement point. Only 1 healthy dog had measurable ampicillin levels at the 12- and 24-hour point and those values were 0.26 and 0.12 mcg/mL. This dog's samples were considered outliers and were not included in this dog's pharmacokinetic data analysis. We speculated that there was extravasation of some of the ampicillin out of the vein during the injection in this dog, which produced slow absorption of the drug from perivascular tissue.

The CLSI lists the minimum inhibitory concentration breakpoint for susceptible bacteria as less than or equal to 0.25 mcg/mL[13] for infections in sites other than the lower urinary tract. The azotemic dogs all maintained measurable concentrations throughout all sampling time points at a range well above this MIC breakpoint. This suggests that ampicillin can be dosed less frequently in dogs with severe azotemia and still maintain a therapeutic level for susceptible bacteria based on our study in this small population of dogs. The evidence in Figure 1 shows that administration of ampicillin once daily would

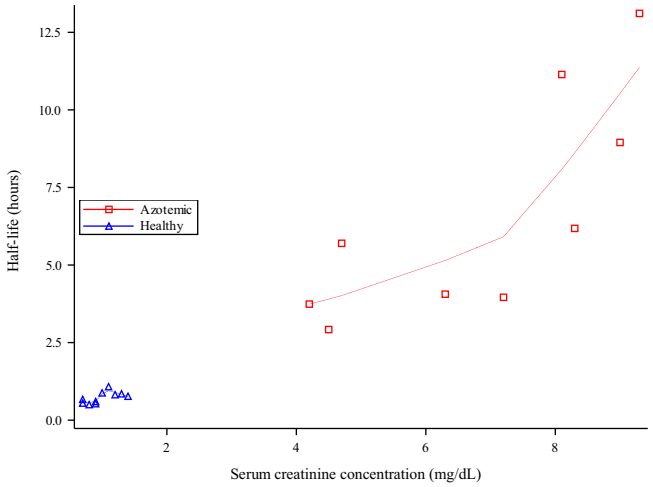

FIGURE 2 Serum creatinine concentration (mg/dL) vs ampicillin half-life (hours) in Healthy dogs (N = 10) blue triangles, and azotemic dogs (N = 9) red squares

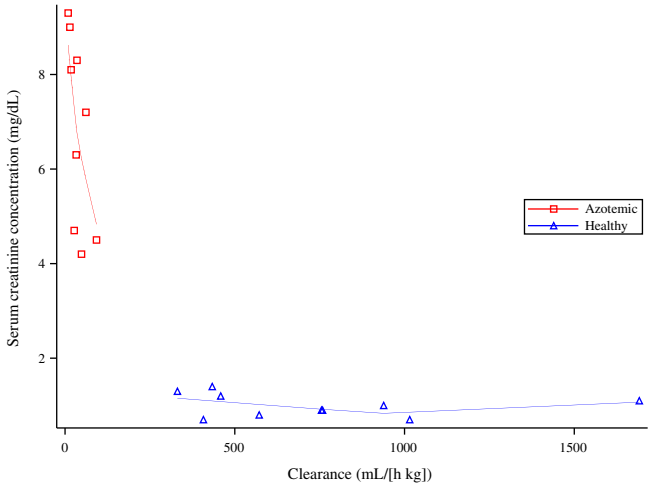

FIGURE 3 Ampicillin clearance (mL/[h kg]) vs serum creatinine concentration (mg/dL) in healthy (N = 10) blue triangles vs azotemic (N = 9) dogs, red squares

reach the therapeutic target of concentrations above the MIC throughout the dose interval. We cannot speculate on the effects of multiple dosing without further study in dogs. However, for many dogs this consideration might be adequate for the first 48 hours of treatment, which is defined as the most critical period in critical care patients.[18]

There are limitations to our study that should be considered. First, the group of azotemic dogs is small and while the pharmacokinetics were similar among the dogs in this group, we might not be able to assume that all dogs with azotemia would have similar results. Additionally, because the dogs were selected from a clinical setting, the cause and severity of azotemia as well as the chronicity of disease varied among the dogs in the azotemic group. Some of the dogs in the group might have had glomerular dysfunction in addition to tubular dysfunction, which might have an impact on elimination of some drug. It is not known if leptospirosis—a clinical indication for ampicillin in azotemic dogs—produces kidney disease that is of the same nature as the dogs in our study. One of the normal dogs had a long terminal half-life and thus the last 2 data points were not included in the half-life determination for this dog. The initial slope for this dog was consistent with the other dogs in the healthy group. We suspect that there might have been some injection of the drug outside of the vein resulting in delayed absorption.

Despite its limitations, this study revealed the magnitude of the plasma concentration differences and pharmacokinetics between healthy dogs and azotemic dogs with impaired renal clearance. Our study demonstrates that 1 cannot assume that commonly employed dosage regimens reported in veterinary formularies can be translated to all clinical dogs without consideration of the effect of the underlying disease on antibiotic pharmacokinetics. If multiple dosing of ampicillin beyond 48 hours is needed in azotemic dogs, our study suggests further study is needed to define the optimal dose adjustment, particularly for a population of dogs with a broader range of increased creatinine concentrations. Fortunately, beta-lactam antibiotics such as ampicillin have a wide therapeutic index. During the first critical 48 hours of antibiotic treatment, little adjustment might be necessary.[18] However, beyond that time, drug accumulation might occur and put the dog at greater risk of adverse effects. Additionally, measuring GFR in the diseased population might further aid development of therapeutic recommendations.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Approved by the Cummings School of Veterinary Medicine IACUC and their Clinical Science Research Review Board.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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