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Short communication

Pharmacokinetics of a novel spot-on formulation of praziquantel for dogs

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ABSTRACT

Praziquantel (PZQ) is an anthelmintic drug used both in humans and animals that can be administered through various routes. There are transdermal formulations for cats, but only oral or subcutaneous dosage forms for dogs. Given the fact that the cat's skin and the dog's skin have different characteristics, which in turn affect bioavailability, we developed a PZQ spot-on formulation for dogs. This study was aimed at determining the plasmatic behavior of topically administered PZQ (Labyes®) in adult dogs.

Dogs were administered PZQ (14.5 mg/kg PZQ, from a solution of 100 mg/ml). Blood samples were drawn before treatment onset and at the following time points after PZQ administration: 1, 2, 4, 6, 12, 24 and 48 h. PZQ plasma concentration was determined by ultra-high performance liquid chromatography (UPLC) coupled to tandem mass spectrometry (MS/MS). Observed maximum concentration (C_{max}), area under the concentration–time curve from the time of drug administration to infinity (AUC_{inf}) and time to maximum concentration (T_{max}) were calculated for each animal, and mean ± SD for each parameter was obtained. Results were as follows: C_{max} = 56.0 ± 15 ng/ml; AUC_{inf} = 910.2 ± 220 ng* h/ml, T_{max} = 5.0 ± 1.1 h. This is the first study to provide pharmacokinetic data of a praziquantel spot-on formulation for dogs.

1. Introduction

Praziquantel (PZQ), a synthetic isoquinoline-pyrazine derivative, is an anthelmintic used for the treatment of infections caused by various species of trematodes and cestodes in domestic animals. There are available both oral and subcutaneous dosage forms for administration of PZQ to dogs, but not transdermal formulations, which do exist for administration to cats.

The transdermal route of administration achieved via spot-on formulations has several advantages over the oral or subcutaneous forms; among them are the possibility of a controlled administration, the minimization of hazardous animal handling by the operator and a better tolerability for the animal, as it reduces the risk of vomiting and of pill rejection. Furthermore, the simplicity of the administration procedure shall very likely contribute to treatment adherence, thus increasing treatment effectivity. Regarding this matter, a critical analysis of the effectiveness of cystic echinococcosis eradication programs in South America demonstrated that the use of PZQ for 30 years has not been able to eradicate the endemic disease in dogs, nor its transmission to humans, mainly due to the practical complications of a large-scale and periodic treatment (Larrieu and Zanini, 2012).

From a pharmacokinetic standpoint, the transdermal route has the advantage of avoiding the first-pass effect, thus increasing drug bioavailability. It is also an adequate route for antiparasitic drug combinations, a formulation strategy that increases the chance of a thorough deworming in a single application.

The efficacy of transdermal PZQ, either alone or in combination with other endectocides, has been widely demonstrated in cats (Böhm et al., 2015; Jenkins and Romig, 2000; Krüdewagen et al., 2015; Traversa et al., 2009). However, its penetration in dogs remains to be determined.

Species-specific formulations need to be developed, due to the differences between the skin barriers of dogs and cats. Variables such as blood flow, capillary density, hair coat structure and hair growth rate, number and distribution of sebaceous glands and mainly skin thickness (21.16 ± 2.55 μm in dogs versus 12.97 ± 0.93 μm in cats)

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considerably affect drug absorption (Monteiro-Riviere et al., 2008).

In view of the above, we decided to develop a topical formulation for dogs, with characteristics similar to those of the ones already available for cats. We here present the first stage of our development; a demonstration of the systemic bioavailability of spot-on PZQ administered to dogs.

2. Materials and methods

2.1. Ethics committee's approval

This clinical trial was approved by the Ethics Committee of the Technological Platform EBAL (University of Buenos Aires-CONICET-University of La Plata). Informed consent was signed by the caregivers of the animals. The study was conducted according to Good Clinical Practices and International Guiding Principles for Biomedical Research Involving Animals as issued by the Council for the International Organizations of Medical Sciences.

2.2. Selection and inclusion of experimental subjects

Seven mixed-breed healthy adult dogs (5 males and 2 females), from the S.A.D.A.C. Animal Shelter located in Quilmes, Province of Buenos Aires, Argentina, were included in this trial. Mean ± SD body weight values were 18.03 ± 4.8 kg, ranging from 12.7 to 25.0 kg.

Animals were physically examined by a veterinarian and health status was determined through the following parameters: nutritional status (appropriate weight for breed, gender and age, with no signs of obesity or of muscle loss), mucous membrane color, capillary refill time, behavioral parameters (aggression, submission, lethargy, tremors), psychomotor performance (normal, inactive, slowed-down), gait (rigidity, ataxia), heart and lung auscultation, abdominal palpation, presence or absence of disease-related signs (fever, vomiting, stool character).

Pregnant or lactating animals were excluded, as were underweight subjects or animals with clinical evidence of any disease, especially parasitic diseases.

Animals were individually housed in 7 suitable stainless steel kennels. Food was available, as provided in the Animal Shelter, in two daily servings, one of them consisting of rice, chicken and beef with bone and the other one of standard dry food; tap water was available *ad libitum*.

Subjects were identified with numbered collars; kennels were also numbered.

A clinical record card was kept, which contained subject number, kennel number, gender, weight, age, hair (short, medium, long), distinctive features, study drug dosage and adverse events.

2.3. Treatment and sample collection

Six of the seven dogs included in the study received a 14.5 mg/kg spot-on dose of PZQ provided by Chemo Group in a solution of 100 mg/ml (0.145 mg/kg). Thus, the volume administered was 0.145 ml/kg. The formulation consisted of a 10% PZQ solution prepared in a diluent that contained several antioxidant substances and a specific enhancer (aprotic polar solvent) (patent pending in the National Institute of Industrial Property, Argentina, May 2016). Animal #7 received no treatment and was used as negative control

PZQ was applied topically directly on the animal's dry skin, in the back cervical area of the neck, pulling the hair apart to avoid significant spillage on the hair coat. This procedure prevents dogs from licking the product from the administration site.

One-milliliter blood samples were drawn either from the cephalic or the femoral vein before and 1, 2, 4, 6, 12, 24 and 48 h after drug administration. Samples were collected in tubes containing dipotassium EDTA as anticoagulant. Within two hours of collection, samples were transferred to the laboratory, where plasma was separated by centrifugation and stored at −20 °C until processed.

2.4. Plasma PZQ quantitation method

Quantitation of PZQ in plasma was performed at a laboratory accredited by the College of American Pathologists. Briefly, plasma samples were pretreated with acetonitrile at a low pH to precipitate proteins. The supernatant was analyzed by ultra high performance liquid chromatography (UPLC) coupled to tandem mass spectrometry (MS/MS) detection. Plasma PZQ identification and quantitation was done by associating the ion mass spectrum obtained with its chemical structure, using the precursor ion $(M\ H)^+$ (*m/z*: 313.1).

2.5. Method validation

In order to validate the plasma PZQ quantitation method, the following parameters were evaluated; acceptance criteria are indicated between brackets: specificity and carryover, recovery, accuracy (percent relative error of less than 15%), precision (coefficient of variation of less than 15%), quantitation limits ($\geq 3 \leq 1000$ ng/ml), linearity (three plots with a minimum of 6 points each and R^2 greater than 0.98), robustness, sample stability through freeze/thaw cycles, short term stability, analytical conditions, storage, autosampler use, stability of the standard and long term stability. All parameters passed the acceptance criteria.

2.6. Data analysis

A non compartmental analysis was performed to calculate individual C_{max}, AUC_{inf} and T_{max}, for each subject. Mean (SD; upper and lower CI 95%) for each parameter were then calculated from the individual values.

3. Results

Fig. 1A shows the individual plasma PZQ concentration versus time profiles obtained for each experimental subject. Also, mean (SD) values are represented in Fig. 1B.

Calculated mean values (SD; 95% CI) of praziquantel pharmacokinetic parameters were as follows: C_{max} = 56.0 ng/ml (15; 40.4–71.7), AUC_{inf} = 910.2 ng* h/ml (220; 679–1141), and T_{max} = 5.0 h (1.1; 3.8–6.1).

4. Discussion

We here present the first study to provide pharmacokinetic data of a praziquantel spot-on formulation for dogs. Our results show that the formulation has a T_{max} of 5 h, with a range between 4 and 6 h, and that there are detectable plasma concentrations after 48 h of the administration. The maximum plasma concentration was 56.0 ± 15 ng/ml, attained after administering a dose of 14.5 mg/kg. Mean value of the area under the concentration-time curve from timepoint 0 to infinity was 910.2 ± 220 ng h/ml.

PZQ has been formulated both as a single drug and in combination with other antiparasitic drugs in spot-on preparations for cats (Krüdewagen et al., 2015; Kvaternick et al., 2014; Tielemans et al., 2014). According to the Profender™ drug monograph submitted by Bayer Laboratories to the European Medicines Agency, this product contains 85.71 mg/ml PZQ, and the obtained C_{max} for a dose similar to the one used in this trial (0.145 ml/kg) was of 61.3 ± 44.1 ng/ml (EMA, 2008), a value comparable to the one obtained by us. On the other hand, the T_{max} reported for Profender™ was 18.7 ± 47 h, a much greater value that the one found in our trial.

PZQ can also be found in another spot-on antiparasitic formulation for cats, Broadline™ from Merial laboratories. The dose in this case is

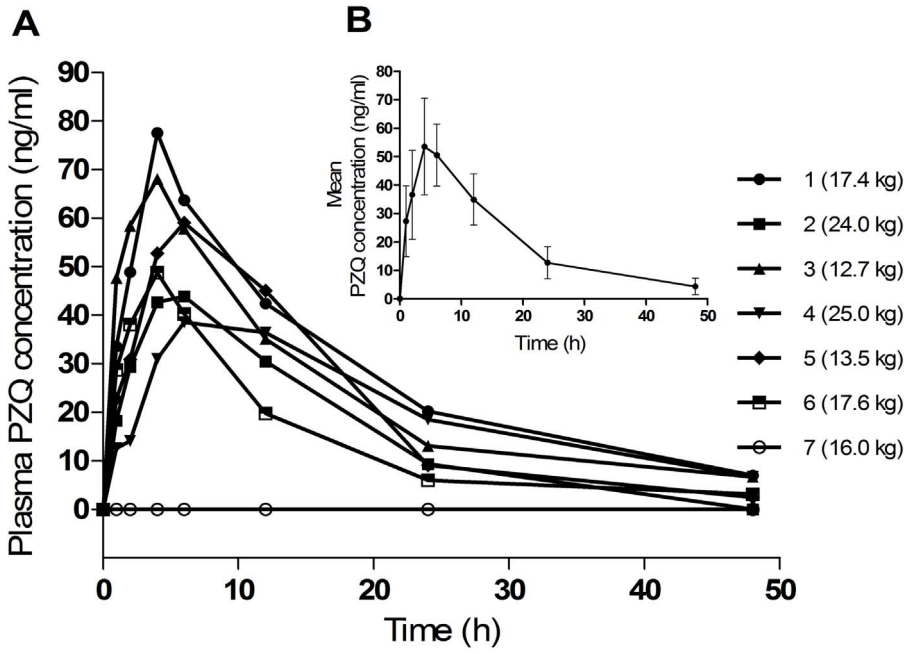

Fig. 1. A) PZQ plasma concentration *vs.* time curves obtained from treating dogs #1 to #6 with a single dose of 14.5 mg/kg PZQ. Dog #7 received no treatment. Body weights are in brackets. B) Mean plasma concentration (± SD) *vs.* time curve.

24.9 mg for cats weighing less than 2.5 kg and 74.7 mg for cats weighing 2.5–7.5 kg. Reported pharmacokinetic PZQ parameters include a T_{max} of 6 h, similar to the one found in this study, and a C_{max} of 157 ng/ml for a dose of approximately 10 mg/kg (EMA, 2014), a much greater value that the one obtained with our formulation.

Even though the relationship between PZQ plasma levels and its antiparasitic efficacy has not been extensively studied in dogs, there are a number of studies in cats which compared PZQ efficacy after an oral and a spot-on administration. The results obtained were encouraging, thus favoring the development of the transdermal route in this species.

Taweethavonsawat et al. (2013) for instance, did a comparative efficacy study in cats infected with *Ancylostoma ceylanicum*. A spot-on and an oral PZQ formulation, in combination with other antiparasitic drugs, were tested. Both products demonstrated to be equally effective against *A. ceylanicum*.

Also, Tielemans et al. (2014) showed that PZQ combined with other antiparasitic drugs was 100% effective against *Echinococcus multilocularis* when administered topically to cats.

The above-mentioned studies reported bioavailability results that are comparable to the ones obtained in our model.

Finally, Xie et al. (2011) compared the pharmacokinetic profile of a PZQ suspension in saline solution with that of a PZQ-loaded solid lipid nanoparticle suspension, both administered subcutaneously to dogs. For the 5 mg/kg dose tested as a saline solution suspension, which represents approximately one third of the dose used in our trial, the authors reported a C_{max} of 47.8 ng/ml, a T_{max} of 1.45 h and an AUC_{inf} around 1040 ng h/ml; plasma concentration at timepoint 48 h was below 10 ng/ml. As expected, these findings may be pointing to the fact that the subcutaneous route of administration has a greater bioavailability and a shorter time to maximum concentration than the transdermal route but it is interesting to point out that AUC_{inf} informed by these authors is within the range we found and that with these pharmacokinetic profile an almost 100% efficacy against *E. granulosus* was reported. Although it seems reasonable that a lower dose may be necessary to achieve effective plasma levels when subcutaneous route is employed, similarities between AUC obtained in the work of Xie et al. (2011) and in our allow us to predict positive therapeutic action of our product. However, this assumption needs to be experimentally evaluated.

Therefore, we can conclude that the plasma PZQ levels found in this trial are comparable to previously reported results. The advantage of a faster absorption rate for the subcutaneous route, as shown by Xie et al. (2011) can be compensated for by the simplicity of the spot-on administration procedure. It is worth mentioning that similar AUC_{inf}, C_{max} and plasma concentration values obtained 48 after the administration for both routes were observed.

5. Conclusion

This is the first report showing the pharmacokinetics of a novel spot-on formulation of praziquantel to be administered to dogs. C_{max} values are similar to those observed after administration of available spot-on formulations for cats. AUC_{inf} was similar to that reported for a subcutaneous administration of an effective saline solution suspension to dogs and therefore may predict clinical efficacy.

Conflicts of interest

Jorge Dale and Carlos Corrales work on Labyes S.A., Guillermo Di Federico perceived fees paid from Labyes S.A. for his professional work in this study. María Laura Gutierrez, Juan Mauricio Minoia, Paula Schaiquevich and Silvia Wikinski are employees of the governmental agency CONICET and have no conflicts of interest.

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