Acta Veterinaria-Beograd 2025, 75 (1), 1-13
UDK: 615.33.015
DOI: 10.2478/acve-2025-0001

*Research article*

# PHARMACOKINETICS AND BIOAVAILABILITY OF DOXYCYCLINE FOLLOWING PARENTERAL ADMINISTRATION IN RABBITS

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(Received 10 December 2024, Accepted 18 February 2025)

Doxycycline, a member of the tetracycline family, is widely used in various species to treat infections caused by bacteria susceptible to this antibiotic. However, data on the pharmacokinetics of this drug in rabbits are scarce. The aim of this study was to investigate the pharmacokinetics of doxycycline after intravenous and extravascular (intramuscular and subcutaneous) administration in rabbits. A randomized crossover study (n = 5) was employed, with a dosage of 20 mg/kg. The $V_{ss}$ was 0.64 L/kg, indicating moderate distribution of the antibiotic in rabbits. The peak concentrations ($C_{max}$) and the times to peak plasma concentration ($t_{max}$) obtained after extravascular administration were very similar, with not significant differences between the intramuscular and subcutaneous routes. The bioavailabilities of extravascular administrations were low ($F_{intramuscular}$=6.01 %, $F_{subcutaneous}$=7.30 %), limiting its efficacy in the treatment of doxycycline–susceptible bacterial infections in rabbits. However, other formulations of doxycycline or other routes of administration may need to be tested to achieve better bioavailability to ensure adequate dosing regimens and clinical efficacy.

**Keywords**: bioavailability, doxycycline; intramuscular; pharmacokinetics; rabbits.

## INTRODUCTION

Rabbits are becoming increasingly important in veterinary medicine, both for their high nutritional value as a meat source [1] and for their growing popularity as pets [2]. Despite this, rabbits are still considered to be a group of "minor species", i.e. species of minor economic importance for which there are not enough approved antibacterial drugs because they are not of sufficient economic interest to pharmaceutical companies.

Respiratory diseases are the second most common cause of morbidity and mortality in rabbits after gastrointestinal diseases. These lagomorphic mammals of the Leporidae

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family are often susceptible to infections by various microorganisms such as pathogenic bacteria *Pseudomonas spp, Staphylococcus aureus, Salmonella spp, Clostridium spp, Escherichia coli, Bordetella bronchiseptica or Pasteurella multocida* (the most common respiratory disease in domestic rabbits) [3,4]. Mycobacteriosis has also been reported in domestic rabbit populations [5]. The pathogens listed are susceptible to antibiotics frequently used in veterinary medicine, such as broad–spectrum penicillin, lincosamides, sulfonamides, macrolides, fluoroquinolones, aminoglycosides, and tetracyclines [5–8].

In this species, due to the sensitivity of the gut microbiota the administration of antimicrobials, especially orally, is not well tolerated and can cause significant dysbiosis by creating an imbalance in the gastrointestinal microflora, which implies the development of pathogenic bacteria [9]. Parenteral administration is an option to reduce this negative effect. In this context, antibiotics of the tetracycline group are more respectful of the rabbit microbiota, so that their administration, especially those of newer generations, does not alter the gastrointestinal balance and is more protective against the growth of pathogenic bacterial populations [10].

Tetracyclines have been widely utilized in veterinary medicine for the treatment of both farm and companion animals. Their popularity is due to their broad antimicrobial spectrum, strong efficacy, and relatively low risk of side effects. Moreover, tetracyclines are now included as Group D in the EMA classification and can therefore be used in all food–producing species [11]. Doxycycline, a second–generation tetracycline, is distinguished by its high lipid solubility compared to the first–generation of tetracyclines. Doxycycline is currently available on the market in various forms, including calcium salt, monohydrate salt, and hyclate salt. Of these, the hyclate salt is most commonly used in veterinary medicine because it offers better water solubility than the monohydrate form [12,13]. The pharmacokinetics of doxycycline has been studied in various livestock species, including calves, donkeys, horses, sheep, goats, pigs, and rabbits [14–26]. In rabbits, pharmacokinetic data for doxycycline have been reported in three main studies, focusing primarily on enteral administration. Two of these studies involved oral administration (using gelatin capsules and microcapsules), while another investigated rectal administration via suppositories, and compared these results with pharmacokinetic data from intravenous (IV) administration [22,27,28]. Nevertheless, no studies have been conducted on the pharmacokinetics of doxycycline following intramuscular (IM) or subcutaneous (SC) administration in this species. These studies are essential to determine appropriate dosing strategies to achieve therapeutic efficacy while preventing the rapid emergence of bacterial resistance. Given that domestic rabbits may harbor bacteria in their microbiome that are phenotypically resistant to antimicrobials, in addition to genes encoding antimicrobial resistance (AMR), it is important to consider this in the context of the growing threat of antibiotic resistant bacteria, including resistance to tetracyclines [29].

Given the paucity of data on the pharmacokinetics of doxycycline in rabbits, this study aimed to determine the disposition kinetics of doxycycline following IV and extravascular (SC and IM) administration, and identify the most suitable route of administration based on pharmacokinetical parameters.

## MATERIALS AND METHODS

### Animals

The research took place at the Animal Facility of the University of Murcia (Murcia, Spain). Five healthy New Zealand rabbits (three females and two males) from the Animalarium of the University of Murcia, with an average weight of 4.92 ± 0.55 kg and an age of 1.4 ± 0.3 years, were selected for the study. The animals were in good health, as confirmed by physical examinations. Each rabbit was housed individually in cages and allowed a 15–day acclimatization period to familiarize them with the experimental environment and handling procedures, during which no medication was administered. Throughout the study, the rabbits had free access to food and water and were fed a pelleted diet. The experimental procedures were approved by the Bioethics Committee of the University of Murcia (protocol CEEA 758/2021).

### Experimental design

A randomized crossover study was designed with three phases, each separated by a wash–out period of at least 15 days. During the study, each rabbit was randomly assigned to receive a single dose of doxycycline (20 mg/kg) by IV, SC and IM injection. The medicines used were Vibravenosa 20 mg/ml solution for injection (HOSPIRA INVICTA, Madrid, Spain) for IV administration and DFV Doxivet Injectable (200 mg/ml, DIVASA–FARMAVIC, Barcelona, Spain) for extravascular administration. Different formulations of doxycycline were used in the study, as DFV Doxivet Injectable cannot be administered by IV, and the only medicine marketed in Spain with doxycycline for IV administration is Vibravenosa.

For IV injections, the dose was administered into the marginal ear vein of one ear, while blood samples were taken from the opposite ear. The IM injection was delivered into the semimembranosus muscle, and the SC dose was administered into the skin between the shoulder blades. Blood samples (0.5–1 mL) were collected at different time points: 0 (pre–treatment), 0.083 hours (post–IV administration only), and subsequently at 0.167, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 8, 12, 24, 48, 72, and 96 hours after drug administration. A 24–gauge needle was inserted into the marginal ear vein, allowing blood to drip into a 2–mL heparinized syringe for collection. Blood samples were centrifuged at 1500g for 10 minutes within 30 minutes of collection. The resulting plasma was collected immediately and stored at – 40 °C until HPLC analysis could be performed.

### Analytical methods

Doxycycline was quantified by a previously published fluorescence detector HPLC assay [30]. Doxycycline pure substance (for quality controls) and internal standard (danofloxacin) were bought from Cymit Química (Barcelona, Spain).

### Method Validation

The method validation was carried out following the FDA's 2018 guidelines [31] for bioanalytical method validation. Several key parameters were assessed, including accuracy, precision, linearity, lower limit of detection (LOD), lower limit of quantification (LOQ), selectivity, recovery and carryover. The specific protocols used to validate these parameters, as well as the acceptable variation coefficients, are detailed in a previous publication by our research team [30].

### Pharmacokinetic analysis

Non–compartmental parameters were calculated utilizing the WinNonlin™ software (Pharsight Corporation; Mountain View, CA, USA). A summary of the abbreviations and descriptions for each pharmacokinetic parameter is provided in the footnote of Table 1. The formula used to determine bioavailability is as follows: F (%) = (AUC of intramuscular or subcutaneous administration / AUC of intravenous administration) × 100.

### Statistical analysis

Statistical analyses were performed using Jamovi software version 2.3.28, specifically the Solid edition. Pharmacokinetic data were calculated using the arithmetic mean and standard deviation, except for half–lives (which were reported as the harmonic mean) and $t_{max}$ (categorical variable) which was expressed as the median value and range [32]. Normality of the data was assessed using the Shapiro–Wilk test. For normally distributed data sets, a paired t–test was employed to evaluate differences; conversely, the Wilcoxon signed rank sum test was used for data that did not meet normality assumptions. A statistical significance threshold of $p < 0.05$ was used to assess the relevance of the findings.

## RESULTS

### Animals

None of the animals showed systemic adverse effects such as fever, lethargy or diarrhea following administration of doxycycline by the various parenteral routes. There was no evidence of discomfort or inflammation at the injection sites.

### Analytical method

Peaks for doxycycline and danofloxacin appeared on the chromatogram at 7.9 and 5.3 minutes, respectively. The peaks of doxycycline and danofloxacin were clearly distinguished. After analysis of the blank samples, there were no interferences at the same retention times for doxycycline and danofloxacin. Linear regression equation was

y = 1506.9·X. LOD was 0.035 μg/mL and LOQ was 0.1 μg/mL. The CV precision values for plasma samples were <4.6 % and <5.9 % for within–day and between–day precision, respectively. Accuracy ranged from – 4.7 % to 9.3 %. Recovery of doxycycline from plasma was 85.7 %. These results indicate that our method could be effective for the quantification of doxycycline in rabbit plasma by HPLC.

**Table 1.** Pharmacokinetic parameters (mean ± SD) of doxycycline in rabbits (n = 5) after IV, IM and SC administration of a single dose of 20 mg/kg.

| Parameters (units) | Intravenous | | | Intramuscular | | | Subcutaneous | | |
|---|---|---|---|---|---|---|---|---|---|
| $C_0$ (μg/mL) | 57.81 | ± | 32.11 | | | | | | |
| $\lambda_z$ ($h^{-1}$) | 0.200 | ± | 0.151 | 0.027 | ± | 0.004[a] | 0.029 | ± | 0.015[a] |
| $t_{½\lambda z}$ (h)* | 3.38 | | | 25.91[a] | | | 23.79[a] | | |
| $V_Z$ (L/kg) | 0.79 | ± | 0.45 | | | | | | |
| $V_{ss}$ (L/kg) | 0.64 | ± | 0.32 | | | | | | |
| Cl (L/h/kg) | 0.12 | ± | 0.05 | | | | | | |
| $AUC_{0–24}$ (μg·h/mL) | 185.93 | ± | 70.17 | 4.92 | ± | 1.11[a] | 5.91 | ± | 1.88[a] |
| $AUC_{last}$ (μg·h/mL) | 185.93 | ± | 70.17 | 7.35 | ± | 2.63[a] | 8.42 | ± | 3.98[a] |
| $\%AUC_{extrap.}$ | 2.73 | ± | 2.05 | 35.26 | ± | 7.11[a] | 39.14 | ± | 6.91[a] |
| $AUC_{0–\infty}$ (μg·h/mL) | 191.21 | ± | 71.47 | 11.11 | ± | 3.13[a] | 14.02 | ± | 6.59[a] |
| MRT (h) | 5.20 | ± | 1.44 | 38.21 | ± | 5.86[a] | 40.96 | ± | 16.58[a] |
| MAT (h) | | | | 31.07 | ± | 9.24 | 35.39 | ± | 16.47 |
| $C_{max}$ (μg/mL) | | | | 0.57 | ± | 0.19 | 0.50 | ± | 0.23 |
| $t_{max}$ (h)[Φ] | | | | 0.75 (0.50–0.75) | | | 1.50 (1.50–4.00) | | |
| F (%) | | | | 6.01 | ± | 1.05 | 7.30 | ± | 1.72 |

[a] There are significant differences with the intravenous route ($p<0.05$).
[b] There are significant differences with the intramuscular route ($p<0.05$).
* Harmonic mean
[Φ] Median value and range.

**$C_0$:** concentration of the drug in the serum immediately after intravenous administration; **$\lambda_z$:** the slowest elimination rate constant; **$t_{½\lambda z}$:** half–life associated with the terminal slope ($\lambda_z$) of a semilogarithmic concentration versus time curve; **$V_z$:** apparent volume of distribution calculated according to the method of the area; **$V_{ss}$:** apparent volume of distribution in the steady state; **Cl:** total clearance of the drug from the plasma in the body; **$AUC_{0–24}$:** the area under the plasma concentration versus time curve from zero to 24 hours; **% $AUC_{extrap.}$:** % AUC extrapolated; **$AUC_{0–last}$:** the area under the curve up to the last quantifiable point in time; **$AUC_{0–\infty}$:** the area under the plasma concentration versus time curve from zero to infinity; **MRT:** the mean residence time; **MAT:** the mean absorption time; **$C_{max}$:** the peak or maximum plasma concentration after extravascular administration of the drug; **$t_{max}$:** the time after extravascular administration to peak or maximum plasma concentration; **F:** The proportion of the administered dose that is available systemically (bioavailability).

## Pharmacokinetics

Plasma concentrations of doxycycline were detected above the lower limit of quantification (LOQ) for up to 24 hours following IV administration and for up to 48 hours after extravascular administration. Figures 1 and 2 illustrate the mean plasma concentrations (± SD) of doxycycline after IV and IM/SC administration, respectively. Table 1 presents the non–compartmental pharmacokinetic parameters. All parameters exhibited significant differences ($p<0.05$) between intravenous and extravascular administration. There were no significant differences in any pharmacokinetic parameter between the two extravascular routes.

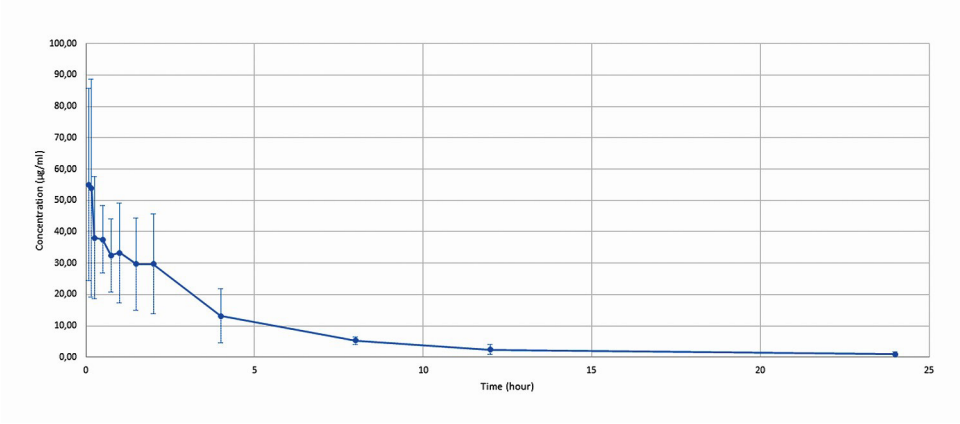


**Figure 1.** Plasma concentration–time profile of doxycycline after an intravenous dose of 20 mg/kg body weight. The values are mean ± SD (n = 5).

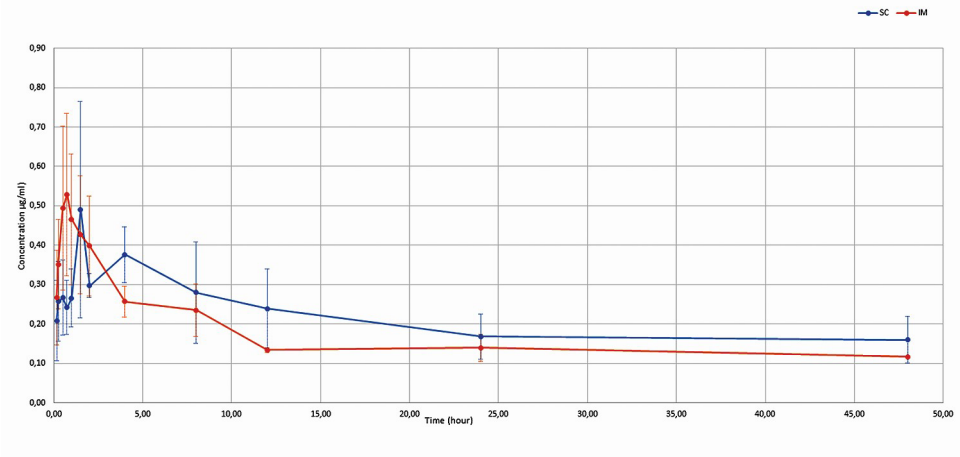


**Figure 2.** Plasma concentration–time profile of doxycycline after an intramuscular and subcutaneous dose of 20 mg/kg body weight. The values are mean ± SD (n = 5).

## DISCUSSION

Rabbits are less commercialized than pigs and cattle. Only a handful of drugs are approved for use in these animals. As a result, many antimicrobials administered to rabbits are considered off–label, with dosing regimens, withdrawal times and indications often derived from data established in other species [33,34]. Therefore, in order to optimize the dosing of doxycycline, it is essential to measure the concentrations of this antibiotic in plasma or other biological fluids. Currently, there is limited information available on the pharmacokinetics of doxycycline in rabbits. Doxycycline is significantly more lipophilic than other tetracyclines, resulting in extensive tissue distribution and a prolonged half–life [12]. These pharmacokinetic characteristics make it suitable for daily administration as a single dose, which is particularly attractive in the veterinary field. In addition, doxycycline has excellent tissue penetration and achieves therapeutic levels in most organs and tissues [35,12,36]. Previous studies of tetracyclines in rabbits have shown that tissue concentrations are significantly elevated, with the highest levels in the kidneys and lungs between 2 and 24 hours after oral and IV administration [37,38].

After IV administration of doxycycline, the half–life was 3.38 hours. This value is lower than that obtained in another study with doxycycline hyclate ($t_{½}$ = 8,83 ± 2,10 h) [27]. This difference may be due to the use of analytical methods with different sensitivities, the use of different formulations of doxycycline, or the use of different rabbit breeds. Taking into account the higher lipophilicity of doxycycline compared to other tetracyclines, the results obtained for other tetracyclines, such as tetracycline hydrochloride administered intravenously to rabbits, showed a lower half–life (1.20 ± 0.26 h) than for doxycycline [39]. Similarly, other pharmacokinetic studies of oxytetracycline in this species show values of 1.32 hours [38] and 2 hours [37]. In other species, similar half–lives have been reported for cats (4.56 h), sheep (2.81 h) and lactating goats (2.14) [40,19,20].

The half–life obtained after IV administration was significantly different from that obtained after other parenteral routes such as IM (25.9 h) and SC (23.79 h). After both extravascular routes, the half–life values of doxycycline were prolonged compared to those observed after IV administration, which can be attributed to the duration of the absorption phase and to the different drug formulations used in the study. Mean residence time (MRT) values showed a similar trend. The MRT values after extravascular administration were significantly longer than those observed after intravenous administration, indicating that the transit time of the molecules is prolonged due to the absorption process. This observation suggests that doxycycline may follow a flip–flop pharmacokinetic model in which absorption is the rate–limiting step for plasma clearance [41]. However, it could also be explained by the increase in volume of distribution following the extravascular routes (approximately 3–5 times higher). Different studies with the same formulation of doxycycline in goats [19] and sheep [20] following the same routes of administration indicate that absorption is

often the rate–limiting factor in the overall disposition and elimination of doxycycline and suggest the existence of a flip–flop pharmacokinetic model.

The Vss value obtained was 0.64 L/kg, suggesting a moderate distribution of this antibiotic in rabbits. Very similar values were obtained with the same formulation in goats ($V_{ss}$=0.62 L/kg) and sheep ($V_{ss}$=0.63 L/kg) [19,20]. In the case of other tetracyclines in rabbits, such as tetracycline and oxytetracycline, the values of $V_{ss}$ were also similar (0.71 and 0.86 L/kg, respectively) [39,38]. The moderate distribution of doxycycline after intravenous administration may be due to its higher binding affinity for plasma proteins compared to other tetracyclines [12]. In addition, doxycycline hyclate, which is more water soluble, has a reduced ability to cross biological membranes and a lower affinity for adipose tissue.

The peak concentrations ($C_{max}$) and the time to peak plasma concentration ($t_{max}$) achieved after extravascular administration were similar, with no significant differences between the IM and SC routes. The bioavailability of doxycycline after IM and SC injections was very low, with mean values, of 6.01 and 7.3 %, respectively. Low bioavailability for this antibiotic has also been reported after oral administration in goats (31.39 %; [25]), sheep (35.77 %; [15]), and pigs (21.20 %; [42]), after IM administration in goats and sheep (45.60 % and 31.00 %, respectively) [19,20] and after SC administration in sheep (53.66 %; [20]). As mentioned above, our research group obtained low bioavailability values after IM administration of doxycycline in small ruminants. In these studies, doxycycline was irritating and caused discomfort at the injection site, which may have contributed to reduced absorption.

Drugs can be divided into two groups based on their antibacterial activity: time–dependent and concentration–dependent. For concentration–dependent drugs, an increase in antibiotic concentration results in faster bacterial killing. In contrast, time–dependent drugs maintain a constant kill rate regardless of any increase in drug concentration [43]. Tetracyclines such as doxycycline are traditionally considered to be time–dependent in their pharmacodynamics. Their efficacy is primarily determined by the time that the drug concentration at the site of action remains above the minimum inhibitory concentration (MIC), often referred to as T > MIC [15]. However, other publications suggest a concentration–dependent effect on bacterial growth, with $AUC_{0–24}$ /MIC being the parameter that best correlates with clinical efficacy [44,45]. In a study investigating the effects of doxycycline on *M. gallisepticum*, the drug showed time–dependent behavior [46]. However, another study on *M. hyopneumoniae* found that its effect was concentration dependent [45]. These results suggest that the antibacterial activity of doxycycline may be different against gram–positive and gram–negative pathogens. At lower concentrations the drug exhibits time–dependent killing, whereas at higher concentrations it exhibits concentration–dependent kinetics [45]. In light of these findings, the efficacy of doxycycline is primarily related to maintaining plasma concentrations that exceed the MIC by 1–5 times for 40–100% of the dosing interval and achieving an $AUC_{0–24}$ /MIC ratio of ≥ 125 [47]. However, $AUC_{0–24}$ /MIC ratios of less than 125 have been required for this antimicrobial to achieve bacteriostatic (59)

and bactericidal (98) activity against *Haemophilus parasuis* in pigs [48]. Importantly, this study reports total plasma concentrations of doxycycline, but only free doxycycline has antibacterial activity and all PK/PD ratios are expressed in terms of free plasma concentration [49,50]. Therefore, this should be taken into account when estimating PK/PD ratios. To date, no data are available on the plasma protein binding rate of doxycycline in rabbits. Some minimum inhibitory concentration (MIC) values for doxycycline in mammalian species of veterinary interest have been reported in the literature. In the case of *Staphylococcus aureus* and *Salmonella spp*. the MIC determined was < 0.25 μg/ml and 2 μg/ml respectively [51]. Other values were 0.053 μg/ml for *Bordetella bronchiseptica* and 0.517 μg/ml for *Pasteurella multocida* μg/mL [52]. However, there are no data on the minimum inhibitory concentrations of doxycycline against bacterial pathogens isolated from rabbits. Therefore, future research is needed to determine the MICs of doxycycline in susceptible microorganisms isolated from rabbits in order to establish AUC $_{0–24}$ /MIC ratios specifically in this species to improve therapeutic success.

## CONCLUSION

In conclusion, our results suggest that doxycycline hyclate has a moderate tissue distribution and that the bioavailability of the formulation studied was very low after IM and SC administration in rabbits, limiting its use for the treatment of bacterial infections susceptible to this antimicrobial. However, other formulations of doxycycline or other routes of administration may need to be tested to achieve better bioavailabilities to ensure adequate dosing regimens and clinical efficacy.

### Authors' contributions

JM, EB, PM, MTY, and EE participated in the conceptualization, methodology, validation, investigation, original draft, writing, and manuscript review. MTY and JM participated in the acquisition of data and analysis. JM and PM participated in the interpretation of data. PM, EB, MTY, and EE supervised, reviewed, and editing of the manuscript. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy of the integrity of any part of the work are appropriately investigated and resolved.

### Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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## FARMAKOKINETIKA I BIORASPOLOŽIVOST DOKSICIKLINA NAKON PARENTERALNE PRIMENE KOD KUNIĆA

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Doksiciklin, član porodice tetraciklina, se široko koristi kod različitih životinjskih vrsta za lečenje infekcija izazvanih bakterijama osetljivim na ovaj antibiotik. Međutim, podaci o farmakokinetici ovog leka kod kunića su oskudni. Cilj ovog rada bio je da se ispita farmakokinetika doksiciklina nakon intravenske i ekstravaskularne (intramuskularne i subkutane) primene kod kunića. Primenjena je randomizovana unakrsna studija (n = 5), sa dozom od 20 mg/kg. Vss je bio 0,64 L/kg, što ukazuje na umerenu distribuciju antibiotika kod kunića. Maksimalne koncentracije ($C_{max}$) i vreme do pika koncentracije u plazmi ($t_{max}$) dobijene nakon ekstravaskularne primene bile su veoma slične, bez značajnih razlika između intramuskularnog i subkutanog puta. Bioraspoloživost ekstravaskularne primene bila je niska ($F_{intramuskularno}$=6,01%, $F_{subkutano}$=7,30%), ograničavajući njegovu efikasnost u lečenju bakterijskih infekcija osetljivih na doksiciklin kod kunića. Međutim, druge formulacije doksiciklina ili drugi načini primene možda će morati da se testiraju da bi se postigla bolja biodostupnost da bi se obezbedili adekvatni režimi doziranja i klinička efikasnost.