Comparative study on the effects of three insecticides (fipronil, imidacloprid, selamectin) on developmental stages of the cat flea (Ctenocephalides felis Bouché 1835): a light and electron microscopic analysis of in vivo and in vitro experiments

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Abstract The effects of three insecticides (fipronil, imidacloprid and selamectin) on developmental stages of cat fleas (Ctenocephalides felis) were studied in vivo, in vitro and by means of light and electron microscopy. The results were documented by video. Adult fleas were attached to the skin of dogs that had been treated 7 days before with one of the three compounds. Furthermore, adult fleas were exposed exclusively to the hair and skin debris of such treated dogs or were placed on filter papers that had been impregnated with one of these three compounds or with the blood of treated dogs. Larval fleas were exposed to hair of treated dogs, to debris obtained by combing treated dogs, to dried blood samples of treated dogs or were placed onto filter papers impregnated with one of the three compounds. In these experiments with adult and larval fleas, it was noted that none of the three insecticides had a repellent effect on adult or larval fleas. Imidacloprid was the only compound that acted exclusively by body contact, and was apparently taken up by adult and larval fleas via the thin, non-sclerotized intersegmental membranes of the flea’s body, shown when flea stages were exposed to hairs taken from dogs treated with one of the compounds or placed onto drug-impregnated filter papers. Imidacloprid killed larvae and adult fleas within 1 h, while it took at least 24 h until all adult fleas had died on fipronil- or selamectin-treated dogs, thus allowing longer feeding periods, increasing the risk of transmission of flea-derived diseases. Flea larvae covered with debris from dogs topically treated 7 days before with fipronil, imidacloprid or selamectin died, like the untreated control, within 16–28 h after exposure. This was, however, probably mainly due to a drying effect. Adult and larval fleas exposed to filter papers impregnated with the blood of treated dogs survived longer than 7 days, as did the untreated controls. All three drugs apparently acted on nerves and muscles and thus stopped motility.

Introduction

Fleas threaten the health of humans and animals due to bite reactions and transmission of diseases. Clinical signs of flea infestation in dogs can be papular dermatitis, seborrhea and pruritus, being followed in some cases by secondary bacterial dermatitis. Repeated infestations may lead in addition to allergic dermatitis in dogs due to sensitization to components of flea saliva. Similar reactions are also described in other hosts such as cats or man (Feingold and Benjamini 1961). Since the life-cycle of cat fleas is rather short, giving rise to a new generation within 3 weeks, human dwellings may become crowded with fleas within a short time. Thus efficient control measures are needed in order to avoid spreading of a flea population (Boch and Supperer 2000; Mehlhorn et al. 1993). Fipronil, imidacloprid and selamectin belong to three different groups of insecticides being directed against the adult fleas on their animal hosts. All three are applied as spot-on treatment to the skin of dogs or cats and protection lasts for at least 4 weeks. The present study investigates the method of uptake of the compounds, their effects on the adult flea stages and attempts to understand described larvicidal activities.

Materials and methods

Fleas

Larval and adult cat fleas (Ctenocephalides felis) were reared under laboratory conditions.

Dogs

A total of 12 male and female beagle dogs weighing 10–15 kg and aged 1.5–2 years were treated in this study during repeated experiments.
Treatment of dogs

Nine animals were treated with one of the three compounds, using the commercially available formulations of fipronil (Frontline Top Spot, Merial), imidacloprid (Advantage, Bayer) or selamectin (Revolution/Stronghold, Pfizer) as recommended on their product labels. Three other dogs served as untreated controls. All animals were kept in separate cages without contact with each other.

In vivo experiments

Seven days after the application of the drugs, the dogs were shaved to obtain four circular hairless skin regions measuring 20 cm in diameter (two on each side of the body). The clipped hair was stored and used for in vitro studies. On the clipped region of the left side, the superficial fatty layer of the skin was removed by repeated swabbing with alcohol, while the right side remained untreated. Two clean covers of plastic petri dishes, each containing approximately 100 adult fleas, were fastened with tape to these hairless zones on either side of each dog’s body. The behaviour of the fleas was observed and monitored during the first hours. The first petri dish was then removed 1 h later, and the second was removed after another 24 h. The morphology of the obtained fleas was studied 1 and 24 h after first exposure. Flea movements were recorded on video tape. All fleas were collected and fixed for light and electron microscopy.

In vitro experiments

Hair

Separated groups of 100 adult and 20 larval fleas were repeatedly exposed to the clipped hair of the treated and untreated dogs and observed for at least 4 days.

Detritus

Groups of 50 larvae and 100 adult fleas were exposed to detritus obtained by combing treated (= 7 days before) and untreated dogs. The detritus was placed in plastic petri dishes containing flea larvae and their regular food or adult fleas were placed on filter papers.

Remnants of blood

Groups of 50 larval and 100 adult fleas were exposed to filter papers impregnated with dried blood obtained either from treated or untreated dogs at day 7 after the treatment with either one of the drugs.

Pure compounds

Filter papers were impregnated with 100–300 μl of one of the original products, a 10% dilution of the original using alcohol, or pure water as untreated control. These filter papers were air-dried for 24 h and placed into plastic petri dishes or open-air boxes (covered by gauze to avoid flea escape). One hundred adult fleas or 20 larvae were placed onto the filter papers and observed for at least 4 days.

Light and electron microscopy

The flea stages were collected from the different experiments and fixed with 5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) at 4 °C, then further processed, embedded, and prepared for light and electron microscopy using standard laboratory methods described elsewhere (Mehlhorn et al. 1999). For light microscopy, semi-thin sections were coloured with methylene blue and studied with an Olympus photomicroscope, while the electron micrographs were taken using Zeiss electron microscopes (EM-9-S, EM 902 A).

Results

In vivo experiments

In order to find out how long adult fleas survive on treated dogs and how they take up the insecticides, groups of 100 adult fleas were attached (in petri dishes) to the shaved flanks of dogs that had been treated 7 days before with 1 of the 3 compounds or left untreated (control animals). It was noted that in the case of imidacloprid, the fleas started sucking blood, stopped 3–5 min later, started tectonic trembling movements and became motionless. After 1 h of exposure, all fleas on imidacloprid-treated dogs had died.

In the case of fipronil and selamectin treatment, the fleas fed during the first hour of exposure, as did the fleas on the control dog. After the first hour of exposure, these fleas were still highly active and motile. Twenty-four hours later, about 96–99% of the fleas from the fipronil- and selamectin-treated dogs were dead, while the remainder were still able to jump away. Considering the fleas on those flanks from which the lipid skin layer had been removed with alcohol, there were again differences in the behaviour of fleas obtained from dogs treated with each of the three compounds.

In the case of imidacloprid-treated dogs, the fleas on the defatted areas showed no reduction in feeding and the fleas were still alive 1 and 24 h after exposure.

In contrast to this finding, the fleas on fipronil- and selamectin-treated dogs showed identical behaviour to those on non-defatted areas: the fleas sucked constantly and died within 24 h. These series of experiments indicate that imidacloprid is apparently present and active on the skin and can be removed by alcohol, while the two other compounds are taken up during blood-meal. The light and electron microscopic studies of all treated fleas showed compound-dependent degenerations (Figs. 1–12). Imidacloprid led to a quick complete destruction of the ganglia of the head and thorax within 20 min and introduced disaggregation of the fibres of the muscle cells. The latter showed at first large gaps between the fibres and finally (after 1 h) the mitochondria became swollen or were even disrupted. In fleas that had fed on flanks from which the imidacloprid had been removed with alcohol, no or only small damage was seen in the ganglia or in the muscles. In fleas that had fed on dogs being treated with fipronil or selamectin, slight damage was seen after 1 h in nerves and muscles, being mostly restricted to mitochondria that had swollen or disrupted aspects. After 24 h, dead or dying fleas showed an overall cell-lysis, not restricted to muscle and nerve cells, but being also found, for example, in epidermal, intestinal or sexual system cells. There were no differences in timing or intensity of damage seen in fleas that had engorged on defatted spots or on not-defatted