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Research paper

Injectable fipronil for cattle: Plasma disposition and efficacy against *Rhipicephalus microplus*

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ABSTRACT

Fipronil is a phenylpyrazole class insecticide. It is widely used as an insecticide in agriculture and in the control of ectoparasites in veterinary medicine. The application of fipronil in an injectable form (subcutaneously) becomes an innovation, since there is no commercially available preparation containing fipronil herein. The present study aimed at fipronil usage, applied subcutaneously in cattle, to control *Rhipicephalus microplus.* The assessing criteria used in the research have been the construction of the plasma concentration curve and efficacy studies. A method using High Performance Liquid Chromatograph with ultraviolet detection was developed for determination of fipronil in bovine plasma samples, providing a fast and simple process with good reproducibility and low limit of quantification. The validation of the analytical method showed linearity, selectivity, precision, accuracy, sensitivity and stability, thus proving it as suitable for routine analysis. This method showed to be an important investigative tool in the analysis of fipronil plasma concentration in cattle. Fipronil administered via subcutaneous in bovine reached the systemic circulation (C_{max} = 378.06 ± 137.44 ng/mL), was quickly absorbed (t_{max} = 10 ± 0.87 h), and its elimination occurred slowly ($t_{1/2}$ = 12 days), while maintaining quantifiable blood plasma levels (23.79 ± 12.16 ng/mL) for up to 21 days after the treatment with a 1 mg/kg dosage. The *in vivo* efficacy tests proved that fipronil applied subcutaneously in a single dose of 1 mg/kg in cattle exhibited a mean efficacy of 82.41% against *R. microplus*. The potential of subcutaneous injection as an alternative treatment route in cattle encourage the development of an injectable formulation of fipronil.

1. Introduction

Fipronil (FIP) is a broad-spectrum phenylpyrazole recommended for agricultural, phytosanitary and veterinary uses (Tingle et al., 2003). Is very effective against grasshoppers, mosquitoes, fleas and ticks both larval and adult stages (Chanton et al., 2001; Aajoud et al., 2003). Its mechanism of action involves neurotransmitter aminobutyric acid (GABA) block (Raugh et al., 1990) via chloride channels resulting in insect death (Postal et al., 1995). GABA plays an important role in neural transmission of both vertebrates and invertebrates, however FIP presents some selectivity since it's binding with GABA receptor is weaker in vertebrates, being much more toxic to these parasites than for mammals (Hainzl and Casida, 1996; Matsuda et al., 2001; Payne et al., 2001; Hovda and Hoser, 2002).

Since the main tick species, that compromises the health and productivity of cattle in Brazil is the *Rhipicephalus microplus*, its control has become a focus of attention of the veterinary pharmaceutical industry as well as government agencies, and educational and research institutions. Possible innovations include the development of new pharmaceutical forms that promote a broader spectrum of action, ease of use, and safety for the environment and the applicator (Taylor, 2001).

Currently, FIP is commercially available only in topical forms (pour-on) for tick control in cattle. Efficacies against *R. microplus* after single treatment of topical formulation in cattle have been demonstrated (Davey et al., 1998). The injectable route has some advantages over topically, as the preference of the owner and/or veterinarian by injection application method and the issue of environmental impact. Furthermore, topical products would be more exposed to environmental degradation, like mechanical removal by action of rain or degradation of photosensitive drugs by action of sunlight. In the specific case of FIP, Davey et al. (1999) demonstrated that the persistence time against reinfection after a single

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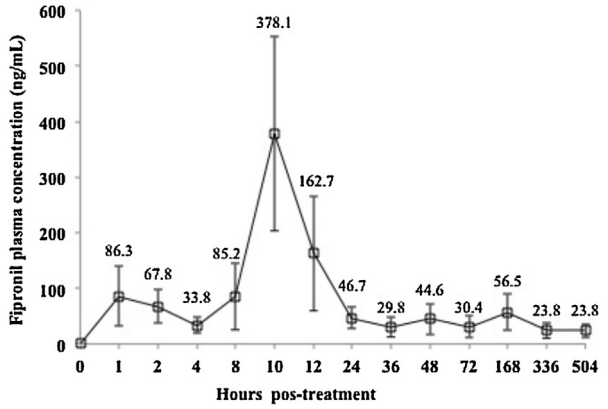

Fig. 1. Mean ± SE concentration of fipronil in plasma of cattle treated with a single subcutaneous injection of fipronil solution at dose of 1 mg/kg body weight.

application of FIP "pour-on" reduced significantly after exposure to sunlight.

The therapeutic action of drugs is dependent on their effective concentration at the site of action for a given period of time. Once a drug concentration at the site of action is in equilibrium with the same concentration in the bloodstream, for most drugs, the measurement of drug concentration in plasma becomes its measurement at its site of action (Shargel et al., 2004). The drug availability from the dosage form plays a critical role in a drug's clinical efficacy (Shargel et al., 2004). Therefore, the drug's plasma profile from dosage form studies is crucial in assessing the performance of new formulations.

Thus, this study was designed to determine the potential of subcutaneous injection of FIP as an alternative treatment route in cattle. The aim of the study was to investigate the plasma disposition and efficacy of FIP in cattle after subcutaneous administration.

2. Material and methods

2.1. Pharmacokinetics analysis

A dose of 1 mg/kg b.w. of FIP solution in glycerol formal/propylene glycol was administrated by single sub-cutaneous injection to 12 male zebu calves, which have been fed with hay free of larvae tick and kept individually in stalls for 30 days before treatment. As a result, these animals remained free of ticks throughout the experimental period. Blood was collected in heparin tubes by jugular venipuncture of cattle before and at 1, 2, 4, 8, 10, 12, 24, 36 and 48 h and 3, 7, 14, 21 and 28 days after administration. Plasma was obtained by centrifugation at 756*g* for 10 min at 4 °C and stored at −20 °C until analysis. Animal procedures were conducted in accordance with accepted standards for good clinical practice from The European Agency for the Evaluation of Medicinal Products (VICH, 2000).

The plasma concentration *vs.* time curves obtained after each treatment in individual animals was fitted with the PK Functions for Microsoft Excel program (Allergan, Irvine, CA 92606, USA). Pharmacokinetic parameters for each animal were analyzed using compartmental model analysis. The maximum plasma concentration (C_{max}) and time to reach maximum concentration (t_{max}) were obtained from the plotted concentration time curve of each drug in each animal. The trapezoidal rule was used to calculate the area under the plasma concentration time curve (AUC).

2.2. Analytical procedure

The plasma concentrations of FIP were analyzed by high performance liquid chromatography (HPLC) with ultraviolet (UV) detection following solid phase extraction (SPE) procedure according to a previously described method (Cid et al., 2012a).

Briefly, plasma samples were subjected to the SPE clean-up using Oasis® HLB cartridges (Waters, Massachusetts, USA) using methanol as eluent solvent. The chromatographic separation was performed using a Symmetry C18 column (5 μm, 4.6 × 250 mm) (Waters, Massachusetts, USA) maintained at 25 °C. The mobile phase consisted of acetonitrile: water (60:40, v/v) with a flow rate of 1.0 mL/min. The UV wavelength was set at 210 nm and the injection volume was 10 μL.

2.3. Efficacy studies

To evaluate the *in vivo* efficacy of the FIP applied subcutaneously was used the methodology developed by Holdsworth et al. (2006) and recommended by WAAVP – World Association for advancement of Veterinary Parasitology (2006) – for testing acaricide in ruminants.

Twelve zebu calves, each weighing 250 ± 5 kg, were randomly divided into two groups of six animals per group. One group of six calves designated as an untreated group served as a negative control group to which the treated group was compared. The second group of calves was treated with FIP solution in glycerol formal/propylene glycol at dose of 1 mg/kg b.w. by single subcutaneous injection. All calves were weighed on certified scales one day before application of the test material to ensure proper dosing. Throughout the study all animals were held in an open-sided barn under ambient conditions, except that a roof prevented direct sunlight or rainfall from reaching the animals. During the study each animal was held in an individual stanchion on wooden pallets so that ticks could be collected every 24 h.

The RS strain of *R. microplus*, courtesy of Veterinary Research Institute Desiderius Finamor (Rio Grande do Sul) was used for artificial infestation of animals. This was chosen to carry out the clinical efficacy trial because it showed greater susceptibility to FIP in an *in vitro* assay (Drummond et al., 1973) comparing three distinct populations (Cid et al., 2012b).

All calves (both groups) were artificially infested with approximately 2500 larval *R. microplus* ticks that were 2–4 weeks old on days −23, −21, −19, −17, −15, −13, −11, −9, −7, −5, −3 and −1 before FIP treatment. This procedure and interval ensures an infestation with all stages of the tick (larva, nymph and adult) during treatment. Throughout the whole experiment, animals were kept individually in stalls with pallets so that ticks could be collected daily. The collection of naturally detached engorged females was performed by manual recovery from the waste produced after washing the stalls.

On days −3, −2 and −1, engorged females recovered from each animal were quantified and weighed, thus ranking the animals in accordance with the number of ticks recovered. On the day of treatment (day 0), each treated calf was injected subcutaneously with the test material at dose of 1 mg/kg according to b.w. From day 1 to day 23 post-treatment (P.T.), engorged female ticks detached from each animal were collected from the floor of the stall and counted daily. Fipronil efficacy in each animal of each day was calculated using the formula: Efficacy = 100 − [100 × (A × D)/(B × C)]. Where: A = mean ticks of untreated group before treatment (days −3, −2 and −1); D = mean ticks of treated group on the day of treatment (day 0), B = mean ticks of untreated group on the day of treatment (day 0), C = mean ticks of treated group before treatment (days −3, −2 and −1).