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Comparative pharmacokinetics of a new oral long-acting formulation of doxycycline hyclate: A canine clinical trial

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Doxycycline hyclate (PubChem CID: 54686183)
Poly(ethyl acrylate-co-methyl methacrylate-co-trimethylammonioethyl methacrylate chloride) 1:2:0.2 (EUDRAGIT RL100®, PubChem CID: 104804)
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ABSTRACT

Doxycicline is used in dogs as treatment of several bacterial infections, mycoplasma, chlamydia and rickettsial diseases. However, it requires long treatments and several doses to be effective. The aim of this study was to determine the pharmacokinetics of four formulations of doxycycline hyclate, administered orally, with different proportions of excipients, acrylic acid–polymethacrylate-based matrices, to obtain longer therapeutic levels than conventional formulation.

Forty-eight dogs were randomly assigned in five groups to receive a single oral dose (20 mg/kg) of doxycycline hyclate without excipients (control) or a long-acting formulation containing doxycycline, acrylic acid polymer, and polymethacrylate in one of the following four proportions: DOX1(1:0.25:0.0035), DOX2(1:0.5:0.0075), DOX3 (1:1:0.015), or DOX4(1:2:0.0225). Temporal profiles of serum concentrations were obtained at several intervals after each treatment.

Therapeutic concentrations were observed for 60 h for DOX1 and DOX4, 48 h for DOX2 and DOX3 and only 24 h for DOX-C. None of the pharmacokinetic parameter differed significantly between DOX1 and DOX2 or between DOX3 and DOX4; however, the findings for the control treatment were significantly different compared to all four long-acting formulations.

Results indicated that DOX1 had the most adequate pharmacokinetic–pharmacodynamic relationships for a time-dependent drug and had longer release times than did doxycycline alone. However, all four formulations can be effective depend on the minimum effective serum doxycycline concentration of the microorganism being treated. These results suggest that the use of any of these formulations can reduce the frequency of administration, the patient's stress, occurrence of adverse effects and the cost of treatment.

1. Introduction

In dogs, doxycycline (DOX) is used for controlling infections caused by *Staphylococcus spp*, *Streptococcus spp* (Ross and Jones, 2004), *Haemophilus spp, Bordetella bronchiseptica* (Speakman et al., 2000), *Mycoplasma spp, Borrelia burgdorferi, Campylobacter jejuni,* and *Fusobacterium spp* (Cunha et al., 1982; Holmes and Charles, 2009). It is the choice for treatment of infections caused by *Leptospira spp* (Levett and Edwards, 2009), *Brucella canis* (Holmes and Charles, 2009), *Haemobartonella canis* and numerous tick-borne diseases, most importantly, Erhlichia canis (McClure et al., 2010). Nevertheless, these diseases have importance not only in canine health but also in human public health, because they are zoonotic diseases (McClure et al., 2010).

DOX has a bacteriostatic effect by protein synthesis inhibition (Holmes and Charles, 2009); recently, anti-inflammatory and anti-neoplastic roles have been discovered through the inhibition of matrix metalloproteases (Lee et al., 2009; Zeng et al., 2011). Doxycycline has a better clinical efficacy at low concentrations, such as at 2 to 4 times the microorganism inhibition concentration (MIC) level for susceptible microorganisms. Therefore, the inhibition of microorganisms by the drug occurs in a time-dependent manner (Cunha et al., 2000). However, suitable treatment with DOX requires 5 mg/kg dose, twice a day, during prolonged periods ranging from 21 days to years, depending on the microorganism in treatment (Bharti et al., 2003).

In spite of the benefits of treatments with DOX, its use has been limited to some extent because of adverse reactions. Oral administration can generate adverse effects such as gastric irritation, with vomiting and risk of ulcerations; tissue irritation following subcutaneous or intramuscular injection usually appears (Smith and Leyden, 2005; Xiao et al., 2013).

In veterinary medicine, a long-acting injectable formulation of DOX has been evaluated for treatment in cattle (Vargas-Estrada et al., 2008), small ruminants (Vargas et al., 2008) and dogs (Gutiérrez et al.,

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2012). An oral formulation for horses (Zozaya et al., 2013) and a subgingival system for the localized treatment of periodontitis in beagle dogs (Polson et al., 1996; Zetner and Rothmueller, 2002) have also been evaluated. The injectable drug showed an increase in half-life in dogs (133.61 ± 6.32 h), but it caused inflammation and pain at the injection site that lasted 30 days, an aspect that can cause refusal by the dogs' owners (Gutiérrez et al., 2012).

In recent decades, there has been an increased interest in the pharmaceutical industry in the development of controlled-release formulations, which include the election of the excipients according to the properties of the drug which needed modification or improvement. In this case, both acrylic acid polymer and polymethacrylate have many advantages that achieve a sustained-release. The Carbopol® is an insoluble acrylic acid polymer with gel-forming ability and mucoadhesive properties; this last property prolongs the residence time of the formulations at the site of drug absorption and reduces contact and irritation on the absorption surface (Blanco-Fuente et al., 1996; Goskonda et al., 1998; Rajesh et al., 2012). In addition, Eudragit®, a polymethacrylate polymer that has mainly been used for film-coating, also creates an inert matrix structure that allows the diffusion of the drug through pores serving as a sustained release drug and binding agent (Karthikeyini et al., 2009; Vasantha et al., 2011; EVONIK, 2013). In other studies reported that Eudragit L100 were effective stabilizers of the drug in solid dispersion and the precipitation inhibition in solution (Chauhan et al., 2013).

With regard to above the goals of this study were to determine the pharmacokinetics of doxycycline hyclate administered orally in experimental formulations with different proportions of acrylic acid and polymethacrylate-based matrices. Four (4) nonirritating, long-acting formulations of doxycycline with acrylic acid polymer and polymethacrylate were developed. The hypothesis is that long-acting formulation of doxycycline would have the potential to increase the duration of therapeutic blood concentrations of the drug, reduce the frequency of administration and decrease adverse reactions compared with immediate-release products.

2. Materials and methods

2.1. Materials

Doxycycline hyclate (Indukern, Mexico), polymethacrylate (EUDRAGIT RL100®; Evonik, Germany) and acrylic acid polymer (Carbopol® 971 P NF polymer; Lubrizol, Mexico) were acquired from the manufacturing companies.

2.2. Pre-formulation stage

The physical and chemical characteristics of the doxycycline hyclate powder (Indukern, Mexico) were obtained by scanning electron microscopy, particle size distribution, infrared spectroscopy (Spectrometer FTIR Perkin-Elmer RX-I model, using the potassium bromide pellet method), x-ray diffraction (Siemens D5000 powder diffractometer with copper anticathode l = 1.5406 Å and Software Diffract AT 3.3 on 35 kV and 30 mA) and differential scanning calorimetry (DSC 321 METTLER TOLEDO with a heating rate of 10 °C/min with nitrogen atmosphere). Moreover, the rheological properties were evaluated, included bulk density, tapped density, true density, Carr compressibility index, Hausner ratio, porosity percentage, angle of repose, and flow velocity. The wet percentage of the powder was measured with a thermobalance (OHAUS MB 2000). All techniques were performed according to the US Pharmacopeia (USP 30, 2007).

2.3. Animals

Forty-eight (48) healthy adult dogs (2 to 8 years old) of different breeds and both sexes were included in this study. The mean body weight of the dogs was 17.75 kg (range, 15 to 30 kg). The dogs were vaccinated and were determined to be healthy on the basis of physical examination findings. The dogs had not been medicated with any antibacterial medication for at least 30 days. During the study, all dogs received water ad libitum and were fed a commercial diet twice daily.

This study was approved by the Institutional Subcommittee of Research, Care and Use of Experimental Animals according to the Mexican Official Regulation NOM-062-ZOO-1999. The study was conducted at the Facultad de Medicina Veterinaria of the Universidad Nacional Autónoma de México, Mexico City. The owners of the dogs included in this research project gave written consent for their dog's participation in the study.

2.4. Long-acting drug preparation

For long-acting formulations, doxycycline hyclate, acrylic acid polymer, and polymethacrylate[g] were mixed in the following four different ratios: DOX1(1:0.25:0.0035), DOX2(1:0.5:0.0075), DOX3(1:1:0.015), and DOX4(1:2:0.0225). After mixing, the formulations were granulated manually by wet granulation process with ethanol (Faure et al., 2001). Granulation process includes one or more powder particles to form multiparticle entities called granules. Granules were formed by the addition of ethanol onto excipients and doxycycline until obtained homogeneous mixture. The agitation resulting in the system along with the wetting of the components within the formulation results in the aggregation of the primary powder particles to produce wet granules (Faure et al., 2001). To obtain homogenous granules, the mixture was passed through an N° 20 mesh and final size was 0.85 mm average. The granulation liquid (ethanol) was removed by drying with stove BINDER (BINDER GmbH, Germany) at 37 °C during 24 h. Excipient proportions were based on previous research (Karthikeyini et al., 2009; Kulkarni et al., 2010), manufacturing recommendations (Lubrizol Advanced Materials, 2011; EVONIK, 2013) and the handbook of pharmaceutical excipients (Rowe et al., 2012). The granules were inserted in conventional gelatin capsules according to the animals' body weight. Each dog was given one capsule administered by hand into the dog's mouth.

2.5. Study design

In a crossover study, dogs were randomly assigned (in five groups, four groups of ten and control group with 8) to receive a single oral dose (20 mg/kg) of doxycycline hyclate without excipients (control) or any of the four long-acting formulations. Each dog received the treatment assigned and the control treatment with a washout period of 30 days between both treatments.

The dose of 20 mg of doxycycline/kg represents the cumulative dose for 2 days of treatment, according to the recommended dosage of 5 mg of doxycycline/kg twice a day (Bharti et al., 2003; Holmes and Charles, 2009; Levett and Edwards, 2009). After administration, blood samples (3 mL) were obtained by venipuncture from each animal at 0, 1, 2, 4, 8, 12, 24, 36, 48, 60, 72 and 96 h after drug administration. The serum was immediately separated from each sample by centrifugation and was stored at −20 °C until analyzed.

To evaluate acute toxicity, the animals were supervised for three days after the treatment ended. Dogs were monitored for signs of discomfort, diarrhea, or vomiting during and after each experiment.

2.6. Serum doxycycline concentration determination

The serum doxycycline concentrations were determined by modified agar diffusion analysis (Okerman et al., 2004) with Bacillus cereus (ATCC 11778, American Type Culture Collection, Manassas, Va.) as a test organism on a Mueller-Hinton dehydrated growth medium (BIOXON, Becton Dickinson, Mexico City, Mexico). The drug concentrations were determined with linear regression analysis by a comparison of the diameters of the inhibition halos with the standard curve (200, 20, 10, 5, 2.5, 1.25, 0.625, 0.3125, and 0.1562 μg/mL) prepared in pooled