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Original Research

Pharmacokinetics of Ertapenem in Sheep (*Ovis aries*) with Experimentally Induced Urinary Tract Infection

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Sheep are commonly used as animal models for human biomedical research, but descriptions of their use for studying the pharmacokinetics of carbapenem antimicrobials, such as ertapenem, are unavailable. Ertapenem is a critical antimicrobial for human infections, and the description of the pharmacokinetics of this drug is of value for research using ovine as models for human diseases, such as urinary tract infections (UTI). There are currently no ovine models for comparative biomedical research of UTI. The objective of this study was to report the pharmacokinetics of ertapenem in sheep after single and multiple dosing. In addition, we explored the effects of an immunomodulatory drug (Zelnate) on the pharmacokinetics of ertapenem in sheep. Eight healthy ewes (weight, 64.4 ± 7.7 kg) were used in an ovine bacterial cystitis model of human cystitis with *Pseudomonas aeruginosa*. After disease confirmation, each ewe received 1 g of ertapenem intravenously once every 24 h for 5 administrations. Blood was collected intensively (14 samples) during 24 h after the first and last administration. After multiple-dose administration, the volume of distribution was 84.5 mL/kg, clearance was 116.3 mL/h/kg, T1/2$_{(\lambda_z)}$ was 1.1 h, and the extraction ratio was 0.02. No significant differences in pharmacokinetic parameters or time points were found between groups treated with the immunostimulant and controls or after the 1st or 5th administration of ertapenem. No accumulation was noted from previous administration. Our ovine pharmacokinetic findings can be used to evaluate therapeutic strategies for ertapenem use (varying drug dosing schedules and combinations with other antimicrobials or immune modulators) in the context of UTI.

Abbreviations: AUMC$_{inf}$, the area under the first-moment curve from time 0 to infinity; UTI, urinary tract infection

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Ertapenem is a member of the carbapenem family and as such has activity against gram-positive and gram-negative aerobic as well as anaerobic bacteria. This drug demonstrates bactericidal characteristics through binding to penicillin-binding proteins, thereby inhibiting bacterial cell wall synthesis. Due to its high degree of protein binding and stability against renal dehydropeptidase enzymes, ertapenem can be dosed once daily in humans.[13] Similar among carbapenem antimicrobials, ertapenem demonstrates a postantibiotic effect, where bacterial growth is suppressed after concentrations fall below the minimal inhibitory concentration of the organism.[12] These characteristics allow for favorable use of ertapenem for the treatment of complicated urinary tract infections (UTI) in people, often with excellent efficacy.[30,33]

UTI are a leading cause of nosocomial and resistant bacterial infections in human healthcare. New approaches to treat infection and models could be beneficial to develop treatment strategies and combat the high rate of resistance to infection. Complicated UTI are often associated with immunosuppression, renal disease, renal transplantation, or physical objects, such as urinary calculi and indwelling urinary catheters.[19] Animal models of infection are critical for these types of studies, but traditional models, such as mice, do not work well for all aspects of urinary tract diseases, most notably due to size and the inability to place human urinary catheters. In addition, sample collection could be impaired in a murine model of cystitis due to indwelling catheterization, because only a limited volume of urine would be produced, and traditional methods of sample collection, such as cystocentesis, could induce artifact in the form of transient hematuria, which would be suboptimal for a longer-term study.[8] Sheep research models are used for many human diseases including respiratory disease, hemophilia, and polycystic kidney disease.[4,11,15] Sheep possess many attributes that are advantageous for research models, including body size, laboratory disposition, low cost, and longevity, and they can be catheterized for long periods of time, thus allowing for the collection of large volumes of urine. We do not advocate the treatment of ovine patients with carbapenem antibiotics but do recognize that—because of their attributes—sheep may serve as a model of infectious urinary tract disease for human biomedical research.

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Although ertapenem is used in human medicine for community-acquired pneumonia as well as mixed and complicated UTI, there are no reports of its use among veterinary species for human biomedical research.[13] Drug efficacy is inherently related to drug exposure for carbapenem antibiotics, because time-dependent exposure exceeding the MIC for 40% and 20% of the dosage interval is necessary to achieve a bactericidal or bacteriostatic effect.[6] Therefore the development of a sheep model for testing various ertapenem dosing schedules or combination therapies will rely heavily on the comparative pharmacokinetics of ertapenem. One of the primary steps in developing an animal model for human disease that requires ertapenem for treatment is a description of the pharmacokinetics of this drug in sheep (*Ovis aries*). The purpose of this study was to define the pharmacokinetics of ertapenem after single and multiple dosing in sheep undergoing experimental complicated catheter-associated cystitis with and without the use of an immunomodulator.

Materials and Methods

Experimental animals. All aspects of this project were reviewed and approved by the Iowa State University IACUC (log no. 3-15-7965-O). Eight ewes (weight, 64.4 ± 7.7 kg) were sourced from a commercial breeder and used for this study. Ewes were housed in a climate- and humidity-controlled room for the entirety of the study, with the first 72 h used for acclimation prior to initiation of the study. Animals were randomly assigned by body weight to 1 of 2 groups, an ertapenem-only group (n = 4) and an ertapenem-and-immunomodulator (Zelnate [ZN], Bayer HealthCare, Animal Health, Shawnee Mission, KS) group (n = 4). The ewes were housed in individual pens since arrival, and the study took place in the same individual pens for each ewe. After acclimation, no alterations to feeding or handling were made for this study. During the prestudy time period, all ewes were trained to be restrained by a halter placed on the head and tied to the wall of the pen. Criteria for enrollment in this study included a normal physical exam that yielded vital signs within the normal limits of an adult ewe, no previous history of medical illness as well as no recent history of a previously administered medication. Both before and during the study, all sheep were fed a diet that either met or exceeded the National Research Council requirements for maintenance of ewes.

At 24 h prior to initiation of the study, ewes were restrained, and the skin of the neck was aseptically prepared by using 4 alternating scrubs of chlorhexidine surgical scrub and 70% isopropyl alcohol. Prior to catheter placement, the skin at the catheter site was infiltrated with 2% lidocaine, and a 14-gauge, 5.5-in. catheter (MILACATH, MILA International, Florence, KY) were aseptically placed in each jugular vein. After catheter placement, an injection port was added, and the catheters were sutured to the skin and wrapped for security.

UTI was induced as described previously.[24] After an acclimation period, a Foley catheter was placed aseptically, the bulb was inflated with sterile saline, and ewes were inoculated with *Pseudomonas aeruginosa* strain ATCC 15442. The catheter was clamped for 4 h during the inoculation procedure and then left unclamped. Blood collection for the study began 72 h after inoculation.

Experimental design and sample collection. At 24 h prior to collection of samples, ewes in the immunomodulator group received 2.0 mL SC of ZN, a *STING* pathway activator;[7] control ewes received a similar volume of 0.9% saline administered via the same route. At time 0, 1 gof ertapenem (Invanz, Merck, NJ) was given as a 60-s bolus through the jugular catheter designated for drug administration; blood was collected through the other jugular catheter, with predosing samples collected before drug administration and then at times 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 48, 72, and 96 h after drug administration. Ertapenem was administered at a dose of 1g every 24 h for 5 doses. At 96 h, the fifth intravenous dose of ertapenem was given, and additional samples were collected at 96, 96.25, 96.5, 96.75, 97, 97.5, 98, 99, 100, 102, 104, 106, 108, and 120 h to describe pharmacokinetics at presumed steady state, defined according to human pharmacokinetic data.[2,9,13]

At each sampling time point, blood was collected from the catheter by using a 12-mL syringe and placed into sodium heparin–treated tubes (Vacuum phlebotomy tube, Becton Dickinson, Franklin Lakes, NJ) The samples were then centrifuged at 1500 × *g* for 10 min. The plasma was collected, transferred to cryovials, and then stored at −80 °C until analysis.

Sample analysis. Standards were made in 0.1mM 2-[N-morpholino]ethanesulfonic acid (MES) buffer containing 0.24M sodium fluoride at pH 6.5, refrigerated, and used within 3 d to ensure the stability of ertapenem.[16,21] Frozen samples were thawed in cold water, vortexed, pipetted, and immediately returned to –80 °C freezer to minimize degradation of ertapenem.

Analytical standards for plasma were prepared in 200 µL of blank ovine plasma at ertapenem concentrations of 0.25, 0.5, 1, 2, 5, 10, 20, 50, 100, 200 µg/mL. Quality control samples were prepared in 200 µL blank ovine plasma at ertapenem concentrations of 1.5, 30, and 150 µg/mL. Standards and quality controls were kept on ice when not in use. A 25-µg/mL solution of the internal standard, ertapenem-d4, was made in 0.1 mM MES buffer containing 0.24 M sodium fluoride at pH 6.5, and 10 µL was added to standards, quality controls, blank plasma, and samples.

We modified a previously published method for plasma sample preparation.[20] For the standards, quality controls, blank and samples, an aliquot of 200 µL of plasma was transferred into a microfuge tube, and 0.25 µg ertapenem-d4 internal standard was added to each tube. A 200-µL portion of chilled 0.1 mM MES buffer, containing 0.24 M sodium fluoride, was added to each sample, followed by 600 µL of chilled acetonitrile. Samples were mixed by using a vortex mixer and then centrifuged for 5 min at 2700 × *g*. The supernatant was decanted into another tube, to which was added 600 µL of chilled dichloromethane; the tube then was mixed by using a vortex mixer and centrifuged for 5 min at 2700 × *g*. A 100-µL portion of the top layer was transferred into a microvial containing a glass insert. Samples were then centrifuged for 20 min at 1500 × *g* in a chilled centrifuge. Standards and samples were kept on ice during the extraction procedure. Data points with concentrations above the range of the standard curve were diluted with blank plasma and reextracted to achieve a concentration within the range on the curve.

Ovine plasma concentrations of ertapenem were determined by using LC–MS/MS. A TSQ Quantum Discovery Max triple quadrupole was coupled to a Surveyor Pump with a chilled Autosampler. (Thermo Scientific, San Jose, CA). The mobile phases consisted of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile. The mobile phase began at 15% B with a linear gradient to 70% B at 2.5 min, followed by reequilibration to 15% B. A Kinetex phenyl hexyl column was used (100 mm × 2.1 mm, 2.6-µm particles) from Phenomenex (Torrance, CA) with the column temperature set to 35 °C. The injection volumes were 10 µL for plasma and 2 µL for urine. The following ions were used for identification: ertapenem (m/z 476) 68, 114, and 432 and ertapenem-d4 (m/z 480) 68, 114, and 436. The retention

time for both compounds was 1.8 min. The 432 and 436 ions were used for quantitation of ertapenem and ertapenem-d4, respectively.

Calibration curves were calculated by using the Quan Browser module of Xcalibur software (Thermo Scientific, San Jose, CA) and quadratic fit. All correlation coefficients (R^2) exceeded that of 0.99. The calibrators were within a tolerance of 15% of the nominal value, except for the lower limit of quantitation, which was less than 20%. The quality controls were within a tolerance of ±15% of the nominal value. The limit of detection (LOD) was 0.1 µg/mL for ovine plasma. The limit of quantitation, which was based on the calibration curve, was 0.25 µg/mL for ovine plasma.

Pharmacokinetic analysis. Pharmacokinetic analysis of total ertapenem plasma concentration was completed using a noncompartmental module in commercial software (Phoenix WinNonlin 8.0, Certara, Princeton, NJ). Figures showing time plotted against concentration of ertapenem were produced by using a commercial program (Prism 8, GraphPad Software, La Jolla, CA). (Figures 1 and 2).

The following standard pharmacokinetics parameters were generated for individual ewes maximum observed ertapenem concentration (µg/mL); last observed ertapenem concentration (µg/mL, C_{last}); time to last observed ertapenem concentration (min; T_{last}); area under the ertapenem concentration–time curve from time 0 to infinity ([µg/mL]×h; AUC_{inf}); area under the ertapenem concentration–time curve from time 0 to last measurement ([µg/mL]×h, AUC_{last}); and ertapenem mean residence time (h; = $AUMC_{inf}/AUC_{inf}$; where $AUMC_{inf}$ is the area under the first-moment curve from time 0 to infinity [µg/mL×h^2]). Area parameters (AUC, AUMC) were calculated by using the log-linear trapezoidal rule. Ertapenem elimination half-life (h) was calculated as $T1/2_{(\lambda_z)} = \ln(2)/\lambda_z$, where λ_z is the slope of the terminal phase of the natural logarithm of concentrations compared with time curve; ertapenem systemic clearance (mL/h/kg) was dose/AUC_{inf}; the volume of distribution (mL/kg) of ertapenem during the elimination phase was calculated as dose / $AUC_{inf} \times \lambda_z$; the volume of distribution (mL/kg) of ertapenem at steady-state was equal to systemic clearance × mean residence time. The extraction ratio (E_{body} = systemic clearance / cardiac output) was calculated as reported previously,[27] first calculated for each individual ewe and then combined for a mean value, with ewe cardiac output described[27] as 180 × body weight (in kg)$^{-0.19}$.

Statistical analysis. Statistical analysis was completed using Prism 8.0.2 (GraphPad Software, La Jolla, CA). The respective data distributions for all pharmacokinetic parameters and group time point concentrations were assessed for normality by means of the Shapiro–Wilk test. Comparisons between the 2 treatment groups were performed with unpaired *t* tests for parametric statistics and Mann–Whitney tests for nonparametric statistics as previously described.[22] A *P* value less than 0.05 was considered statistically significant.

Results

Ewe health. Ertapenem was well tolerated by all 8 ewes during the study. No changes in appetite, behavior, or stool consistency were noted. No adverse reactions were noted at the catheter sites.

Pharmacokinetics. No ewe had detectable ertapenem in plasma prior to commencement of the study. The average time course of ertapenem can be found in Figure 1. Geometric mean, median, minimum and maximum profiles after single-dose administration are presented in Table 1. Among individuals there

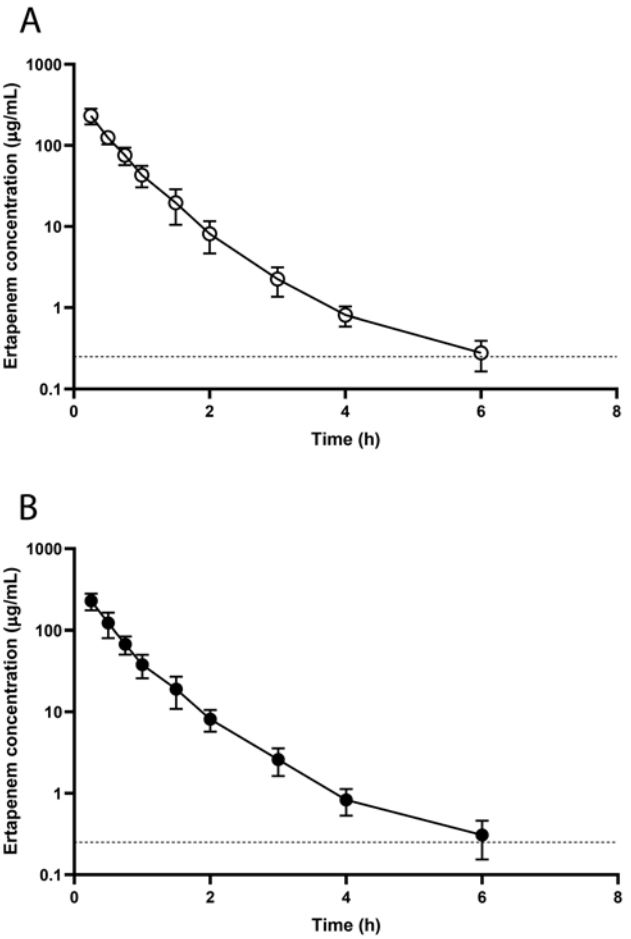

Figure 1. (A) Plasma concentration (mean ± 1 SD [error bars]) after a (A) single initial intravenous administration and (B) multiple-dose administration of ertapenem to sheep. The dashed line illustrates the limit of quantification for the assay (0.25 µg/mL).

appears to be limited variation of time compared to concentration data for ertapenem in plasma. All ewes had no detectable concentrations of ertapenem in plasma after 6 h for the single-dose study. No statistically significant differences were noted among time point concentrations when compared for the presence or absence of the immunomodulator or for single compared to multiple dosing.

After initial dosing, ertapenem C_{max} (mean ± 1 SD) was 232.6 ± 50.8 µg/mL, AUC_{last} was 144.0 ± 24.8 h×µg/mL, mean residence time was 0.7 ± 0.08 h, volume of distribution at steady state was 78.3 ± 16.1 mL/kg, systemic clearance was 109.6 ± 17.9 mL/h/kg and $T1/2_{(\lambda_z)}$ was 1.3 ± 0.6 h.

Ertapenem concentrations in plasma immediately prior to administration of the 5th dose were below the limit of detection. The geometric mean, median, minimum, and maximum plasma concentration profiles for each group after administration of the 5th dose are presented in Table 2, and no detectable concentrations were noted after 6 h in the multiple-dose study.

After multiple dosing (Figure 2), C_{max} of ertapenem was calculated as 228.0 ± 52.8 µg/mL, AUC_{last} was 138.7 ± 34.0 h×µg/mL, mean residence time was 0.7 ± 0.06 h, the volume of distribution at steady state was 84.5 ± 13.1 mL/kg, systemic clearance was 116.3 ± 24.4 mL/h/kg, and $T1/2_{(\lambda_z)}$ was 1.1 ± 0.6 h.

No accumulation was noted, given that the majority of 8-h time points and all 10, 12, and 24 h timepoints demonstrated

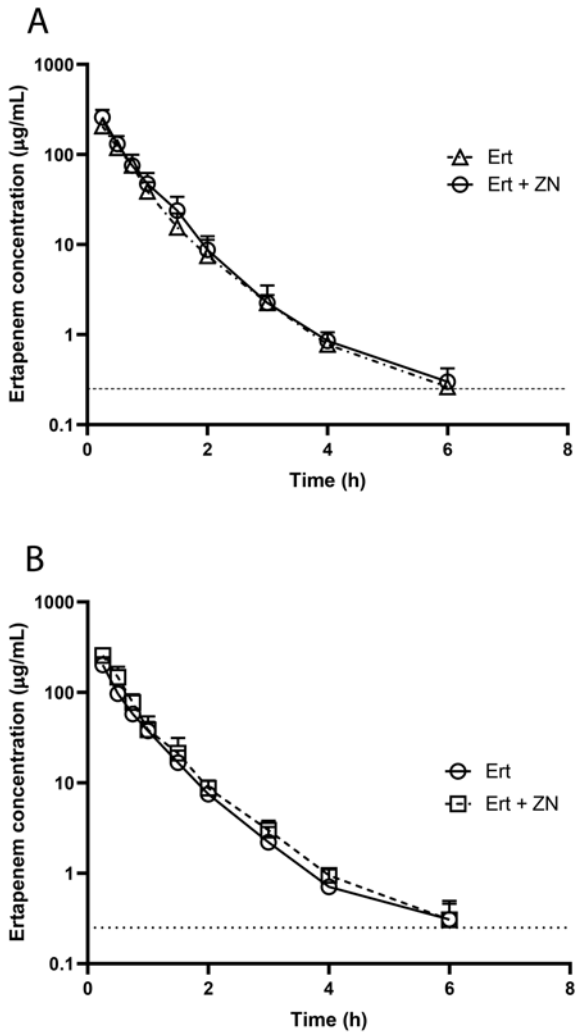

Figure 2. Plasma concentration (mean ± 1 SD [error bars]) after (A) a single intravenous dose and (B) multiple intravenous doses of ertapenem in sheep treated without the immunomodulator (Ert) or with the immunomodulator (Ert + ZN). The dashed line illustrates the limit of quantification for the assay (0.25 µg/mL).

ertapenem concentrations below the limit of detection. The extraction ratio for ertapenem in our ewes was 0.02 ± 0.004.

Statistical analysis. When parameters were compared with the values for the 1st and 5th administration of ertapenem, no significant differences were found for $T1/2\lambda_{z)}$ ($P = 0.44$), C_{max} ($P = 0.86$), AUC_{last} ($P = 0.73$), Vz ($P = 0.65$), systemic clearance ($P = 0.88$), AUMC ($P = 0.97$), mean residence time ($P = 0.47$), and Vss ($P = 0.41$). No significant differences in drug concentrations or pharmacokinetics between ewes were found regardless of the presence or absence of immunomodulator.

Discussion

Before using sheep as a model for human diseases with regard to carbapenem administration, it is important to understand the pharmacokinetics of ertapenem in this species. With the increase of resistance in nosocomial infections in human hospitals requiring carbepenem therapy, additional animal investigations will need to be developed for translational studies. Sheep used in this study displayed ideal characteristics as subjects for a pharmacokinetic study. By commencement of this study, sheep were rapidly accustomed to halter restraint. Intravenous catheters maintained patency and allowed for ease of sample collection. Although ruminant urine pH differs from that of humans, it can be manipulated by dietary factors, such as the addition of ammonium chloride.[14,26] This manipulation may be ideal for other investigations of ertapenem in experimentally infected sheep. The body weight of the sheep in this study (65.2 ± 7.7 kg) was similar to the weights of human patients in several human pharmacokinetic studies (e.g., 76.2 ± 9.3 kg and 73 kg).[31,33] This similarity in body weights allow for translational dosing for sheep when ertapenem is dosed at 1 g/patient, as is common for humans.[1,9,33] However, this similarity in total body mass does not account for differences in anatomy, such as the relatively larger size of the ruminant gastrointestinal system.

Our analysis revealed a short half-life for ertapenem, similar to what is described in human patients. The pharmacokinetic parameters and concentration at individual timepoints also demonstrated no differences after single or multiple administrations of ertapenem in sheep. This is noteworthy as our study sheep appear to have linear pharmacokinetics, again similar to humans. When ertapenem is administered to people, it can be given as either a 30-min or 5-min infusion, typically once daily.[31] The C_{max} of our study in sheep (232.6 ± 50.8) is similar to that after a 5-min bolus in humans (195.9 ± 34.0).[31]

The volume of distribution at steady state for ertapenem—after adjustment for bodyweight for our ewes—was 5.5 ± 0.14 L, which is descriptively similar to what is reported for human outpatients with complicated UTI (4.85 ± 1.8 L) but less than what is reported for healthy female human volunteers (7.5 ± 0.9 L).[13,33] In addition, the elimination half-life in our ewes was less than reported in either of those human studies. Although some of the pharmacokinetic parameters of ertapenem in sheep seemed lower than what is reported in human patients, it is important to note that specific disease status can alter ertapenem pharmacokinetics, such as sepsis can increase distribution volume and UTI can decrease this parameter. For example, in patients with severe sepsis treated with ertapenem, lower C_{max} and AUC as well as larger volume of distribution were observed when compared with healthy human volunteers.[2] A decreased volume of distribution has been noted in human outpatients with complicated UTI when compared with healthy volunteers, potentially a result of decreased clearance.[33] Furthermore, age may also present an effect on the PK of ertapenem as in humans elderly people have higher AUC values when compared with younger persons.[17]

The extraction ratio of ertapenem in our current study sheep would be classified as low, because it is less than 0.05.[27] This value is in the range of extraction by glomerular filtration (that is, 2% of cardiac output) and in agreement with previous descriptions for humans.[18] Although our results suggest some species-specific differences in the pharmacokinetics of ertapenem in sheep and humans, it is important to note that analytical method sensitivity can have a profound effect on pharmacokinetics, as recently illustrated in the comparative pharmacokinetics of fentanyl in large animal species.[25] The limit of quantification of our assay was 0.25 µg/mL, and in the human literature limits of quantitation of 0.125 µg/mL have been reported.[13] As noted for fentanyl concentrations in large animal species, when comparing pharmacokinetic parameters it is important to consider analytical sensitivity as a lower limit of quantification can lead to the reporting of a longer elimination half-life. As such, it is possible that our analytical limits would yield decreased half-life and other parameters due to the higher limits of quantitation.

Adverse effects with ertapenem administration in people are primarily of concern for the nervous system. Several human

Table 1. Pharmacokinetic parameters of ertapenem in sheep after single dosing

Parameter	Unit	Geometric mean	Median	Minimum	Maximum
C_{max}	µg/mL	228.1	216.5	182	324
T1/2 (λz)	h	1.09	1.13	0.65	2.7
AUC_{last}	(µg/mL)×h	142.0	146.5	112.3	176.7
Clearance	mL/h/kg	108.3	109.1	131.4	81.2
AUMC	(µg/mL)×h^2	97.3	97.1	73.4	137.9
Mean residence time	h	0.69	0.70	0.55	0.78
Volume of distribution at steady state	mL/kg	76.8	78.1	52.7	113.4
C_{last}	µg/mL	0.28	0.3	0.125	0.7
T_{last}	h	5.9	6	4	8
λz	1/h	0.59	0.61	0.25	1.06

patient undergoing peritoneal dialysis have developed seizures after the administration of ertapenem.[23,32] In addition, seizures have been reported in elderly patients receiving ertapenem.[1,29] Among humans, stroke, low Hgb, and a low platelet count were identified as risk factors for seizures when administered ertapenem.[10] Less common adverse effects, such as thrombocytopenia, have also been reported in human patients.[5] Although safety evaluation was not a primary goal of this study, none of the sheep given ertapenem had overt seizure activity or neurologic disease, and none displayed the hematologic abnormalities associated with ertapenem in people.

Table 2. Pharmacokinetic parameters of ertapenem in sheep after multiple dosing

Parameter	Unit	Geometric mean	Median	Minimum	Maximum
C_{max}	µg/mL	222.3	217.5	136	324
T1/2 (λz)	h	1.01	0.86	0.56	2.16
AUC_{last}	(µg/mL)×h	134.6	136.3	77.8	183.8
Clearance	mL/h/kg	114.3	112.4	92.5	170.9
AUMC	(µg/mL)×h^2	42.9	46.9	47.2	138.7
Mean residence time	h	0.71	0.72	0.61	0.79
Volume of distribution at steady state	mL/kg	77.2	72.7	67.1	105.6
C_{last}	µg/mL	0.26	0.25	0.25	0.3
T_{last}	h	4.73	5	3	6
λz	1/h	0.58	0.75	0.19	1.35

Of note, organisms susceptible to ertapenem are typically inhibited by in vitro concentrations of less than or equal to 4 µg/mL.[18] The time above the minimal inhibitory concentration required for bacteriostasis for ertapenem in people is approximately 30% of the dosing interval.[28] Given the shorter elimination half-life of ertapenem in sheep (1 h) when compared with human patients (approximately 3.5 h), future studies involving sheep models of infection may need to use an increased dosage (greater than the 1 g/ewe we used here) to maintain plasma concentrations above 4 µg/mL for 30% of the day with once-daily dosing.

A limitation of our study was the relatively small sample size; however for veterinary pharmacokinetic studies, a sample size of 4 to 6 animals typically is sufficient to describe the pharmacokinetics of a test drug.[3] Additional studies are required to explore the pharmacodynamics of ertapenem in sheep, as well as the potential synergistic effects of ertapenem and immunomodulators on bacteriuresis and resistance development.

In conclusion, our study established the pharmacokinetics of ertapenem in sheep used as a model for human biomedical research. No drug accumulation was reported after 5 d of dosing, which is consistent with the short elimination half-life of this antimicrobial in sheep. Likewise, the absence of notable difference between ertapenem clearance after single and multiple dosing is indicative of first-order elimination in sheep. This ovine model can be used to evaluate pharmacokinetics for therapeutic strategies for ertapenem use (varying drug dosing schedules and combinations with other antimicrobials or immune modulators) in the context of UTI.

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