Veterinary Parasitology 233 (2017) 25–31

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Research paper

Initial evaluations of the effectiveness of spinetoram as a long-acting, oral systemic pulicide for controlling cat flea (*Ctenocephalides felis*) infestations on dogs

W. Hunter White[a], Kari L. Riggs[a], Michelle L. Totten[a], Daniel E. Snyder[a,*], Christine M. McCoy[a], David R. Young[b]

[a] Elanco Animal Health, A Division of Eli Lilly & Company, Greenfield, IN, USA
[b] Young Veterinary Research Services (YVRS), Turlock, CA, USA

ARTICLE INFO

Article history:
Received 25 July 2016
Received in revised form
14 November 2016
Accepted 19 November 2016

Keywords:
Dog
Spinetoram
Spinosyn
Flea
Ctenocephalides felis

ABSTRACT

Spinetoram is a semi-synthetic, spinosyn class natural product derived from fermentation by the actinomycete, *Saccharopolyspora spinosa*. Based on LD_{50} (50% lethal dose) values against adult cat fleas (*Ctenocephalides felis*) using an *in vitro* contact assay, spinetoram was approximately 4-fold more potent than spinosad. Subsequently, two parallel-arm, randomized block design laboratory studies were conducted to evaluate the effectiveness of orally administered spinetoram against experimental *C. felis* infestations on dogs, when administered as a single dose or multiple doses over a 6–12 h interval. In the first study, 16 mixed-breed dogs were allocated to two treatment groups of eight dogs each, based on pre-treatment flea retention rates: negative (placebo) control; and a single dose of spinetoram at 30 mg/kg. In the second study, 32 mixed- and pure-breed dogs were allocated to four treatments groups of eight dogs each, based on pre-treatment flea retention rates: negative (placebo) control; a single dose of 60 mg/kg; three sequential 20 mg/kg oral doses evenly administered over a 6 h period; and three sequential 20 mg/kg oral doses evenly administered over a 12 h period. In both studies, treatments were administered to dogs in a fed state in order to enhance absorption of spinetoram. Therapeutic efficacy was assessed 24 h after treatment and persistent efficacy was assessed 48 h after each subsequent flea infestation. The duration of effectiveness was assessed at approximate weekly intervals beginning on Day 5 through Day 56 in the first study, or through Day 105 in the second study. In both studies, treatment efficacy was ≥99% (geometric means) through 44 d, with ≥99% efficacy continuing through 72 d for all three treatments in the second study. Efficacy remained ≥90% for at least 8 weeks with a single 30 mg/kg dose; through 13 weeks with three sequential 20 mg/kg doses; and through 15 weeks with a single 60 mg/kg dose. For all time points and in both studies, spinetoram-treated groups had significantly fewer live fleas relative to their respective negative control group ($p < 0.05$). The pharmacokinetic profile in dogs revealed that the mean plasma concentration of spinetoram required for effectiveness against fleas was maintained for at least 3 months regardless of whether the 60 mg/kg total body dose was administered as a single bolus or in three sequential 20 mg/kg doses administered over a 6–12 h period of time. The results of preliminary *in vitro* and *in vivo* studies demonstrate that orally administered spinetoram was well tolerated, and provides long lasting effectiveness against *C. felis* infestations on dogs.

1. Introduction

The cat flea (*Ctenocephalides felis felis*) is the most common flea species infesting dogs and cats globally (Dobler and Pfeffer, 2011), and is classified as a public health pest by many governmental agencies including the United States Centers for Disease Control and Prevention. Fleas are responsible not only for general discomfort to the host animal, but the physical irritation can lead to excessive scratching with secondary medical issues such as pruritus, dermatitis and infection (Bruet et al., 2012). Additionally, dogs and cats can develop an allergic hypersensitivity reaction to the saliva of *C. felis*, resulting in flea allergy dermatitis (Dryden, 2009; Lam and Yu, 2009). Finally, fleas in general and cat fleas in particular, are known

* Corresponding author at: Elanco Animal Health Research and Development, 2500 Innovation Way, Greenfield, IN 46140, USA.
E-mail address: snyder_daniel_e@elanco.com (D.E. Snyder).

http://dx.doi.org/10.1016/j.vetpar.2016.11.018
0304-4017/

vectors of zoonotic pathogens that include the flea tapeworm (*Dipylidium caninum*) as well as causative agents of flea-borne spotted fever in cats (*Rickettsia felis*) and cat scratch fever, or bartonellosis (*Bartonella helselae*) (Beugnet and Marié, 2009).

While there are an increasing number of effective options that are available to pet owners for controlling infestations on dogs and cats, fleas remain problematic and of great concern to both consumers and veterinary practitioners (Pfister and Armstrong, 2016). Topical treatments, usually applied directly to the animal's skin along the dorsum, are favored by many owners because of general widespread availability and affordability in commerce, often without a veterinary prescription, the ease of administration to cats in particular, and because they frequently contain additional chemical entities that either repel fleas (*e.g.*, pyrethrins and synthetic pyrethroids) or render flea eggs sterile and interfere with flea larval development (*e.g.*, insect growth regulators). However, topical flea control products are not without their limitations, both founded and perceived, which can include: (i) safety concerns due to dislodgable residues and inadvertent exposure to people and in particular, children or other co-habitating pets (Jennings et al., 2002; Craig et al., 2005); (ii) misuse with subsequent toxic effects on non-target species, for example the use of synthetic pyrethroid-containing dog products on cats (Gleadhill, 2004); (iii) the improper application by consumers, a factor that is often cited as a primary culprit behind perceived lack-of-effectiveness (Coles and Dryden, 2014); and (iv) cosmetic/aesthetic concerns from topical formulations that can leave the fur at the treatment site with a wet, oily or discolored appearance (Sabnis et al., 2007), or with an unpleasant odor for excessive periods of time following treatment. On the other hand, orally administered flea control products have the advantage of ease of administration, and minimal safety concerns for other pets or small children in the household. While fleas must bite the animal and begin feeding in order to gain exposure to an oral systemic agent, this has been shown to occur with some topically applied agents as well (Melhorn et al., 2001; McCoy et al., 2008).

An organophosphate cythioate was the first compound to exhibit an oral systemic killing effect against adult-stage fleas (Gordon, 1995), but its level of effectiveness and poor safety profile precluded commercialization as a flea control drug. Lufenuron, a benzoylurea-class molecule, was the first oral systemic flea preventive to be commercialized for dogs (Hink et al., 1994; Blagburn et al., 1995; Smith et al., 1996) as well as cats (Hink et al., 1991; Blagburn et al., 1994), despite the fact that the mechanism of activity is limited to developmental inhibition of immature flea stages with subsequent interruption of the flea life-cycle, and no flea adulticidal activity. The introduction of nitenpyram provided somewhat of a solution to shortcomings inherent with lufenuron, delivering a very rapid onset of therapeutic effectiveness against adult-stage fleas following a single oral treatment, although persistent activity was limited to 24–48 h (Dobson et al., 2000; Rust et al., 2003; Schenker et al., 2003). A combination of these two treatments, while effective, proved inconvenient for the user and involved repeated dosing with nitenpyram as frequently as every-other day (Dryden et al., 2001; Miller et al., 2001). The first commercialized oral systemic treatment designed to provide therapeutic efficacy with up to 30 d of continuous killing of adult and immature fleas on dogs and cats contained the fermentation-derived natural product spinosad (Snyder et al., 2007, 2013; Blagburn et al., 2010; Paarlberg et al., 2013; Wolken et al., 2015). The subsequent emphasis on research into oral systemic agents has resulted in the recent introduction of three new compounds belonging to the isoxazoline class of chemistry, afoxolaner (marketed for dogs under the trade name NexGard™), fluralaner (marketed for dogs under the trade name Bravecto®) and sarolaner (marketed for dogs under the trade name Simparica™), all of which provide excellent therapeutic and persistent effectiveness against flea as well as tick infestations on dogs (Beugnet et al., 2014, 2015a,b; Halos et al., 2014; Hunter et al., 2014; Meadows et al., 2014; Rohdich et al., 2014; Shoop et al., 2014; Becskei et al., 2016; McTier et al., 2016; Six et al., 2016a,b).

Spinetoram is a member of the spinosyn-family of fermentation-derived insecticides produced by the actinomycete, *Saccharopolyspora spinosa* (Martz and Yao, 1990), and it is the second spinosyn-class molecule to be commercialized for crop protection purposes (Sparks et al., 2008; Kirst, 2010). Spinetoram is a semi-synthetic mixture consisting of two primary active factors, 3′-*O*-ethyl-5,6-dihydro-spinosyn factor J and 3′-*O*-ethyl-spinosyn factor L, present in an approximate 3:1 ratio, respectively (Sparks et al., 2008; Kirst, 2010). The discovery of spinetoram represented advancement over spinosad for crop protection, in terms of having a superior activity profile against lepidopteran insects, an increased spectrum of activity against other crop pests and an improvement in photo-stability that contributed to a longer duration of pest control under field conditions (Sparks et al., 2008). Spinetoram possesses a favorable toxicity and safety profile in mammals, and is considered by the EPA to be a reduced risk pesticide that is toxicologically identical to spinosad (Chloridis et al., 2007; Sparks et al., 2008). This favorable safety profile and inherently potent insecticide activity contributed to the commercialization of spinetoram as a monthly topical flea control product for cats (marketed under the trade-name Cheristin® for Cats). Because of demonstrated superiority to spinosad in terms of insecticide activity and stability in the crop protection sector, spinetoram was evaluated in a series of *in vitro* and *in vivo* studies against the cat flea (*C. felis*) in order to assess potential utility of the molecule as a long-duration, oral systemic treatment for controlling flea infestations on dogs.

2. Materials and methods

2.1. In vitro *adult flea bioassay*

Fleas were acquired from commercial vendors (EL Labs, Soquel, CA or Kansas State University, College of Veterinary Medicine, Manhattan, KS) either as pupae (pre-emergent adults) that were held at approx. 25–27 °C and >90% relative humidity until adult emergence, or as newly hatched, unfed adults that were held under similar conditions until utilized for an assay. Adult fleas were used within 1–2 d of emergence or receipt.

Technical grade spinosad and spinetoram were acquired from Dow Agrosciences (Indianapolis, IN). Compounds were prepared for testing by dissolution in acetone to achieve a stock concentration of 30 μg/ml, with subsequent dilutions in acetone to yield solutions with compound concentrations of 15, 3, 1.5, 0.3, 0.15, 0.03 and 0.015 μg/ml. Using a micropipet, 50 μl of solution from each concentration was placed into the bottom of a 10 ml glass test tube, immediately followed by approx. 40–50 white hairs approx. 6 mm in length that were clipped from insecticide-naïve beagles. Inclusion of hair in the tube provided a more natural environment for fleas, minimizing "concussive" self-damage from agitation and jumping observed in previous experiments where no dog hair had been used. Using a pipet tip, the compound solution was then "swabbed" along the convex bottom of the test tube. Tubes were left uncapped until all of the acetone had evaporated, resulting in deposition of compound over the 1.5 cm^2 convex surface area at the bottom of the test tube. This procedure yielded testing levels of 1000, 500, 100, 50, 10, 5 and 1 ng/cm^2. Three replicates (n = 3) were tested at each concentration and for each compound. Control tubes were treated with 50 μl of acetone only.

Newly emerged, unfed adult fleas (1–2 d of age) were placed into a large, deep stainless steel container. Using a low-vacuum device, 15 (±5) fleas were captured and dispensed into each treated glass test tube. Test tubes were sealed with a plastic cap, and