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PROGRAMA DE MAESTRIA Y DOCTORADO EN CIENCIAS DE LA PRODUCCION Y DE LA SALUD ANIMAL

DESARROLLO DE VALORES FARMACOCINETICOS, FARMACODINAMICOS Y CLINICOS DE UN PREPARADO DE LIBERACION SOSTENIDA Y LARGA ACCIÓN DE DOXICICLINA HICLATO EN EQUINOS

T E S I S

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CONTENIDO

1. LISTA DE ABREVIATURAS

AUC	área bajo la curva
AUC0-24	área bajo la curva en el intervalo de 0 a 24 horas
AUCDox-a	área bajo la curva de la solución acuosa de doxiciclina
AUCDox-pol	área bajo la curva de la formulación de doxiciclina en una matriz de poloxámero
Cmax	concentración máxima
CMI	concentración minima inhibitoria
Dox	doxiciclina
Dox-a	solución acuosa de doxiciclina
Dox–β	formulación de doxiciclina en una matriz de β ciclodextrina
Dox-pol	formulación de doxiciclina en una matriz de poloxámero 407
Dox-SR	formulación experimental de liberación modificada y larga acción
EV	endovenosa
F	biodisponibilidad
Frel	biodisponibilidad relativa
GI	gastrointestinal
HPLC	cromatografía líquida de alta definición
IM	intramuscular
LA	larga acción
MF	meglumina de flunixin

MDA	método microbiológico cuantitativo/cualitativo de difusión en agar
MMP	metaloproteinasas matriciales
Pen G	penicilina G procaínica
PD	farmacodinamia
PK	farmacocinética
PK/PD	relación farmacocinética/farmacodinamia
PO	per os
RA	reacciones adversas
SC	subcutánea
Tmax	tiempo máximo
T½ el	vida media de eliminación

2. RESUMEN

Doxiciclina (Dox) es una tetraciclina semi-sintética de segunda generación con ventajas farmacológicas sobre las tetraciclinas naturales. Es utilizada en el tratamiento de una amplia variedad de enfermedades bacterianas en caballos. Sin embargo en la actualidad no existe una formulación farmacológica específicamente diseñada para esta especie. De acuerdo a su relación farmacocinética/farmacodinamia (PK/PD), la Dox es considerada un antibacteriano tiempo-dependiente y que fundamenta su efectividad clínica en el mantenimiento de concentraciones plasmáticas sostenidas sobre la concentración mínima inhibitoria (CMI) durante un tiempo prolongado entre dosificaciones. El objetivo de la primera parte de esta tesis fue el desarrollo de dos formulaciones de larga acción (LA) de doxiciclina hiclato y la definición de sus valores farmacocinéticos después de la administración por vía oral en caballos adultos clínicamente sanos no dietados. Con base en los resultados obtenidos, se eligió la formulación con el mejor perfil PK/PD para llevar a cabo la segunda parte de esta tesis. Se condujo un estudio clínico longitudinal, de no inferioridad y multicéntrico en un contexto no hospitalario para demostrar la efectividad de la formulación experimental de liberación modificada y larga acción de doxiciclina hiclato al 20% (Dox-MR) que fue elegida, para la prevención o tratamiento de heridas infectadas en caballos adultos, en comparación con el tratamiento con Penicilina G procaínica (Pen G) con o sin tratamiento concomitante con meglumina de flunixin (FM). Se concluyó que el tratamiento con Dox-SR o con Dox SR + FM son igualmente efectivos que el tratamiento con Pen G para prevenir o tratar infecciones en heridas en caballos en un escenario clínico.

Palabras clave: doxiciclina, farmacocinética, caballos, pasta oral, heridas

3. ABSTRACT

Doxycyline (Dox) is a semisynthetic antibacterial drug with pharmacological advantages over its parent drug (tetracycline) in the treatment of various bacterial diseases in horses. Yet, at present a horse-customized pharmaceutical formulation is not available. Based on its pharmacokinetic/pharmacodynamic (PK/PD) ratios, Dox is considered a time-dependent antibacterial drug and ideally is expected to achieve sustained plasma drug concentrations both at or slightly above the minimal inhibitory concentration (MIC) for as long as possible between dosing intervals. Hence, the objective of the first part of this study was to formulate several long-acting (LA) doxycycline hyclate pastes for oral administration and define their pharmacokinetics in non-fasted adult horses, in an attempt to obtain values that would better comply with its pharmacokinetic/pharmacodynamic (PK/PD) ratios. A poloxamer based preparation showed improved PK as compared to the aqueous dissolution of the drug. In accordance with the results obtained, the formulation with the best PK/PD profile was chosen to conduct the second part of this study. A multicenter longitudinal non-inferiority clinical trial, in a non-hospital environment in horses was done to assess the efficacy of the chosen modified release long-acting formulation of doxycycline hyclate 20% (Dox-MR) in preventing or treating infected wounds, in comparison to treatment with procaine penicillin G (Pen G) with and without flunixine meglumine (FM). It was concluded that treatment with either Dox-SR or Dox-SR + FM, are as effective as treatments with Pen G to either prevent or treat wound infections in horses in a clinical setting.

Keywords: doxycycline, horses, pharmacokinetics, oral paste, wounds

4. TRABAJOS GENERADOS POR ESTA TESIS

Artículos en revistas indexadas

Publicados

Zozaya, H., Gutiérrez, L., Bernad, M.J., Sumano, H. (2013) Pharmacokinetics of a peroral single dose of two long-acting formulations and an aqueous formulation of doxycycline hyclate in horses. *Acta Veterinaria Scandinavica* , 55. 21 http://www.actavetscand.com/content/55/1/21.

Zozaya, H., Gutiérrez, L., Tapia, G., Sumano, H. (2014) Clinical efficacy of a long acting doxycycline experimental formulation in wound infections in horses, *Wulfenia Journal* 21(7);166 – 177.

En preparación

Zozaya, H., Gutiérrez, L., Miranda J., Sumano, H. Pharmacokinetics of a multiple dose of a stabilized long acting formulation of doxycycline in adult horses

5. CAPITULO 1

5.1 MARCO TEORICO

5.1.1. Antibioticoterapia en equinos

La necesidad de utilizar antimicrobianos en pacientes equinos en situaciones en las que la vida o la función zootécnica del animal están comprometidas es frecuente (Haggett y Wilson, 2008; Hollis y Wilkins, 2009). Las entidades clínicas en esta especie en las que resulta indispensable utilizar fármacos antimicrobianos son múltiples y muy variadas siendo las más importantes, infecciones primarias o secundarias en el árbol respiratorio superior e inferior, sistema cardiovascular, sistema gastrointestinal incluyendo peritoneo, sistema nervioso central (SNC) , músculos, huesos y articulaciones, estructuras umbilicales e infecciones septicémicas en neonatos, piel, aparato genitourinario, córnea y otras estructuras oculares, entre otras (Sellon y Long, 2013).

Sin embargo la gama de fármacos antibacterianos que se pueden utilizar con seguridad en la práctica clínica diaria en equinos es reducida, si se compara con otras especies. Esto puede atribuirse en gran parte a la frecuencia de presentación de reacciones adversas (RA) asociadas a su uso (Sumano et al., 2000).

La administración de antibacterianos en equinos por vía parenteral presenta algunas limitaciones. No es muy conveniente utilizar un antimicrobiano que, por su relación farmacocinética/farmacodinamia (PK/PD), requiera ser administrado varias veces al día y durante varios días por vía intramuscular (IM) debido a la alta probabilidad de producir RA locales, desde dolor, inflamación y edema , hasta miositis y/o mionecrosis clostridial (Vengust et al., 2002). La miositis clostridial a su vez, puede traer consigo consecuencias graves en el animal, como laminitis, edema pulmonar y muerte (Peek et al, 2002). Estos esquemas de tratamiento además, aumentan el riesgo para el médico o la persona que lo administra porque requiere un mayor manejo del animal,

lo que a su vez tiene como consecuencia inconsistencia en los esquemas de tratamiento y por ende, falla terapéutica (Zozaya et al., 2008).

El vehículo de formulaciones de antibacterianos llamados de larga acción (LA) utilizadas en los preparados de oxitetraciclina, amoxicilina y otros, como propilenglicol y polivinilpirrolidona, muy útiles en bovinos y porcinos para reducir la frecuencia de las aplicaciones, están contraindicadas en equinos por el dolor e inflamación severa que producen en el sitio de la aplicación si son administrados por vía intramuscular (IM) o subcutánea (SC) (Sumano, et al., 2000). Por otro lado, si se utiliza la vía endovenosa (EV), fácilmente producen colapso circulatorio o choque de velocidad medicamentoso (Dowling y Russell, 1999).

Es claro que no es deseable una reacción inflamatoria en el sitio de la aplicación, no sólo por las consecuencias mencionadas anteriormente, sino porque disminuye la absorción del fármaco y puede haber, por lo tanto, falla terapéutica (Ahmed y Kasraian, 2002).

Adicionalmente, todos los antibacterianos tienen el potencial de ocasionar cambios en la población microbiana intestinal del ciego y colon del caballo, esto es, proliferación de *Clostridium difficile* y *Salmonella* spp, independientemente de la vía por la que son administrados. La enterocolitis asociada al uso de antimicrobianos puede tener consecuencias fatales para el animal (Gustafsson, 2004).

Finalmente, otro factor que limita la selección de un antibacteriano adecuado en equinos, es que existe poca información documentada con respecto a valores PK, relación farmacocinética/farmacodinamia (PK/PD) y pruebas clínicas tanto en caballos adultos como en potros de la mayoría de estos fármacos (Morley et al., 2005; Zozaya et al., 2013).

Por lo mencionado anteriormente, las preparaciones farmacéuticas específicamente diseñadas para uso en equinos y más aún en nuestro país, son escasas.

5.1.2. Doxiciclina en equinos

Un ejemplo de un fármaco antibacteriano que ha sido utilizado sin diseño farmacológico en equinos es la doxiciclina (Dox). Este fármaco es una tetraciclina semisintética de 2ª generación, con ventajas farmacocinéticas y farmacodinámicas sobre las tetraciclinas naturales, como mayor liposolubilidad, mejor penetración a los tejidos, mayor volumen de distribución, mejores propiedades antimicrobianas y vida media más prolongada (Chopra y Roberts, 2001; Haggett y Wilson, 2008; Riviere y Spoo, 2001). Debido a los mecanismos de biotransformación y excreción, la doxiciclina también produce menos efectos adversos en el tubo gastrointestinal (GI) del caballo. Según Agwuh y McGowan (2006) solamente un 18% de la dosis administrada se excreta a través de las vías biliares, mientras que el resto del fármaco se inactiva en la pared intestinal mediante quelación con iones Ca^{+2} y Zn^{+2}, mecanismo mediante el cual se previene el ciclo enterohepático. Se sabe que en perros la doxiciclina se biotransforma hasta en un 40% y se excreta principalmente por bilis y secreciones intestinales (<5% y 75%, respectivamente) en forma de un metabolito microbiológicamente inactivo (Barza et al., 1975).

El espectro antibacteriano de la doxiciclina es amplio: actúa contra una gran variedad de bacterias Gram + y Gram –, clamidias, rickettsias, micoplasmas, espiroquetas y algunos protozoarios de importancia clínica en equinos (Chopra, 2001; Jacks et al., 2003). Tiene una alta efectividad contra bacterias intracelulares como *Rhodococcus equi* y *Lawsonia intracellularis* (Pusterla y Gebhart, 2013) y muestra mayor actividad que otras tetraciclinas contra algunas bacterias anaerobias (Aronson, 1980). Es el tratamiento de elección para fiebre del Potomac causada por *Neorickettsia risticii* (Lewis et al., 2009) y enfermedad de Lyme causada por *Borrelia burgdorferi* (Chang et al., 2005). La mayoría de los miembros de las enterobacterias (*Escherichia coli* y *Vibrio cholerae*) son sensibles a la doxiciclina (Dubey, 2001; Kyriakis, 2002). En el ser humano, es el antimicrobiano de elección para al tratamiento de *Leptospira*, sp. (Edwards y Levett, 2004). Sin embargo, la eficacia

de doxiciclina contra la uveítis causada por leptospirosis en equinos es aún desconocida (Weese, 2009).

A diferencia de las tetraciclinas naturales, la doxiciclina atraviesa la membrana celular a través de transporte pasivo (Plump, 2002). Se une a la subunidad ribosomal 30S, lo que inhibe el crecimiento bacteriano (Kyriakis, 2002; Womble et al., 2007) interfiriendo con la unión de la RNA-aminoaciltransferasa al sitio receptor en el complejo RNA mensajero, inhibiendo a su vez la incorporación de aminoácidos a la cadena peptídica en crecimiento y como consecuencia la síntesis de proteínas (Shaw y Rubin, 1986). Se cree que la doxiciclina también se une a los ribosomas 50S y altera la permeabilidad citoplasmática de los microorganismos sensibles (Plump, 2002). En algunos microorganismos ejerce efecto bacteriostático, mientras que en otros y a mayores dosis, tiene efecto bactericida. (Shaw y Rubin, 1986; Riond y Riviere, 1989). Los microorganismos resistentes poseen un plásmido R que produce una disminución en la absorción del fármaco. (Cunha et al. 1982; Riond y Riviere, 1989).

La doxiciclina presenta acciones independientes de su efecto antibacteriano. A dosis subantimicrobianas impide la proteólisis a través de la inhibición de la acción de las metaloproteinasas matriciales (MMP), incluyendo las colagenasas, gelatinasas y estromelisinas así como la acción de citocinas inflamatorias y óxido nítrico (NO) (Watts et al. 2007; Wilcox et al., 2012; Serra et al., 2013). Se ha documentado tanto *in vitro* como en estudios clínicos en seres humanos y perros que la doxiciclina tiene efectos condroprotectores, (Krakauer y Buckley, 2003; Nganvongpani et al., 2009) ya que inhibe la proliferación e hipertrofia de condrocitos (Sapadin y Fleischmeyer,2006) . En seres humanos se reporta su uso como potente inductor de la reparación tanto de heridas crónicas en piel y úlceras varicosas (Wilcox et al., 2012; Serra et al., 2013) como de úlceras corneales (Smith y Cook, 2004).

La doxiciclina se utiliza frecuentemente en potros y caballos adultos en forma de solución acuosa al 10% preparada al momento, poco antes de la administración por vía oral (PO) mediante sonda

nasogástrica a dosis de 10 mg cada 12 h. Este método presenta varias desventajas como costo, consumo de tiempo, desperdicio del principio activo, necesidad de conocimientos técnicos, mayor manejo del animal, así como riesgo y estrés, tanto para el animal mismo como para el veterinario y el caballerango. Estos factores causan inconsistencias en el esquema de tratamiento y por ende, fallas terapéuticas

.5.2 JUSTIFICACION

Dada la susceptibilidad de los patógenos causantes de infecciones en caballos y por la escasa disponibilidad de formulaciones antibacterianas diseñadas para esta especie, resulta indispensable contar con una presentación farmacéutica de doxiciclina (Dox) de diseño exclusivo para ser usada en la práctica clínica diaria especializada. Por sus bases PK/PD, idealmente Dox debe ser diseñada en una formulación de liberación modificada y LA. Adicionalmente y dada la capacidad irritante de la Dox si es administrada por vía parenteral, el preparado debe ser en forma de pasta para administración PO, no debe dañar al epitelio GI y no debe ser rechazada por el caballo. Se persigue un intervalo de dosificación de 24 horas para poder tener la opción de suspender un esquema de tratamiento dado y así poder evitar consecuencias adversas por una acción más prolongada de lo deseado *v.g.*, colitis y diarrea, que en equinos puede ser mortal. Es evidente, además, que debe lograr valores PK congruentes con su PD. Finalmente se busca que pueda utilizarse con buenos resultados terapéuticos en infecciones causadas por bacterias susceptibles.

5.3 HIPOTESIS

HIPOTESIS GENERAL

Es factible elaborar un preparado de doxiciclina hiclato de liberación modificada y larga acción para uso en equinos que cumpla con la relación PK/PD y que resulte efectivo en casos clínicos en esta especie.

HIPOTESIS PARTICULARES

Dada la biocompatibilidad de los polímeros que se usan para lograr LA es factible lograr formulaciones que se administren PO y que no irriten mayormente el tubo GI.

Es factible llevar a cabo una fase de evaluación PK en caballos adultos clínicamente sanos y no dietados para elegir el preparado que logre la congruencia PK/PD de Dox.

Es factible utilizar la formulación elegida en la fase anterior para llevar a cabo un estudio clínico longitudinal, de no inferioridad y multicéntrico en un contexto no hospitalario para determinar su efectividad en la prevención o tratamiento de infecciones en heridas en caballos adultos

5.4 OBJETIVOS

OBJETIVO GENERAL

Elaborar un preparado de doxiciclina hiclato de liberación modificada y larga acción para uso oral en equinos con el que se obtenga una relación PK/PD óptima y que pueda utilizarse en estudios clínicos en esta especie.

OBJETIVOS PARTICULARES

Elaborar prototipos de doxiciclina con base en la utilización de polímeros y matrices similares.

Determinar la formulación con mejor perfil PK/PD en caballos adultos sanos clínicamente y no dietados (véase Capítulo 2).

Dada la congruencia PK/PD del preparado de la fase anterior, utilizarlo para llevar a cabo un estudio clínico longitudinal, de no inferioridad y multicéntrico en un contexto no hospitalario para

determinar su efectividad en la prevención y tratamiento de infecciones en heridas en caballos adultos (véase Capítulo 3).

6. CAPITULO 2

6.1. PRIMER ARTICULO PUBLICADO

Farmacocinética de una dosis única por vía oral de dos formulaciones de larga acción y una formulación acuosa de doxiciclina hiclato en caballos

Zozaya, H., Gutiérrez, L., Bernad, M.J., Sumano, H. (2013) Pharmacokinetics of a peroral single dose of two long-acting formulations and an aqueous formulation of doxycycline hyclate in horses. *Acta Veterinaria Scandinavica* , 55. 21
http://www.actavetscand.com/content/55/1/21.

7. CAPITULO 3

7.1. SEGUNDO ARTICULO PUBLICADO

Eficacia clínica de una formulación experimental de larga acción de doxiciclina en infecciones en heridas en caballos

Zozaya, H., Gutiérrez, L., Tapia, G., Sumano, H. (2014) Clinical efficacy of a long acting doxycycline experimental formulation in wound infections in horses, *Wulfenia Journal* 21(7), p.166 – 177.

8. CAPITULO 4

8.1. DISCUSION

Debido a la concordancia que existe entre el método microbiológico cuantitativo/cualitativo de difusión en agar (MDA) y la cromatografía líquida de alta definición (HPLC) utilizada en este estudio para determinar las concentraciones séricas de doxiciclina (Dox), se considera lo suficientemente confiable para asumir que las concentraciones obtenidas a través de este último método son biológicamente activas (ver Capítulo 1) Debido a que el MDA determina la(s) fracción(es) activa del fármaco, ofrece datos clínicamente significativos. En este caso, tal suposición ha sido validada mediante un método puramente químico. Esto permite hacer especulaciones veraces en cuanto a la relación entre concentraciones séricas e intervalos de dosificación para patógenos específicos. Las concentraciones séricas obtenidas después de la administración oral de Dox en una matriz de β ciclodextrina (Dox−β) fueron notablemente bajas (concentración máxima (Cmax) = 0.2 ± 0.0 μg/mL; área bajo la curva (AUC) = 1.5 ± 0.1 μg · h/mL). Estos valores no se encuentran en el rango considerado efectivo para la mayoría de los patógenos encontrados en equinos (Bryant et al., 2000; Jacks et al., 2003), por lo que este preparado no es mencionado en esta sección. Se ha determinado la farmacocinética de la solución acuosa de Dox en caballos adultos a un rango de dosificación de 3 a 20 mg/kg. (Bryant et al., 2000; Davis et al., 2006; Schnabel et al., 2010; Winther et al., 2011). Davis et al. (2006) obtuvieron una Cmax = 0.9 ± 0.2 μg/mL con un tiempo máximo (Tmax = 1.6 ± 1.3 h) después de la administración PO de una dosis única de 20 mg/kg. En comparación, en este estudio se obtuvo una Cmax de 0.3 ± 0.1 μg/mL con un Tmax = 2.2 ± 0.4 h administrando la solución acuosa de Dox (Dox-a) PO a una dosis única de 10 mg/kg . Se puede atribuir la diferencia encontrada entre estos dos estudios a la dosis

administrada y a la variabilidad biológica, pero principalmente al hecho de que Davis et al. (2006)

dosificaron caballos dietados. Por el contrario, los valores de Cmax y Tmax para la formulación de

Dox en una matriz de poloxámero 407 (Dox-pol) fueron de 1.3 ± 0.6 μg/mL y 5.9 ± 1.6 h,

respectivamente. Un valor mayor de Cmax con una dosis igual o menor, puede ser el resultado de

acumulación sérica del fármaco debido a una absorción con cinética de orden 0, producida tanto

por los polímeros incluidos en la fórmula del preparado, como por una mayor superficie de

absorción en el tubo GI. Esta afirmación podría explicar a su vez, el mayor Tmax observado. El

AUC obtenido por Davis et al. (2006) administrando 20 mg/kg fue de 13.3 ± 2.7 μg.h/mL, mientras

que el AUC obtenido con la formulación Dox-pol con la mitad de la dosis fue de 17.0 ± 2.2

μg.h/mL, lo cual suele suceder con preparaciones LA que presentan una cinética de tipo *flip flop*

(Baggott, 1992). Esta cinética también explicaría la biodisponibilidad relativa (Frel) con la Dox-

pol, que alcanzó un valor de 548%. A su vez, con el fin de demostrar farmacocinética de tipo *flip

flop* debe considerarse el perfil general de la curva concentración vs tiempo del fármaco. En

ocasiones, como es en este caso, no es tan sencillo concluir que la velocidad de absorción es menor

que la velocidad de eliminación. Si al administrar un fármaco por vía extravascular, la vida media

de eliminación (T½ el) es mayor en comparación con la vía endovenosa (EV), se sugiere que existe

una cinética *flip flop* (Yañez et al., 2001). Sin embargo, esto no es posible en el caso de Dox, ya que

su administración por vía EV está contraindicada en caballos (Riond et al., 1989; Riond et al.,

1992). Por lo tanto, con base en información publicada (Boxenbaum, 1998) y en la siguiente

ecuación:

$$\text{Velocidad de absorción} = Vz (KC + (\Delta C / \Delta t))$$

En donde Vz es el volumen de distribución terminal exponencial, K es la constante de velocidad de

eliminación terminal una vez que el fármaco ha sido absorbido en su totalidad, C es la

concentración plasmática al tiempo t y ΔC es la variación en concentración plasmática durante el intervalo Δt.

La velocidad de absorción tomando los datos de concentración plasmática vs tiempo para Dox-pol a las 4 y 12 horas es de ΔC/Δt = 0.0176 μg/mL/h. En el punto medio de este periodo (8 h), KC = 0.1339 μg/mL/h. Ya que KC > ΔC/Δt, la velocidad de absorción ≈ velocidad de eliminación. Por lo tanto, se puede afirmar que existe una condición *flip flop* y la formulación Dox-pol aquí descrita puede considerarse de liberación modificada y LA.

El polímero utilizado como matriz – vehículo de liberación fue poloxámero 407, un co-polímero tribloque de óxido de polietileno - óxido de polipropileno – óxido de polietileno. Se ha demostrado que aumenta la solubilidad y permeabilidad, lo que resulta en una mayor biodisponibilidad (F) oral, como lo reportado por Kahn et al. (2012) utilizando atorvastatina, carbamacepina y otros fármacos con baja solubilidad.

Tomando en consideración los índices de permeabilidad y solubilidad de doxiciclina, existe controversia en cuanto a la clasificación de este fármaco en el Sistema de Clasificación Biofarmacéutica (BCS) en humanos[1]. Anteriormente, Amidon et al. (1995) clasificaron a la Dox en Clase IV esto es, poco soluble y poco permeable. Sin embargo, más recientemente, Chavda et al. (2010) la incluyeron en la Clase I, considerándola altamente soluble y altamente permeable, lo cual sin embargo, no es congruente con el valor de F absoluta para Dox descrito en caballos de 2.4% a 17% (Winther et al., 2011; Davis, 2006). En este estudio, no se determinó la F absoluta de las dos formulaciones de larga acción (Dox-pol) debido al riesgo de toxicidad cardiovascular (Riond et al., 1992) al determinar la cinética por vía EV. La Frel para Dox-pol y Dox-β en caballos no dietados, fue de 548% y 48%, respectivamente, en comparación con Dox-a. Aun cuando se ha demostrado una absorción oral disminuida de Dox en caballos no dietados (Lakritz et al., 2000; Davis, 2006) consideramos que pudiera haber otros factores que aumentaran la F de Dox administrada en la

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[1]http://www.fda.gov/AboutFDA/CenterOffices/OfficeofMedicalProductsandTobacco/CDER/ucm128219.htm

formulación Dox-pol: mucoadhesividad lograda por los polímeros poloxámero, carbopol y goma xantana incluidos en la formulación (Hoon et al., 2005; Kumar et al., 2010), probablemente circulación enterohepática, considerando que, con algunas excepciones, este fenómeno es considerado como una característica propia de las tetraciclinas y otros fármacos (Agwuh & McGowan, 2006). Además el mayor volumen de fluido del tubo GI del caballo produce una mayor disolución de la Dox, lo que a su vez pudiera permitir su absorción a lo largo de la totalidad del mismo (Davis et al., 2006). Es importante considerar también, que el tiempo de tránsito total de boca a colon menor en caballos adultos es de aproximadamente 40 horas (Van Weyenberg et al., 2006), lo que pudiera confirmar este razonamiento. Sin embargo, es necesario realizar una mayor cantidad de estudios para afirmar estas consideraciones.

La liberación modificada de fármacos tiene varias ventajas, entre las que se pueden mencionar menor manejo del animal, mejor cumplimiento con el tratamiento y por ende, mejor resultado terapéutico (Brayden, 2003). La formulación experimental de liberación modificada y larga acción de doxiciclina hiclato al 20% (Dox-pol) desarrollada en este estudio, administrada PO a una dosis bolo de 10 mg/kg, produjo concentraciones séricas útiles durante 12 - 24 horas, pero no durante más tiempo. Aun cuando se ha afirmado que si se obtienen altas concentraciones de Dox *in vitro*, equivalentes a 8 a 16 veces el valor de una CMI promedio, podría convertir a la Dox en un antimicrobiano concentración-dependiente (Cunha, 2000), su cardiotoxicidad impide su uso a dosis elevadas y la obtención de Cmax altas no es una aproximación segura (Chiers et al., 2004; Yeruham et al., 2002). La doxiciclina es considerada un fármaco antibacteriano tiempo- dependiente. Por lo tanto, se obtiene una relación PK/PD óptima cuando las concentraciones séricas del fármaco se mantienen de 1 a 5 veces por arriba de la CMI del patógeno involucrado durante el 40 a 100% del intervalo entre dosificaciones (Agwuh & McGowan, 2006; Bousquet et al., 1998; McKellar et al., 2004). Los valores de CMI para microorganismos sensibloes considerados en este estudio son de 0.25 a 1.0 μg/mL (Davis et al., 2006; Jacks et al., 2003). En humanos, un índice de PK/PD aceptado para predecir eficacia terapéutica de las tetraciclinas como grupo antibacteriano, es la

relación AUC0-24/CMI (Goue et al., 2009). Si se considera una CMI de 0.25 μg/mL, la relación AUCDox-pol/CMI = 68.02, mientras que la relación AUCDox-a/CMI = 12.4. Con base en lo mencionado anteriormente, es seguro considerar al preparado Dox-pol como una formulación con mejor relación PK/PD que Dox-a para el tratamiento de enfermedades causadas por bacterias susceptibles a la doxiciclina. Se concluye que Dox-pol es una formulación en forma de pasta para administración PO que optimiza el uso de doxiciclina en caballos en términos de congruencia de la relación PK/PD y es probable que también mejore el cumplimento con el tratamiento debido a su facilidad de administración. Este hecho pudiera contribuir a retrasar la aparición de cepas bacterianas resistentes. Sin embargo, y aun cuando no se observaron efectos adversos en el tubo GI, como diarrea, cólico o malestar abdominal, en ninguno de los caballos utilizados en este estudio, se requieren ensayos de dosis múltiples, distribución, toxicología y ensayos clínicos para determinar si esta formulación se puede considerar potencialmente útil en esta especie.

En la segunda parte de esta tesis se utilizó el preparado en un estudio clínico longitudinal, de no inferioridad y multicéntrico en un contexto no hospitalario para determinar su efectividad en la prevención y tratamiento de infecciones en heridas en caballos adultos.

En general, la publicación de ensayos clínicos es limitada si se compara con la de artículos experimentales, debido probablemente, a que es más fácil controlar variables en estos últimos. Sin embargo, el valor y objetivo final de los estudios experimentales es obtener eventualmente, un resultado pragmático que pueda servir como una guía alterna para los clínicos. Para cumplir con tal fin, por lo tanto, se pretendió utilizar un número significativo de casos clínicos similares. No obstante, no existe una regla establecida que define el número experimental y se encuentra una gran variación entre publicaciones (Rohdich et al., 2009; Dutton et al., 2009; Van Loon et al., 2010; Foreman et al., 2012). Este estudio, en el que se utilizaron cuarenta caballos adultos, con edades entre 4 y 13 años con heridas quirúrgicas o traumáticas, se puede considerar entre los ensayos con mayor número experimental. En este contexto, una prueba de G power reveló una potencia de 0.84, lo cual puede ser considerado como apoyo para el uso de Dox-pol. Se utilizó el modelo estadístico

de análisis robusto linear mixto para datos longitudinales ya que el objetivo de este ensayo fue comparar los efectos de dos tratamientos experimentales en una variable de resultado, esto es, los días hasta cicatrización total de la herida. En este contexto, se puede proponer que una determinada herida, con o sin infección, puede ser tratada de igual manera con Penicilina G procaínica (Pen G) o con la formulación Dox-pol. La recuperación del animal está, a su vez, influida por factores como género, edad, condición corporal, localización, manejo, tipo y profundidad de la herida.

Es bien conocido que la inflamación y dolor, en conjunto con la presencia de secreción seropurulenta o purulenta son indicativos de infección en una herida (Waguespack et al., 2006). A su vez, estos factores, como causa de restricción del movimiento, influyen en la cicatrización de las heridas (Hendrickson y Virgin, 2005). De estos tres signos clínicos, el dolor es específico y relativamente fácil de ser evaluado subjetivamente. Por lo tanto, este parámetro fue tomado como punto clave para llevar a cabo este ensayo. Con el fin de asegurar objetividad, precisión y confiabilidad en su evaluación se adaptaron dos escalas de puntuación, *ASEPSIS* descrita por Wilson et al. (1986) y *Composite Pain Score* (*CPS)* diseñada por Bussiéres et al. (2008) a la que se incorporó el sistema de graduación de claudicación de la Asociación Americana de Especialistas en Equinos (AAEP)[2]. Se instruyó a tres médicos veterinarios especialistas en equinos para graduar las características de las heridas y signos de dolor de los caballos involucrados y registrar estos parámetros en las hojas de trabajo de *ASEPSIS* y *CPS*. Consideramos que las hojas de trabajo fueron instrumentos claros, simples y consistentes para garantizar imparcialidad en la evaluación de los animales. A su vez, el hecho de que se hiciera un estudio ciego aumenta la confiabilidad de los resultados obtenidos.

En este ensayo clínico, la tasa de recuperación fue del 100%, a pesar de haber tratado heridas de diferente localización, curso, severidad y tipos. No se encontró diferencia estadísticamente significativa entre el tratamiento con penicilina G procaínica (Pen G) sola o con tratamiento

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[2] http://www.aaep.org/health_articles_view.php?id5280

concomitante de meglumina de flunixin (MF) y el preparado recientemente formulado de doxiciclina (Dox-pol) con o sin tratamiento antiinflamatorio, en prevenir o tratar infecciones en heridas en caballos. Se pueden, sin embargo, mencionar claras ventajas para Dox- pol. Al ser administrado PO y cada 24 h, representa un estrés mínimo para el animal, menos reacciones adversas y mejor cumplimiento del tratamiento en un escenario clínico día a día.

Por otro lado, Dox es bien conocida por sus efectos no-antimicrobianos, esto es, sus propiedades antiinflamatorias (Serra et al., 2012; Wilkins, 2013). Más aún, se ha reportado una sinergia entre Dox y antiinflamatorios no esteroidales (AINEs) en cuanto a la estimulación del proceso de cicatrización en heridas crónicas en humanos (Wilcox et al., 2012). De tal manera, se puede prever su uso en el tratamiento de heridas crónicas con producción excesiva de tejido de granulación, un problema muy común en caballos. Según Wilcox et al. (2012) esta sinergia podría, a su vez, facilitar la distribución de Dox hacia áreas terapéuticas, lo cual pudiera producir mejores resultados clínicos en general. Se requieren, sin embargo, una mayor cantidad de estudios para confirmar esta aseveración y la seguridad de esta formulación en escenarios clínicos extensos y de sobredosificación en caballos.

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CLINICAL EFFICACY OF A LONG ACTING DOXYCYCLINE EXPERIMENTAL FORMULATION IN WOUND INFECTIONS IN HORSES

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ABSTRACT

A multicenter longitudinal non-inferiority clinical study in wounds of horses, in a non-hospital environment was conducted to assess the efficacy of a new sustained release long-acting formulation of doxycycline hyclate 20%, as an oral paste (Dox-SR). Cases were distributed according to sex, age breed, body condition score, wound type, course, location and wound classification based on degree of microbial contamination. Wound management, bacteriological culture of infected wounds and days to complete healing were recorded. Horses were randomly divided into 4 groups and treated as follows: procaine penicillin G (22,000 UI/kg IM q 12 h) (Pen G); Pen G as before plus flunixin meglumine (1.1 mg/kg IV q 24 h) (Pen G + FM); Dox-SR (10 mg/kg PO q 24 h); and Dox-SR plus FM. Three independent, trained clinicians assessed the initial clinical status and their progression on days 0, 7, 14, 21 and 28 on a provided modified Composite Pain Scale and ASEPSIS worksheet. Logistic model for repeated measures analysis showed that there was no statistically significant difference between treatments as far as days to complete wound healing is concerned. It is concluded that treatment with either Dox-SR or Dox-SR + FM, are as effective as treatments with Pen G to either prevent or treat wound infections in horses in a clinical setting.

INTRODUCTION

Pharmaceutical preparations of antibacterial drugs specifically designed for horses in general and particularly for oral administration, are scarce (Davis, 2006). A reduced share of the veterinary pharmaceutical market and anatomical and physiological peculiarities of the gastrointestinal (GI) tract of this species may account for this particular trend (Davis, 2006). One important example of an antibacterial drug that has been used without pharmaceutical design is doxycycline (Dox). This drug is frequently used as an aqueous solution compounded on the spot, just before administration via a nasogastric tube, for the treatment of a variety of bacterial diseases such as diarrhea caused by *Lawsonia intracellularis* in foals (Reed, 2008), Lyme disease caused by *Bordetella burgdorferi* (Chang, et al, 2005); pneumonia due to *Rhododoccus equi* (Giguére, 2012) and a variety of infections caused by susceptible *Streptococcus* spp, *Staphylococcus* spp, *Pasteurella* spp and *Actinobacillus equuli* (Jackss et al, 2003; Bryant et al, 2000), including septic arthritis (Haerdi-Landerer, 2008) septic osteitis, osteomyelitis and physitis (Ramzan and Pilsworth, 2001; Koch and Witte, 2013; Lawrence, 2013). In humans, good results have been obtained with the use of doxycycline orally for treating wound and other soft tissue infections (Hicks, 2012; Rummukainen, 2013).

Pharmacokinetics (PK) of aqueous solutions, such as the ones used above, has been defined in horses (Bryant et al, 2000; Davis et al, 2006; Womble et al, 2007; Smith et al, 2008; Schnabel, 2010; Winther et al, 2011). From these PK studies it becomes clear that maximum serum concentration (C_{max}) values range from 0.22 µg/mL to 0.91 ± 0.25 µg/mL and elimination half-lives ($t_{1/2}\beta$) were 8.7 ± 1.6 h and 11.81 ± 3.51 h, administering doses of 3 mg/kg and 20 mg/kg, respectively (Bryant et al, 2000; Davis et al, 2006). Area under the curve $AUC_{0-\infty}$ (µg/mL· h) was 10.85 ± 7.46 after a dose of 5 mg/kg (Schnabel et al, 2010) and 13.35 ± 2.71 after a dose of 20 mg/kg (Davis et al, 2006).

Dox has been regarded, from a pharmacokinetics/pharmacodynamics (PK/PD) view point, as a time-dependent antibacterial drug (Agwuh and McGowan, 2006). Desirable key variables for doxycycline include that a dose interval should ideally be established at the time when plasma concentrations of Dox reach the lowest minimal inhibitory concentration (MIC) for a particular pathogen. Considering available literature on bacterial sensitivity to Dox, a breaking point of 0.25 µg/mL can be set as the working MIC (Bryan et al, 2000). Based on this assumption, aqueous solutions should be administered orally (PO) at a dose of 10 mg/kg every 12 hours (Bryant et al, 2000) or 20 mg/kg every 12 or 24 hours (Davis et al, 2006; Baker et al, 2008).

Considering the inconvenience associated with manufacturing an aqueous solution of Dox just before administration to a patient, plus the need to administer it through a nasogastric tube twice a day and fasting the horse 8 hours before and 2 hours after Dox administration (Davis et al, 2006), Zozaya et al, (2013) developed a sustained release long acting formulation of Dox as an orally delivered paste

(Dox-SR). This formulation is placed in the posterior part of the oral cavity through the inter-dental space with the aid of a long tipped syringe, in a volume of approximately 25 mL for an adult 500 kg horse. In this PK study in which non-fasted horses were used, the authors obtained a maximum serum concentration (Cmax) of 1.3 ± 0.7 µg/mL with time to reach the Cmax (Tmax) of 5.9 ± 1.7 h, area under the curve (AUC) of 17.0 ± 2.2 µg h/ml and elimination half-life (T½ β) of 4.9 ± 1.0 h with a relative bioavailability of 548% and with a dose of 10 mg/kg, These pharmacokinetic values make Dox-SR an oral paste formulation that optimizes the use of doxycycline in horses in terms of PK/PD ratio congruency (Agwuh and McGowan, 2006), and may also improve prescription compliance, due to its ease of administration. The horses in this study showed neither rejection of this preparation, nor gastrointestinal (GI) adverse clinical signs.

This work is based on the hypothesis that Dox-SR and Dox-SR with concomitant meglumine flunixin are as effective as procaine penicillin G and procaine penicillin G with concomitant meglumine flunixine which are options widely accepted and documented for preventing and treating infections in open wounds in horses (Brumbaugh, 2005; Adam and Southwood, 2006).

MATERIAL AND METHODS

 A multicenter longitudinal non-inferiority clinical study in a non-hospital environment was conducted with 40 field clinical cases of adult (4 – 13 years) client owned wounded horses, complying as best as possible with Guidelines on good Clinical Practice (VICH GL9, 2000[1]). This study received approval by the Ethics Committee of the Postgraduate Studies Division at the *Facultad de Medicina Veterinaria y Zootecnia* of the *Universidad Nacional Autónoma de México.* The horses included in this study presented open surgical and traumatic wounds (lacerations, incisions and puncture wounds) with no synovial structure involvement. The horses were distributed according to sex, age and breed, as well as body condition score[2], use, clinical history, wound type and deepness of lesion, location and wound classification based on degree of microbial contamination as referred in Table 1 (Waguespack et al, 2006). Also wound management instituted upon arrival, additional supportive therapy, results of bacteriological culture of infected wounds and days to complete healing were recorded.

Samples for bacterial culture were taken with a sterile swab moistened with three drops of sterile 0.9% sodium chloride. The swabs were transported to the laboratory in Stuart transport media[3] and were cultured according to standard procedure (CLSI[4]).

The horses were randomly divided in 4 groups according to the Research Randomizer site[5] two positive control groups treated as follows: procaine penicillin G at a dose of 22,000 UI/kg IM q12 h (group: Pen G), and procaine penicillin G (same dose) with concomitant flunixin meglumine at 1.1 mg/kg IV q

24 h (group: Pen G + FM); and two experimental groups: one treated with Dox-SR at 10 mg/kg PO q 24 h (group: Dox-SR), and one last group treated as group Dox-SR plus concomitant flunixin meglumine 1.1 mg/kg as earlier described (group: Dox-SR + FM). Intramuscular injections of procaine penicillin G were applied at the base of the neck; intravenous injections of flunixin meglumine were applied in the jugular vein while Dox-SR was placed in the interdental space aided by a long tipped syringe.

Horses which underwent elective palmar digital neurectomy and whose wounds were classified as clean contaminated, received antimicrobial prophylaxis 60 minutes before starting surgery and 24 h later, once a day (q 24 h) for 2 additional days. Horses that underwent elective castration and whose wounds were classified as clean contaminated as well, received antimicrobial prophylaxis as before but during 7 days. Horses with traumatic wounds classified as contaminated and infected were treated for 5 to 10 days as required based on the ASEPSIS scoring as laid out in table 2.

Three independent equine clinicians received training on the parameters registered in the modified composite orthopedic pain scale (CPS) originally described by Bussières et al. (2008), as well as on wound characteristics registered in the modified ASEPSIS scoring method developed by Wilson et al. (1986). Briefly, the composite orthopedic pain scale (CPS) is a multifactorial descriptive numerical rating scale incorporating physiologic data such as heart and respiratory rate, digestive sounds and rectal temperature; equine-specific behavior such as responses to stimuli, interactive behavior - response to observer and response to palpation of wounded area, appearance of the animal, sweating, pawing on the floor, posture, head movement and posture and appetite. Additionally the five point lameness grading system accepted by the American Association of Equine Practitioners (AAEP)[6] has been incorporated into this CPS to establish a composite grading score as presented in table 3. Pain scoring was done with the animals in their box stalls. Lameness evaluation was done at rest or movement, as required.

To objectively register wound characteristics, a scale was used as described in the modified ASEPSIS scoring method, originally defined in humans to compare antibiotic regimens for their effectiveness in preventing or treating wound infections (Wilson et al, 1986) (Table 2).

The clinicians made an initial examination of the animals included in this study on day 0; their findings on a provided CPS and ASEPSIS worksheets, were recorded. They were blinded to treatment and treatment effectiveness was evaluated by wound healing and decreased signs of pain. Clinicians assessed individual progression recording their weekly findings on the same CPS and ASEPSIS worksheets on days 7, 14, 21 and 28.

Sutured and delayed closed wounds were considered healed when an epithelial line was observed at the suture line, while wounds left to second intention healing were evaluated via measurements with calipers and sequential photographs obtained every week. These wounds were considered healed and treatment stopped when an epithelial layer covered the entire wound surface, which rendered an overall CPS score of 0 to 1 and an ASEPSIS score of 0 to 10.

Constant observations on a daily basis were made by the horses' caretakers in order to record clinical signs of possible local inflammation, abdominal discomfort, colic and diarrhea or any other alteration which might be attributed to the antibiotic treatment. When a particular case showed any of these signs or did not show sign of recovery after 3 days of treatment, the case was regarded as treatment failure and follow up treatment outside the scope of this trial was established. Such a case did not occur.

The effectiveness of treatment on the outcome was analyzed with a robust mixed linear model analysis for longitudinal data (Gill, 2000). All data were previously analyzed on IBM SPSS 18$^{®}$ with the Shapiro Wilk test to determine normal distribution. A first model considered CPS as the dependent variable and included the fixed effects of treatment and examination day. On day 28 a Kruskal-Wallis test was performed in order to determine if there was any statistical difference between treatments ($P < 0.05$) in relation to the reduction of CPS values. In a second model the dependent variable was days to complete healing and included the effects of treatment and interactions between treatment and wound localization, type, management and ASEPSIS score, considering the random effect of horse, sex, body condition score and age to account for the repeated observations on each animal. Wound localization was classified as head and neck, upper body and proximal extremities, and distal extremities. Wound types were divided as follows: incisional skin deep, incisional full deepness, laceration skin deep, laceration full deepness and puncture. Wound management was considered as primary closure, delayed closure and second intention healing. Analysis was done by restricted maximum likelihood (REML) as applied to mixed linear models for experimental data ($P < 0.05$).

RESULTS

Days to complete healing for the Pen G treated horses ranged from 15 to 40 in all wound types (mean = 27.5 days), for the Pen G + FM group, it was 18 to 37 days (mean = 27.5). For the Dox-SR group, the same values were 23 to 50 days (mean = 36.5 days) and the Dox-SR + FM group, 22 to 36 days (mean = 29 days). Comparison of these data revealed no statistically significant difference among the four treatments ($P = 0.999$).

The restricted likelihood reason analysis showed a statistically significant difference ($P = 0.004$) reduction of CPS throughout time in all four groups (0 – 28

days). However, this decrease was not statistically different when comparing the four groups (P = 0.999). Furthermore, the reduction on CPS seen for all groups as depicted in figure 1 was analyzed through a Kruskal-Wallis test and it revealed no statistically significant difference among them (P = 0.903).

As far as wound management (primary closure, delayed closure and second intention healing) is concerned and as expected, sutured wounds in the Pen G groups showed a complete healing in 22.33 ± 8.6 d; while in the Dox-SR groups this value was 19.4 ± 9.75 d. In contrast, for wounds that required second intention healing, Pen G groups needed 35.23 ± 7.4 d and Dox-SR groups needed 40.25 ± 9.2 d When comparing days to complete healing from one type of wound to the other, a statistically significant difference became apparent (P = 0.041). No statistically significant difference was obtained when comparing days to complete healing and linear wounds or wounds healed by second intention (P = 0.664).

In relation to the interaction of wound type (incisional-skin deep, full deepness, laceration skin deep and full deepness and puncture wounds) and treatment, a less number of days to complete healing in puncture wounds when treated with Dox-SR + FM was found (28.54 ± 5.2 d). Yet statistically significance was only obtained when comparing these data with Pen G (P = 0.048), but not when comparing results with Pen G + FM (P = 1) and Dox-SR alone (P = 0.291) groups.

Bacteria cultured from infected wounds were *Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pyogenes, Streptococcus zooepidemicus, Escherichia coli* and *Enterococcus faecalis.* No specific pattern could be identified considering different wound types.

No adverse effects were observed at all in any of the treated horses.

DISCUSSION

In general, the frequency of publication of clinical trials is limited when compared with experimental articles, probably due to the fact that it is easier to control variables in the latter setting. However, the value and ultimate objective of the experimental studies is to have, eventually, a pragmatic impact that can serve as an alternative guide for practitioners' handling infected wounds. In order to better comply with this aim, it is usually preferred to use a significant number of similar clinical cases. However, there is no set rule that establishes a given experimental number, and it varies greatly among published articles (Rohdich et al, 2009; Dutton, Lashnits and Wegner, 2009; van Loon et al, 2010; Foreman et al, 2012). By comparison, the forty adult horses (aged 4 – 13 years) included in this study, with surgical or traumatic wounds rank among the papers with the most cases. In this context a G-power analysis revealed a potency of 0.84, which can be regarded

as supportive for the use of Dox-SR. The statistical model of robust mixed linear analysis for longitudinal data was used because the main purpose of this study was to compare the effects of two experimental treatments on a variable outcome; that is, the days to complete healing. In this context, it can be proposed that a given wound, whether infected or not can be equally treated with Pen G or Dox-SR. The above is, in turn, influenced by factors such as gender, age and body condition, wound location on the horses' body, wound management, wound type and deepness of lesion.

It is well known that inflammation and pain in conjunction with the presence or not of seropurulent or purulent discharge are indicative of wound infection (Waguespack et al, 2006). In turn, infection, inflammation and pain, as a factor that restricts movement, are features with a recognized effect on wound healing (Hendrickson and Virgin, 2005). However, of these three signs, pain can be taken as a specific one and relatively simple to be evaluated subjectively. So, this parameter was taken as a key point for this trial, ensuring objectivity, accuracy and reliability in assessing it. Hence, the ASEPSIS score described by Wilson et al, (1986) and the Composite Pain Score designed by Bussières et al. (2008) with incorporation of the lameness grading score system developed by the AAEP[6], were adapted to this study. Three equine clinicians were trained to grade horses´ wound characteristics and pain signs in working ASEPSIS and CPS score-sheets. In our view the score-sheets provided to them were clear, simple and consistent instruments that minimized bias. Also the fact that they were blinded to treatment increase the reliability of these results.

In this study the cure rate was 100% despite having dealt with numerous wound types, courses, locations and severities. Consequently, no statistically significant difference was found between the widely accepted treatment of procaine Penicillin G with or without flunixin meglumine for preventing and treating wound infection in horses and the newly developed Dox-SR with or without anti-inflammatory treatment. In spite of the above, clear advantages can be pinpointed for Dox-SR; *i.e.*, it is administered orally and once a day with minimum stress for the patient and can result in better treatment compliance in regular clinical settings.

Doxycycline is well-known for its non-antimicrobial effects, that is anti-inflammatory properties (Serra et al, 2012; Wilkins, 2013). Furthermore, a synergistic effect between doxycycline and non-steroidal anti-inflammatory drugs (NSAIDs) in stimulating the healing process has been reported for humans with chronic wounds (Wilcox et al, 2012). This effect can also be envisioned for a common problem in horses, chronic wounds with exuberant granulation tissue. According to Wilcox et al, (2012) this synergy may also facilitate the uptake of doxycycline into therapeutic areas, which could render better clinical outcomes overall. Further studies are warranted to determine the above and the safety of this preparation in large clinical tests and overdosing horses.

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FOOTNOTES

1 http://www.vichsec.org/en/guidelines2.htm
2 Henneke Body Condition Score (1983)
3 Grupo La Rochelle, SA de CV, México, DF
4 http://clsi.org/standards/micro/
5 http://www.randomizer.org/
6 http://www.aaep.org/health_articles_view.php?id5280

ACTA VETERINARIA SCANDINAVICA

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RESEARCH   **Open Access**

Pharmacokinetics of a peroral single dose of two long-acting formulations and an aqueous formulation of doxycycline hyclate in horses

Heidi Zozaya[1], Lilia Gutierrez[1], Maria Josefa Bernad[2] and Hector Sumano[1*]

Abstract

Background: Doxycyline (Dox) is a semisynthetic antibacterial drug with pharmacological advantages over its parent drug (tetracycline) in the treatment of various bacterial diseases in horses. Yet, at present a horse-customized pharmaceutical formulation is not available. Based on its pharmacokinetic/pharmacodynamic (PK/PD) ratio, Dox is considered a time-dependent antibacterial drug and ideally expected to achieve sustained plasma drug concentrations both at or slightly above the minimal inhibitory concentration (MIC) level for as long as possible between dosing intervals. Hence, the objective of this study was to formulate two long-acting (LA) doxycyline hyclate (Dox-h) formulations for oral administration and define their pharmacokinetics in non-fasted adult horses to obtain better bioavailability and longer mean residence time, features needed to comply better with its pharmacokinetic/pharmacodynamic (PK/PD) ratios.

Results: Pharmacokinetic parameters were determined after the oral administration of a single 10 mg/kg bolus dose of two 20% Dox-h formulations: one based on a β cyclodextrin (Dox-β) matrix and a second one on a poloxamer (Dox-pol) matrix. The results were compared with the pharmacokinetics of a single 10 mg/kg bolus oral dose of a freshly made aqueous Dox-h solution (Dox-a). Dox-pol showed the greatest values for relative bioavailability (548%); maximum serum concentration (Cmax) value was 1.3 ± 0.7 μg/mL with time to reach the Cmax (Tmax) of 5.9 ± 1.7 h, area under the curve (AUC) of 17.0 ± 2.2 μg h/ml and elimination half-life (T½ β) of 4.9 ± 1.0 h.

Conclusions: Considering a minimal inhibitory concentration MIC of 0.25 μg/mL, clinically effective plasma concentrations might be obtained for up to 24 h administering Dox-pol. This is an oral paste formulation that might optimize the use of Dox-h in horses in terms of PK/PD ratio congruency, and it is likely that it may also improve prescription compliance due to its ease of administration.

Keywords: Doxycycline, Long-acting, Horses, Pharmacokinetics, Oral-administration

Background

Doxycycline hyclate (Dox-h), a semi-synthetic analog of tetracycline, offers several pharmacological advantages over the parent drug (tetracycline) in horses, mainly higher oral bioavailability, higher tissue penetration, a larger volume of distribution and exhibits a more potent antimicrobial activity [1-5]. Additionally, Dox-h is better tolerated than other tetracyclines in this species; hence, the risk of enterocolitis and diarrhea is milder and/or infrequent [6,7].

The intramuscular and subcutaneous administration of Dox-h can cause extreme local pain, irritation and tissue necrosis and these routes are therefore not recommended [7,8]. The intravenous use should also be avoided, as it can cause supraventricular tachycardia, systemic arterial hypertension, clinical signs of discomfort, cardiovascular collapse and even death in horses [9,10].

The pharmacokinetics of an aqueous solution of Dox-h administered orally (PO) has been determined in adult horses [1,6,11,12] and in foals [2]. In these studies, Dox-h was administered either dissolved in water via nasogastric tube or as a top dressing at doses ranging from 3 mg/kg to 20 mg/kg every 12 (q12h) or every 24 hours (q24h). Davis et al. [6] determined that after administering single or

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multiple doses of Dox-h (20 mg/kg) via nasogastric tube, the time to maximum concentration (Tmax) was 1.6 ± 1.3 x`h, the maximum concentration (Cmax) was 1.7 ± 0.3 μg/mL and elimination half-life (T½β) was 12.07 ± 3.1 h. Plasma protein binding was 81.7 ± 2.4%. These authors concluded that Dox-h administered at a dosage of 20 mg/kg PO q24h will result in drug concentrations adequate for inhibiting intracellular bacteria and bacteria with minimal inhibitory concentration (MIC) equal or higher than 0.25 μg/mL. On the other hand, Bryant et al. [1] concluded that Dox-h at a dose of 10 mg/kg PO q12 h could be appropriate for treating infections caused by susceptible (MIC < 0.25 μg/ml) gram positive microorganisms. Yet, Davis et al. [6] and Womble et al. [2] consider that the therapeutic value of oral Dox-h in adult horses is limited due to its low bioavailability. Winther et al. [12] reported an estimated oral bioavailability of Dox-h of 17% after intragastric administration and 6% after topdressing administration in non-fasted adult horses. Similarly, these authors consider that if the oral bioavailability of Dox-h could be enhanced, this antimicrobial drug might be a valuable resource for the treatment of lower airway infections in horses. Additionally, a high local drug concentration of Dox-h in the stomach causes gastric irritation and nausea in humans [13,14] and it has been shown that retarding the release of Dox-h diminishes the incidence of gastrointestinal adverse side effects [15].

Considering the above, the objective of the present study was to formulate and define the pharmacokinetics of two long-acting (LA) Dox-h formulations intended for oral administration in horses, with the aim of improving its bioavailability and its gastrointestinal tolerance in an attempt to enhance the value of Dox-h as an antimicrobial drug in equine medicine.

Methods

The study was conducted in Mexico City campus of the School of Veterinary Medicine at the National Autonomous University of Mexico (UNAM). The study was approved by the Postgraduate Committee of Research, Care, and Use of Experimental Animals in accordance with its regulations [16].

Ten healthy adult Quarter Horses (three mares and seven geldings) weighing a mean of 450 ± 22.4 kg were included in the study. The animals were considered clinically healthy on the basis of physical examination and standard hematological and biochemical tests. The horses had not been medicated with any antimicrobial agent for at least 30 days before enrollment in the study. They were maintained on a diet of oat hay and feed concentrate and had *ad libitum* access to water throughout the study.

Three preparations were assessed; two experimental LA formulations and an aqueous one. The first LA formulation was the Dox-h in a poloxamer base (Dox-pol; 200 mg/mL), prepared as follows: first Dox-h powder (PARFARM S.A., Mexico) was made soluble in distilled water. Then, a reverse gel copolymer polyoxypropile–polyoxyethylene poloxamer 407 (Lutrol micro 127 MP (BASF Germany) was added and stirred vigorously and constantly at 4°C. The mixture was protected from sunlight and maintained at 2 – 4°C during 24 h. It was then further homogenized to obtain a clear solution. Carbomer 934P (Carbopol 934P, Lubrizol USA) was added to increase viscosity. After stirring continuously during 30 minutes, it was considered ready when a microemulsion was formed. This point could be determined when the mixture clarified. Xanthan gum (Padoquimia S.A., Mexico) was then added to obtain a suspension with a paste-like consistency ready to use. Finally, 35 mL syringes were filled up with the 20% Dox-pol formulation, protected from light and the formulation used within three days after preparation.

The Dox-h–β cyclodextrin (Dox-β) on a poloxamer base formulation was prepared, by first forming complexes of Dox-h 20% (w/v) with β-cyclodextrin (Cerestar Pharmaceutical Excipients, U.S.A.). For this purpose the kneading method was used [17]. The ingredients were first mixed in a mortar to obtain a homogeneous paste. Then, Dox was added slowly. The mixture was further grounded for 30 min and an appropriate quantity of water was added to maintain a paste-like consistency. It was then dried in an oven at 40-50°C for 24 h. The dried complex was pulverized into a fine powder, which was then diluted in water. This mixture was then included in a reverse gel copolymer polyoxypropile–polyoxyethylene poloxamer 407 (Lutrol micro 127 MP (BASF Germany) under constant stirring at 4°C. The preparation was regarded as ready when a micro-emulsion is formed and this could be pin-pointed when the mixture clarified. Xanthan gum (Padoquimia S.A., Mexico) was then added to obtain a suspension with a paste-like consistency ready to use. Finally, 35 mL syringes were filled up with the 20% Dox-β formulation, protected from light and the formulation used within three days after preparation. This experiment does not intend to present stability studies for this preparation, and has no proprietary restrictions.

Finally, an aqueous formulation of Dox-h (Dox-a) was prepared from powdered Dox-h by diluting it in sterile distilled water, obtaining a 20% final solution and immediately administered to the horses.

Individual dose vs. pharmaceutical preparation compliance was calculated to have 5.5, 4.2 and 2.8% error from the set dose of 10 mg/kg in the Dox-β, Dox-pol and Dox-a (1 mL/20 kg of body weight in all cases), as assessed by determining Dox-h concentration in all three preparations, taking 4 random test samples of each group. Determination of Dox-h in by HPLC with UV detection in these pharmaceutical samples was carried out as described by Axisa et al. [18].

A longitudinal crossover (3 x 3 x 4) study design was employed with washout periods of 21 days. Each horse was individually weighed and dosed with the Dox-a preparation via nasogastric tube at a dose of 10 mg/kg in a volume of approximately 30 mL. After the washout period, each horse of the same experimental group was moved to the next group and dosed at 10 mg/kg with either Dox-β (30 mL approximately), or Dox-pol, also at 10 mg/kg in a volume of 30 mL approximately. These latter groups received their dose as a paste, placed in the interdental space aided by a long tipped syringe. In all three groups adverse gastrointestinal drug reactions i.e., colic, diarrhea and other signs of abdominal discomfort were sought for hourly during the day.

Determination of pharmacokinetic values was accomplished through serial blood sampling.

To achieve accurate intervals between administration of the drug and collection of serum, a 16-gauge, permanently heparinized catheter was inserted into a jugular vein and glue-fixed on each horse. Blood samples were obtained before administration of any of the formulations (time 0), and after the administration of each of the preparations, at 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, 24 and 48 h. Blood samples were obtained by removing 5 mL of heparin-containing blood from the catheter, discarding it, and then collecting additional 5 mL of blood, which were placed into 10 mL test tubes with no anticoagulant. Blood was allowed to clot at room temperature (20°C) during 30 minutes and then centrifuged at 1500 RPM for 15 minutes. Serum was harvested, frozen in liquid nitrogen and stored not more than 7 days until analyzed.

Serum Dox concentrations were determined both by high performance liquid chromatography (HPLC) as described by Axisa et al. [18] and through the modified agar diffusion analysis, described by Abd El-Aty et al. [19]. For the former analytical analysis, the intra-assay coefficient of variance was < 1.9 and interassay error was < 1.8. The analytic assay was linear over a range of concentrations from 0.1 to 10 µg/mL. Mean ± 1 SD recovery was $94 \pm 2\%$ ($r = 0.97$). Limit of detection was 0.07 µg/mL, and limit of quantification was 0.1 µg/mL.

For the modified agar diffusion analysis, *Bacillus cereus* (ATCC-11778) was used as a test organism grown on Mueller-Hinton agar (MCD LAB, S.A. de C.V., Mexico City). The intra-assay coefficient of variance was < 4.8 and inter-assay error < 4.6. The analytical assay was linear over a range of concentrations from 0.04 to 10 µg/mL, with a percent recovery of 93 ± 2 and a correlation coefficient (r) of 0.97 ± 0.1. Limit of detection was 0.005 µg/mL and limit of quantification was 0.01 µg/mL. Compliance between both methods to determine serum concentrations of Dox-h was carried out using doxycycline-spiked horse serum samples and processed

by the two analytical techniques. Subtraction of recover percentages revealed an error of no more than 6.2%.

A computerized curve stripping program (PK Analyst for Windows, MicroMath, St. Louis, MO) was used to fit and analyze the concentration-versus-time profiles for each horse and the mean values for each group. Models of best fit ($r \geq 0.99$) were chosen after analysis by use of residual sum of squares and the minimal Akaike's information criterion. The best fit for Dox-a was obtained by using a 2 compartment model with first-order input and first order output in accordance with the following equation:

$$Concentration\ (Time) = Ae^{-a\ Time} + Be^{-\beta\ Time}$$
$$+ Ce^{-KAB\ Time}$$

Pharmacokinetic variables obtained for Dox- a were: elimination rate constant (Kel), absorption rate constant (Kab), area under the curve (AUC), half life of the elimination phase (T ½ β), absorption half life (T½ ab), mean residence time (MRT), mean residence time to infinity (MRT $_{0\ -\ \infty}$), area under the curve to infinity (AUC$_{0\ -\ \infty}$) and area under the moment curve to infinity (AUMC$_{0\ -\ \infty}$).

The best fit for Dox-pol and Dox-β was obtained by using a one-compartment model with first-order input and first order output in accordance with the following equation:

$$Concentration\ (Time) = \frac{Dose\ K_{AB}}{Volume\ K_{AB} - K_{elim}} e^{K_{el}\ Time} - e^{K_{AB}\ Time}$$

The pharmacokinetic values obtained for Dox-pol and Dox-β were: elimination rate constant (Kel), absorption rate constant (KAB), area under the curve (AUC), area under the first moment of the concentration-time curve (AUMC), half life of the elimination phase (T½β), absorption half life (T½ab), time when the concentration reaches its maximum (Tmax), the maximum concentration (Cmax), mean residence time (MRT), mean residence time to infinity (MRT $_{0\ -\ \infty}$), area under the curve to the last time point (AUC $_{0\ -\ \infty}$) and area under the moment curve to infinity (AUMC$_{0\ -\ \infty}$). Table 1 summarizes the pharmacokinetic variables obtained. Data showed no normal distribution for all three groups and are presented as mean ± standard deviation of 10 observations for each parameter. Statistical comparison was made by Kruskal-Wallis and Dunn test.

Relative bioavailability was calculated comparing the long acting formulations with the Dox- a preparation, by using the following equation as described by Sabnis [20]:

$$Relative\ bioavailability\ (Frel) = (AUCDox - pol/AUCDox - a)$$
$$\times\ 100$$

The degree of plasma protein binding of Dox was carried out in vitro as described by Singhvi et al. [21]. Dox-enriched plasma samples were spiked with 0.1, 0.5, 1, 5,

Table 1 Pharmacokinetic variables for doxycycline hyclate (Dox-h) in horses after the oral administration of three experimental formulations

	Dox-a	Dox-pol	Dox-β
	Mean ± SD	Mean ± SD	Mean ± SD
AUC (µg · h/mL)	3.1 ± 0.2 [a]	17.0 ± 2.2 [b]	1.5 ± 0.1 [c]
AUMC (µg · h^2/mL)	35.2 ± 1.2 [a]	208.3 ± 31.1 [b]	12.3 ± 0.1 [c]
AUC$_0$ - $_\infty$ (µg · h/mL)	3.0 ± 0.2 [a]	16.1 ± 4.8 [b]	1.5 ± 0.9 [c]
AUMC$_0$ - $_\infty$ (µg · h^2/mL)	31.2 ± 2.3 [a]	171.3 ± 5.8 [b]	12.3 ± 0.5 [c]
MRT (h)	11.3 ± 4.4 [a]	12.2 ± 4.2 [a]	8.1 ± 2.1 [a]
MRT$_0$ - $_\infty$ (h)	10.2 ± 2.6 [a]	10.7 ± 2.1 [a]	8.1 ± 1.2 [a]
T½β (h)	2.8 ± 0.9 [a]	4.9 ± 1.0 [b]	4.2 ± 0.9 [b]
T½ab (h)	1.2 ± 0.2 [a]	3.5 ± 1.2 [b]	1.4 ± 0.1 [a]
K$_{el}$ (h^{-1})	0.2 ± 0.0 [a]	0.1 ± 0.1 [a]	0.2 ± 0.1 [a]
Kab (h^{-1})	1.6 ± 0.2 [a]	0.2 ± 0.2 [b]	0.5 ± 0.3 [c]
α (h^{-1})	1.2 ± 0.0	-	-
β (h^{-1})	9.2 ± 0.0	-	-
Cmax (µg/mL)	0.3 ± 0.1 [a]	1.3 ± 0.7 [b]	0.2 ± 0.0 [c]
Tmax (h)	2.2 ± 0.4 [a]	5.9 ± 1.7 [b]	3.4 ± 0.6 [c]
Frel (%)*	-	548% [a]	48% [b]

[a,b,c] The values within a row with no common superscript differ significantly (P < 0.05).
* Mean of means.
AUC, Area under the concentration-time curve; *AUC $_0$ - $_\infty$*, Area under the concentration –time to infinity; *AUMC*, Area under the first moment of the concentration-time curve; *AUMC $_0$ - $_\infty$*, Area under the first moment of the concentration-time curve to infinity; *MRT*, Mean residence time; *MRT $_0$ - $_\infty$*, Mean residence time to infinity; *α*, distribution hybrid rate constant; *β*, elimination hybrid rate constant; *A, B,*, zero time intercepts of the distribution and post-distribution phases; *K$_{el}$*, Elimination rate constant from the central compartment; *Kab*, Absorption rate constant; *T½ab*, Absorption half-life; *T½β*, Elimination half-life; *Cmax*, Calculated maximum plasma concentration; *Tmax*, Time of maximum plasma concentration; *Frel*, Relative bioavailability.

10, and 20 µg/mL of Dox and 1 mL was added to commercial ultrafiltration Waters Oasis solid-phase extraction cartridges (Waters Associates, Milford, MA). The ultrafiltrate was centrifuged at $1,200 \times g$ for 30 min at 37°C to further separate plasma proteins. This resulted in an ultrafiltrate volume of at least 200 µL that was frozen until assayed. The resulting filtrates were used to compare the degree of Dox protein binding as compared with unprocessed samples, using the same microbiological analysis. The percentage of protein-bound fraction (B) was calculated according to the following equation: B = (initial plasma concentration – ultrafiltrate concentration)/initial plasma concentration × 100. The CV for this method were <4.2%.

Results

F1

Figure 1 shows the mean ± 1 SD of the serum concentrations of Dox vs time for the three drug preparations (Dox-β, Dox-pol and Dox-a). Table 1 summarizes the pharmacokinetic variables obtained and statistical differences are highlighted.
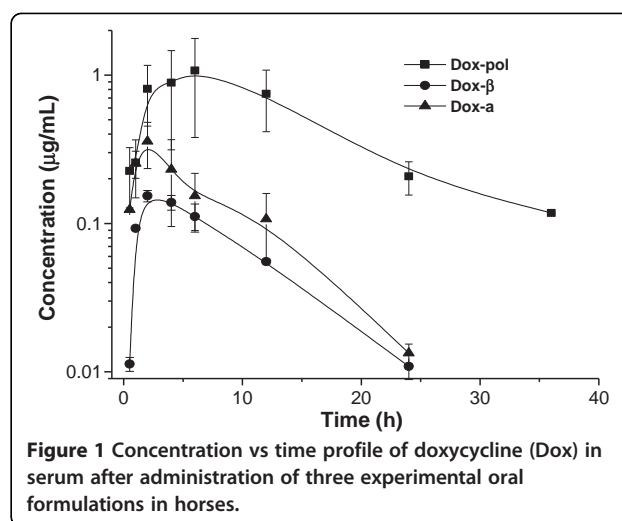
Figure 1 Concentration vs time profile of doxycycline (Dox) in serum after administration of three experimental oral formulations in horses.

Maximum serum concentration (Cmax) was highest in the Dox-pol group (1.3 ± 0.7 µg/mL) at a Tmax of 5.9 ± 1.7 h. Elimination half-life (T½β) in the Dox-pol group was 4.9 ± 1.0 h, a similar value was obtained for Dox-β (4.2 ± 0.9 h), while T½β determined after the administration of Dox-a was 2.8 ± 0.9 h. Dox-β had considerably lower plasma concentrations throughout the established sampling period as compared to the other two groups. The AUC for plasma concentrations was higher for Dox-pol (17 ± 2.2 µg h/ml), intermediate for Dox-a (3.1 ± 0.2 µg h/ml) and lowest for Dox- β (1.5 ± 0.1) (P < 0.01). Plasma protein binding did not differ among groups and was consistently 80.3 ± 1.5%.

Relative bioavailability of the two long-acting preparations as compared to Dox-a was 548% for Dox-pol and 48% for Dox-β (P < 0.01).

Discussion

Agreement between the quantitative/qualitative microbiological agar diffusion technique and the high performance liquid chromatography method used in this trial to determine serum concentrations of Dox, can be regarded as sufficiently reliable to assume that concentrations obtained through HPLC are biologically active. That is, because the microbiological agar diffusion test determines the active fraction(s) of the drug, it offers clinically meaningful data, and in this case such assumption has been validated through a purely chemical method. This allows straightforward speculations on the relationships between serum concentrations and dosing intervals for specific pathogens.

Serum concentrations obtained after the administration of Dox-β were noticeably low (Cmax = 0.2 ± 0.0 mg/mL; AUC = 1.5 ± 0.1 µg · h/mL). These values are not within the range that would be effective for many equine pathogens [1,22], and are only considered marginally for further analysis in this section.

The pharmacokinetics of the aqueous solution of Dox-h administered orally has been determined in adult horses [1,6,11,12] at a dose range from 3 to 20 mg/kg. Davis et al. [6] obtained a Cmax of 0.9 ± 0.2 μg/mL with a Tmax of 1.6 ± 1.3 h, administering a single dose of 20 mg/kg PO. By comparison Cmax obtained with Dox-a in this study, at a single 10 mg/kg dose was 0.3 ± 0.1 mg/mL with a Tmax of 2.2 ± 0.4 h. Differences between these two studies can be safely related to the dose and biological variability, but it relates mainly to the fact that the former study used fasted horses. In contrast, Cmax and Tmax values for Dox-pol were 1.3 ± 0.6 μg/mL and 5.9 ± 1.6 h, respectively, also at a dose of 10 mg/kg. By comparison higher Cmax at a lower or the same dose may be the result of a concentration build-up due to a zero order absorption kinetics caused by the polymers in the preparation along a greater absorption surface area of the GI tract. This would also explain the longer Tmax observed. The AUC obtained administering 20 mg/kg as reported by Davis et al. [6] was 13.3 ± 2.7 μg.h/mL, while the AUC obtained with the Dox-pol formulation at half their dose was 17.0 ± 2.2 μg.h/mL. This finding is not unusual for LA preparations that exhibit flip-flop kinetics [23] and may also explain the relative bioavailability which reaches an unusual 548%. In turn, to demonstrate flip-flop pharmacokinetics, the overall appearance of the serum concentration vs. time profile of the drug must be accounted for. Occasionally, as in this case, the slower rate of absorption as compared to the rate of elimination is not a straightforward conclusion to be drawn. If a much longer apparent elimination half-life following extravascular dosing is observed compared with the IV route, it suggests that flip-flop pharmacokinetics is occurring [24]. However this is not possible with Dox considering that IV administration of this drug in horses is not recommended [9,10]. Thus, applying the following equation and based on information taken from published work [25,26], a flip-flop condition may be demonstrated with the following equation:

$$Rate\ of\ Absorption = Vz\ (KC + (\Delta C/\Delta t)$$

Where Vz is the terminal exponential volume of distribution, K is the terminal disposition rate constant once drug absorption is complete, C is the plasma concentration at time t and ΔC is the change in plasma concentration over the time interval Δt. For Dox-pol plasma concentration-time data at 4 and 12 h , $\Delta C/\Delta t = 0.0176$ μg/mL/h. At the midpoint of this time period (8 h), (K)(C) = 0.1339 μg/mL/hr. Since $KC > \Delta C/\Delta t$, rate of absorption ≈ rate of elimination, a "flip-flop" condition exists and the Dox-pol formulation here described can be regarded as a true long-acting one.

As far as Dox-pol is concerned, poloxamer 407, a polyethylene oxide-polypropylene oxide- polyethylene oxide triblock co-polymer, was used as delivery vehicle-matrix. It has been shown that it enhances solubility and permeability, often resulting in improved oral bioavailability, as reported by Kahn et al. [27] using atorvastatin, carbamazepine and other poorly soluble drugs. Considering the permeability and solubility rates of doxycycline, there is controversial information regarding the classification of this drug in the Biopharmaceutics Classification System (BCS) for humans [28]. Initially Amidon et al., [29] classify this drug in Class IV, that is, poorly soluble and poorly permeable. More recently, however, Chavda et al. [30] include Dox in Class I; that is, Dox is highly soluble and highly permeable, which shows no coherence when analyzing its reported absolute bioavailability in horses i.e., from 2.8% to 17% [12,31]. In this study, absolute bioavailability of Dox for the two long-action formulations (Dox-pol and Dox-β) was not determined because the IV kinetics of the drug is needed, and risk of cardiovascular toxicity was avoided [10]. The F_{rel} for Dox-pol and Dox-β was 548% and 48%, respectively, as compared to Dox-a, in non-fasted horses. Even though decreased oral absorption of Dox has been demonstrated in fed horses [31,32], it is here theorized that other factors could have enhanced bioavailability of Dox administered in the Dox-pol formulation: mucoadhesiveness achieved by poloxamer, carbopol and xanthan gum in the formulation [33-35], perhaps enterohepatic circulation considering that, with few exceptions, this phenomenon is recognized as a common physiological peculiarity of tetracyclines [36], and a greater amount of fluid participating to produce better Dox dissolution in the horses' entire gastrointestinal lumen. This may allow absorption along other surfaces of the GI tract [31]. Additionally, total transit time from mouth to colon in adult horses is of approximately 40 h [37], a fact that complies well with the former reasoning. However, further studies are warranted to define these phenomena.

The benefits of the controlled delivery of drugs include: the maintenance of serum drug concentration at an optimal therapeutic level for a more prolonged time-interval, reduction in handling and consequently, a possible improvement in drug-administration compliance [38]. In this context, the Dox-pol preparation here described was capable of providing with a single oral administration, useful serum concentrations of this antibacterial drug for 24 h, but not longer. Although it has been stated that high concentrations of Dox *in vitro*, equivalent to 8 to 16 times the value of an average MIC could turn this time-dependent antibacterial drug into a concentration-dependent antibacterial drug [39], its cardiac toxicity refrains its use at higher doses. Hence, seeking large Cmax values is an unsafe approach [40,41].

Dox should be considered a time-dependent antibacterial drug in horses. In that context a better PK/PD ratio can be achieved when serum concentrations of the drug are barely above or at the MIC level of the involved pathogen for as long as possible within the dosing interval [36,42]. Values of MIC that can be adopted in this trial can be set from 0.25 to 1.0 µg/mL [6,22] Hence, the length of time in which minimum therapeutic concentrations can be achieved with Dox-pol varies from 12 to 24 h. Additionally, in humans, a PK/PD index accepted as predictor of therapeutic efficacy for tetracyclines as a group is the ratio of AUC0-24 (AUC0-24)/MIC [43]. If a MIC of 0.25 µg/mL is considered, the $AUC_{DOX\text{-}pol}$/MIC ratio is 68.02 and the $AUC_{DOX\text{-}a}$/MIC ratio is only 12.4. Considering the above, it is safe to regard Dox-pol as a drug preparation that possesses better PK/PD ratios to control bacterial diseases in horse as compared to Dox-a.

Conclusions

Dox-pol is an oral paste formulation that optimizes the use of doxycycline in horses in terms of PK/PD ratio congruency, and it is likely that it may also improve prescription compliance, due to its ease of administration. This may contribute to diminish the emergence of bacterial resistance. Nevertheless and although no adverse gastrointestinal reactions (diarrhea, colic, abdominal discomfort) were observed in any of the horses used in this trial, multiple dose, tissue distribution and toxicological studies are needed before clinical trials are set, to assess if this preparation can be regarded as potentially useful in this species.

Authors' contributions

HS and LG participated in the study design, planning and coordination, performed the statistical analysis and helped to draft the manuscript. HZ prepared and administered the formulations, carried out the blood sampling and was in charge of analytical procedures; she also helped to draft the manuscript. MJB participated in the study design and coordination as well as supervision of the chemical soundness of formulations. All authors read and approved the final manuscript.

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