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Pharmacokinetics and adverse effects of doxycycline in the treatment of Ehrlichiosis: theoretical foundations for clinical trials in canines

Monsalve B, Santiago; Gallego L, Vanessa; Molina, Victor Manuel; Rodas G, Juan
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Revisión de Literatura

Pharmacokinetics and adverse effects of doxycycline in the treatment of Ehrlichiosis: theoretical foundations for clinical trials in canines

Farmacocinética y efectos adversos de la doxiciclina en el tratamiento de la Ehrlichiosis: fundamentos teóricos para ensayos clínicos en caninos

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Santiago Monsalve B
Corporación Universitaria Lasallista, Colombia
samonsalve@lasallistadocentes.edu.co

Vanessa Gallego L
Corporación Universitaria Lasallista (CUL), Colombia
samonsalve@lasallistadocentes.edu.co

Victor Manuel Molina
Corporación Universitaria Lasallista, Colombia
samonsalve@lasallistadocentes.edu.co

Juan Rodas G
Universidad de Antioquia, Colombia
samonsalve@lasallistadocentes.edu.co

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Resumen:

Ehrlichia canis is the causative bacterium of canine monocytic ehrlichiosis (CME), a disease of global importance in veterinary and human medicine. Several studies have addressed the therapeutic efficacy of tetracycline hydrochloride and doxycycline hyclate (HD) for the treatment of CME, however the results are still controversial. Doxycycline is the treatment of choice for canine monocytic ehrlichiosis (CME), a well characterized disease that can serve as a model for research in diseases of the Rickettsial order and tick-borne zoonoses. Although the pharmacokinetics and efficacy of the treatment in the acute and subclinical CME phases have been known for decades, some results also indicate that Ehrlichia canis may persist in clinically normal dogs, even after an extensive treatment regimen. The purpose of this review is to (major delve into/further investigate) the pharmacokinetics and side effects of doxycycline in the treatment of canine ehrlichiosis.

Palabras clave: Ehrlichia canis, farmacocinética, hematología, química sanguínea, tetraciclina, (Fuente: CAB).

Abstract:

Ehrlichia canis es la bacteria causante de la Ehrlichiosis monocítica canina (EMC), una enfermedad de importancia global en la medicina veterinaria y humana. Varios estudios han abordado la eficacia terapéutica de hidrocloruro de tetraciclina e hiclato de doxiciclina (HD) para el tratamiento de la EMC, sin embargo los resultados obtenidos siguen siendo controvertidos. La doxiciclina es el tratamiento de elección para la EMC, enfermedad bien caracterizada que puede servir como modelo para las investigaciones en el estudio de las enfermedades del orden de los Rickettsiales y las zoonosis transmitidas por garrapatas. Aunque desde décadas atrás se conoce su farmacocinética y la eficacia en el tratamiento de la EMC en fase aguda y subclínica, algunos resultados también indican que Ehrlichia canis puede persistir en perros clínicamente normales, incluso después de un amplio régimen de tratamiento. El propósito de esta revisión es hacer un acercamiento referente a la farmacocinética y los efectos adversos de la doxiciclina en el tratamiento de la EMC.

Keywords: Blood chemistry, Ehrlichia canis, hematology, pharmacokinetics, tetracycline, (Source: CAB).

Author notes

samonsalve@lasallistadocentes.edu.co

INTRODUCTION

Doxycycline is a bacteriostatic antibiotic member of the tetracycline family that has been used in systemic pathways in human medicine and domestic animals for over 40 years (1,2,3). Due to its higher liposolubility compared to other tetracyclines, doxycycline has a greater distribution volume, which allows for better tissue penetration, despite its high binding percentage to plasma proteins (80% - 90%) (3,4,5). This antibiotic is used in the treatment of multiple canine infections, including those caused by Ehrlichia canis, where the regime is recommended for a period of 28 days (4,5,6). This review presents the pharmacokinetic aspects and possible adverse effects related to the use of doxycycline. The issue addressed is important in the execution of projects related to the treatment of CME with different pharmaceutical presentations of doxycycline.

PHARMACOLOGICAL OVERVIEW

Doxycycline is an amphoteric synthetic compound from the tetracycline group which degrades in distinctly alkaline or acidic media (7,8). Like other tetracyclines, it is a broad spectrum bacteriostatic bacteriostatic drug, that includes action on microorganisms of the order Rickettsiales, Mycoplasmas and Chlamidials (8). In addition, it is used prophylactically against Plasmodium spp., the Protozoa that causes malaria (9).

Tetracyclines enter microorganisms either by passive diffusion, through hydrophilic channels formed by porins, or by energy-dependent active transport processes. Within the bacterial cell, they bind to the 30s ribosomal subunit thereby blocking the binding of aminoacyl-tRNA to the acceptor site of the mRNA ribosomal complex, impeding the protein transcription process (8,9,10,11). Some side effects have been reported in experimental studies of innovative doxycycline formulations (12). However, although tetracyclines are considered safe drugs, they are not exempt from undesirable effects in humans and animals (8), and their toxicity has been reported in different biomodels when administered intravenously (Table 1).

Table 1. Effects of intravenous doxycycline in domestic animals.

Intravenous doxycycline						
Used species	**Symptoms**	**Clinical-pathological effects**	**Cause of death**	**Administered Dose**	**Year**	**Author**
Bovines	Hypersalivation, decrease in muscle tone of the tongue, Dysphagia, dehydration, diarrhea, atrial fibrillation	Dystrophic multifocal calcification and necrosis of muscles, histiocytic and fibroblast infiltrations	Myocardial degeneration and sudden death	Aproximately between 50 and 60 mg/Kg	2004	Chiers K, et al (52)
Equines	Diarrhea and colitis, increased heart rate, increased heart rate	Muscle fasciculations	Fatal colitis, cardiac arrest.	Between 3 and 10 mg/Kg	1992	Riond JL, et al (53)
Felines and canines	Bradycardia, decrease in blood pressure	Abnormalities on the electrocardiogram	Cardiac Arrest	Not reported	1971	Scholkens, et al (54)
Canines	Not reported	No effects	Not reported	1 mg/Kg given in 5 minutes	2003	Bidgood T, et al (36)
Canines	Tissue reactions in the puncture site	No effects	Not reported	10 mg/Kg	2012	Gutierrez L, et al (11)

In general terms, these effects are related to:

a) Irritating capacity causing digestive disorders, mainly vomiting (6).

b) Aseptic abscess after parenteral application (11).

c) Alteration of intestinal microflora (13,14)

d) Calcium chelation (1).

e) Possible toxic effects on renal and hepatic cells on high doses (1)

In addition, the use of doxycycline is contraindicated in pregnant females because it is a teratogenic substance (8). In a study of 386 canine patients (with different etiologies) who received a mean dose of 16

mg / kg doxycycline per day, vomiting was reported in 18.3%, diarrhea in 7%, and anorexia in 2.5% of the canine patients studied (9).

EHRLICHIA CANIS AND THE EFFECTS OF DOXYCYCLINE IN THE TREATMENT

A total of 6 species within the genus Ehrlichia have been reported in humans and animals (Ehrlichia canis, Ehrlichia chaffeensis, Ehrlichia ewingii, Ehrlichia mineirensis, Ehrlichia muris and Ehrlichia ruminantium) based on serological and molecular evidence, however new members of the genus are continuously being Discovered (15).

CME is an infectious disease caused by the Ehrlichia canis bacterium, which is considered to be zoonotic (16,17) and emergent, transmitted mainly by the dog tick (Riphicephalus sanguineus) (18). The etiology of CME arises from infection of Gram-negative bacteria of the genus Ehrlichia (although gram staining is often not useful) classified within the order Rickettsiales and Anaplasmataceae family. (15) In both frequency and experimental studies, infection with acute disease and modification in hematological values by different agents of the Anaplasmataceae family (Ehrlichia canis, Ehrlichia chaffeensis, Anaplasma platys and Anaplasma phagocytophilum) in canine specimens and their detection in known vectors has been demonstrated (19,20,21).

The Ehrlichia genus comprises a group of intracellular, pleomorphic, often spheroid or ovoid species, which are established in monocytes, lymphocytes, neutrophils and platelets, producing intracytoplasmic morulae (22). Infection in the animal spreads via blood or lymphatic cells into infected mononuclear cells (23).

Hemogram. The Ehrlichia canis infection presents leukopenia, thrombocytopenia, anemia, hyperproteinemia, hyperglobulinemia, hypergammaglobulinemia and hypoalbuminemia (Figure 1) (22). After infected, canine patients may suffer from severe acute anemia, which is related to specific antibodies that adhere to molecules found naturally in the erythrocyte wall causing the activation of the cascade complement, resulting in intravascular lysis and opsonization of the Red blood cells in order to facilitate phagocytosis by monocytes in the liver and spleen (immune-mediated hemolytic anemia) (24,25).

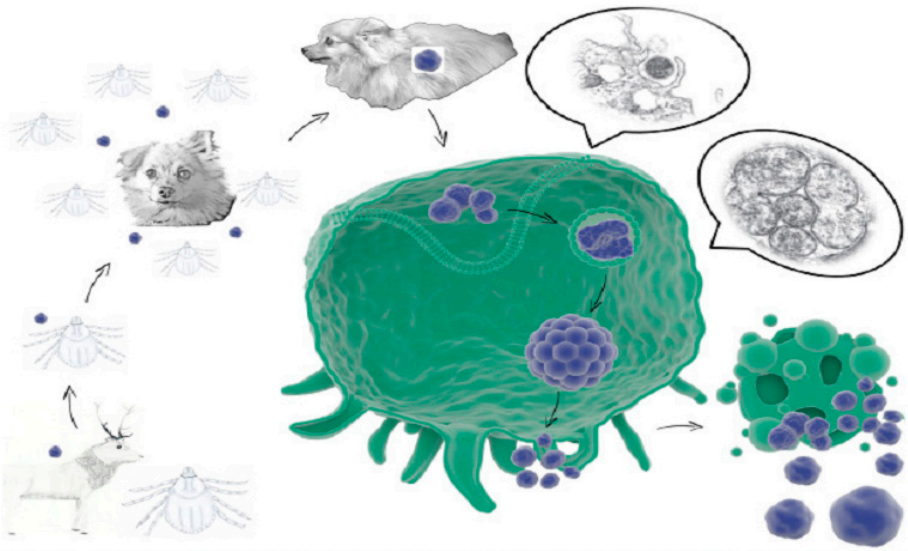

Figure 1. Tick stages can be potential vectors of the disease and transmit it to reservoirs. When canines have contact with the vectors (through the bite), by coexistence or accidental exposure with reservoirs, they acquire the disease. *Ehrlichia spp* enters the monocyte by endocytosis, forms a phagolisosoma, where it enters replication resulting in the conformation of the typical morula and by exocytosis the new bacteria come to circulation repeating the cycle. Adapted from Little et al (18); And Rikihisa (19).

Meningoencephalitis caused by Ehrlichia canis without modified hematological values has been reported in canine patients (26). Acute phase proteins (C-reactive protein) and some antioxidant markers (Haptoglobin and Paraoxonase-1) tend to change significantly in most dogs infected with Ehrlichia canis (27).

Studies in infected dogs show that treatment with doxycycline for 28 days restores erythrocyte, platelet, and hemoglobin levels (5,23).

Blood chemistry. The use of doxycycline, both in animals infected with E. canis and in healthy animals, showed a progressive decrease in serum creatinine concentration suggesting a nephroprotective effect (5).

Certain studies have described that at high doses, the use of doxycycline can lower the rate of glomerular filtration causing states of ischemia in the efferent arteriola, which may lead to an increase in serum creatinine values. One of the theories is the reduction of the oxidative stress, decreasing cytokine levels and inhibiting the activity of matrix metalloproteinases (5,30). The anti-inflammatory and immunomodulatory properties of doxycycline associated with these effects have been determined in different studies (31,32).

An oral dose of doxycycline close to 16 mg/kg/day was given throughout a study in 386 canines with CME. An increase (under normal species-level and dose-dependent levels) was reported in levels of alanine aminotransferase and alkaline phosphatase in 39.4% and 36.4% of the dogs studied, respectively (13). This may indicate that the drug not only causes alteration in filtration, as described by several authors, but could also lead to hepatic alterations due to its lipophilic characteristics and the extensive metabolism of the antibiotic (13).

IMMUNE SYSTEM

Globulins. Following the dose of 10 mg/kg doxycycline, an increase of α2-globulins was reported in the treatment for Ehrlichia canis, whereas gamma-globulins show a statistically non-significant decrease, attributed to the low production of antibodies. After the intervention, an increase in the serum concentration of B-globulins was demonstrated, which could be related to an increase in B lymphocytes in subclinical or chronic disease (5,7).

T lymphocytes. During the treatment of CME, an increase in circulating cytotoxic T lymphocytes (CD3+ and CD8+) and lymph nodes was reported (33,34). However, other studies have shown that the doxycycline regimen decreases the cytotoxic T lymphocyte count. This effect may be related to the antimicrobial activity of doxycycline (5). However these cells may be responsible for immunopathological mechanisms caused by infection (35).

B lymphocytes. B (CD21+) lymphocytes increase after treatment. However, they later and gradually decrease to normal values. The humoral immune response has not been considered a protective mechanism against Ehrlichia canis infection. Therefore, the initial increase in B lymphocytes may be related to therapeutic efficacy (36). Additionally, the increase in beta-globulins following doxycycline treatment could be associated with increased B lymphocytes (5).

Major Histocompatibility Complex (MHC). Following delivery of a dose of oral doxycycline at 10 mg/kg, absolute counts of lymphocytes expressing major class II histocompatibility complex (MHC-II) suggest a direct effect of the active principle on the expression of MHC-II on the surface of peripheral blood lymphocytes. Alterations in the genetic expression of MHC may explain the anti-inflammatory effects of tetracyclines (5).

Studies comprising the expression of MHC class I and II receptors on DH82 (canine cell lines with malignant histiocytoma) cells infected with Ehrlichia canis, found that 46.9% of uninfected cells can express MHC-II molecules, while that these receptors were not detected in Ehrlichia canis-infected cells (37). This indicates that DH82 canine macrophages cell lines infected with Ehrlichia canis are unable to regulate the expression of class II MHC receptors, and suggests the mechanism in which the bacteria could evade the immune system (37).

DOXYCYCLINE PHARMACOKINETICS

In order for doxycycline to exert its bacteriostatic action against Ehrlichia canis, a minimum inhibitory concentration should be reached at the plasma level, which in turn is in balance with that obtained in intracellular spaces (Table 2). The pharmacokinetic processes that determine the concentrations reached in the different organic compartments can be summarized as:

Table 2. Minimum inhibiting concentration determined as bacteriostatic level for treatment of microorganisms of the *Anaplasmataceae* family.

MIC for infection by microorganisms of the Anaplasmataceae family				
Model	**Microorganism**	**MIC**	**Year**	**Author**
Equine, canine, human	*A. phagocytophilum*	0.03 µg/ml	2003	Maurin M, et al (39)
Invitro	*Ehrlichia spp.*	0.125 µg/ml	1990	Brouqui P, et al (55)
DH82 cell lines	*E. canis, E. chaffeensis, A. phagocytophilum*	0.03 µg/ml	2004	Branger S, et al (56)
Canines	*E. canis*	0.024 µg/ml	2005	Davoust B, et al (57)

Absorption. Doxycycline, unlike other molecules in the tetracycline family, has a greater oral absorption with values ranging from 90-95% due to its high liposolubility (8,12,38). It can also bind to plasma proteins up to 88±5% and has a bioavailability of 93%, therefore it could reach plasma concentrations of 2.8 µg/ml on average (9,12).

Food delays the absorption of doxycycline, therefore it is recommended to supply the medication in the absence of food. However, this can cause lesions on the esophageal and stomach mucosa (36,37,39). In addition, the absorption may be reduced by the presence of divalent or trivalent cations (calcium, magnesium, iron, zinc, aluminum and bismuth as salicylate) which chelate the drug formed an inactive complex (36).

The maximum concentration of doxycycline is detectable within 2 hours after delivery, with serum levels of 1.7 µg /ml to 3µg/ml, which corresponds to relative doses between 5 and 10 mg/kg (5,11).

Distribution. The lipophilic characteristics and the high volume of distribution (>1.0 L/kg) of doxycycline (as well as for other tetracyclines), facilitate a wide distribution throughout the organism (40,41) allowing the accumulation of these substances in various tissues (12,42).

Certain factors, such as protein binding and lipid solubility, influence the passage of tetracyclines from the blood into interstitial space and tissues. (4) The free medication diffuses freely to tissues and liposolubility allows it to pass through membranes. High protein binding causes this pharmaceutical to have a long half-life (15.1 hours) compared to other tetracyclines (43). On the other hand, this active principle can be 5 times more liposoluble than oxytetracycline, a property that allows it to cross the cell membrane easily (43,44).

Clearing. Clearance is defined as the volume of plasma that is purified from one drug per unit of time (37). The metabolism of doxycycline, like other tetracyclines, is due to an oxidation at the level of the cytochrome p450 hepatic enzyme complex that allows its renal and/or hepatic excretion (8,9,45). Bile duct doxycycline may have enterohepatic recirculation that prolongs its half-life in the body by delaying its metabolism. In fact, it has been reported that the half-life of doxycycline is 16 ± 6 hours. Therefore, its average time is higher than the other tetracycillins, which could influence its therapeutic action and posology around 24 hours (8,9,43). Other authors (5) indicate that the half-life of doxycycline is in the range of 16-18 hours depending on the plasma concentration (the use of doxycycline every 12 hours can be considered therapeutically viable).

Elimination. Doxycycline, unlike other tetracyclines, can be eliminated by mechanisms other than the renal route. Concentrations of doxycycline in bile close to 50 µg/ml (25 times higher than plasma concentrations obtained) (42). The elimination of doxycycline by the urinary route is 20%, 75% of the doxycycline passes from the blood to the intestinal lumen by passive diffusion, and another 5% from the bile and is then excreted in the faeces (9,12, 46).

The elimination half-life (t1/2e) is the time it takes for the plasma concentration of a medication to be reduced by half and is inversely related to the elimination constant, so the faster the elimination of the component, the greater the Elimination constant (Ke) and smaller elimination half-life (36).

t1/2e= 0.693/Ke

The reported half-life of doxycycline in dogs is 16-18 hours (8,9,10,47) with an elimination rate using doses of 10 mg/kg is 5.65 ± 2.76 mL/kg/min (339mL/kg/Hour) (46). However, in studies with 6 dogs using intravenous doxycycline hyclate at a dose of 5 mg/kg body weight, a t1/2e of 10.36 hours was obtained (38). On the other hand, using oral doxycycline polyphosphate at 10 mg/kg was reported in a group of 4 canine individuals with a t1/2e between 10.7 and 11.8 hours (48). The clearance data indicates that the rate is about 1.7 mL/kg/min (102 mL/kg/hr), which should be taken into account when using a drug encapsulated in a microencapsulated system, as lower values may indicate a bioaccumulation process. Some authors consider that this storage could affect the rate of glomerular filtration and lead to hepatic overload (and consequently a possible insufficiency of this organ) (8,12,42). The pharmacokinetic criteria obtained after the administration of intravenous doxycycline has been reported in different studies obtaining similar results (Table 3).

Table 3. Pharmacokinetic aspects after administration of intravenous doxycycline in canine specimens: T1/2a mean distribution half-life, T1/2b mean elimination half-life, Vd (ss) steady-state distribution volume, Vc Central compartment volume, PB Protein binding percentage, Cl Clarification, AUC 0-∞ Area under curve from 0 to infinity.

Pharmacokinetic criteria obtained after intravenous administration in dogs				
Pharmacokinetic criteria	Unit	Riond (3) 5mg/kg	Bidgood (46) 10mg/ml	Gutierrez (11) 10mg/Kg
T1/2a	Hours	0.25 +/- 0.04	0.08 +/- 0.05	0.28 +/- 0.01
Vd(ss)	L/Kg	0.93 +/- 0.14	0.65 +/- 0.08	11.34 +/- 1.34
T1/2b	Hours	6.99 +/- 1.09	4.56 +/- 0.57	7.44 +/- 0.06
Vc	L/Kg	0.34 +/- 0.05	Not reported	6.37 +/- 0.76
Cl	ml/min/Kg	1.72 +/- 0.17	1.66 +/- 2.21	2.23 +/- 0.13
PB	%	91.40 +/- 0.93	91.75 +/- 0.63	Not reported
AB	%	53.87 +/- 5.18	Not reported	Not reported
AUC 0 - ∞	h*µg/ml	Not reported	12.09 +/- 3.22	97.34 +/- 7.45

DOXYCYCLINE HYCLATE IN THE EXPERIMENTAL TREATMENT OF CANINE EHRLICHIOSIS

Although several studies have addressed the therapeutic efficacy of tetracycline hydrochloride or HD for the treatment of Ehrlichia canis infection in dogs, the efficacy of these drugs remains controversial (49,50).

Breitschwerdt et al (50) evaluated the efficacy of oral HD in the treatment of acute Ehrlichiosis. 8 dogs were experimentally infected with doses of 5.6 mg/kg and 6 mg/kg, in this study, twice a day for 14 days (two study groups). The result obtained was the non-amplification of gene segments for Ehrlichia canis after the treatment. In this case, doxycycline appears to have been uniformly effective in eliminating the infection in treated dogs (50). However, a study that included the use of doxycycline in canine specimens classified in the 3 phases of CME (acute, subclinical and chronic) at a dose of 10 mg/kg per day for 28 days, reported 8 dogs in the acute and subclinical phase of CME that did not show amplified gene segments for Ehrlichia canis by PCR. However there was an intermittent persistence of bands in the electrophoresis of two individuals with chronic Ehrlichiosis and in ticks that were experimentally fed (51). Another study evaluated the persistence

of Ehrlichia canis in 5 dogs treated with doxycycline for 7 days at a dose of 10 mg/kg per day, and the amplification of DNA segments of the bacteria in blood, kidney, lymph nodes, liver and spleen in 3 specimens of the 5 dogs studied was determined. However, in this investigation, in a group of 8 dogs treated for 14 days, no positive results were observed for Ehrlichia canis by PCR (49). The results of these investigations confirm the efficacy of doxycycline to counteract the acute and subclinical CME of the disease. Several studies indicate that Ehrlichia canis may persist in clinically normal dogs, even after a broad regimen of doxycycline treatment (49,51)

THERAPEUTIC PERSPECTIVES

Different publications show the need to delve into clinical trials to determine the possible causes of post-treatment CME flare-ups in the tetracycline family. Persistent Ehrlichia canis infections are an indicator of the importance of continued clinical monitoring, including after a response to antibiotic treatment. All dogs used in studies with experimental infections show signs of acute severe CME, and blood parameters usually return to normal levels after doxycycline treatment. The study of the use of new formulations (microencapsulated or nanostructures) is also important. This is in reference to the treatment to counteract the presence of Ehrlichia canis in canine patients (Investigation with products coated with liposomes, biopolymers, biopolymers, drug delivery systems of doxycycline, etc.), which provide greater specificity and effectiveness in treatment, and an evident decrease in the multiple undesirable effects, typical of this type of antibiotic. New strategies in pharmaceutical pharmaceutical technology could favor the controlled release of the medication, increase its half-life, distribution and the bioavailability necessary to produce bacteriostatic effects where optimal therapeutic action and medical resolution of the canine patient.

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