Disinfestation of Experimentally Infested Cat Fleas, *Ctenocephalides felis*, on Cats and Dogs by Oral Lufenuron

Yumi NISHIDA, Chiyako HAGA, Kenji ODA, and Toyoaki HAYAMA

Division of Animal Husbandry, Research Institute for Animal Science in Biochemistry and Toxicology, 3-7-11 Hasimotodai, Sagami-hara, Kanagawa 229, Japan

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ABSTRACT. *In vivo* efficacy of orally administered lufenuron, an insect growth regulator, in disinfesting cat fleas was evaluated, using flea-free cats and dogs which were purchased and infested every 10 days with cat fleas from a colony kept in our laboratory. Lufenuron was orally administered as a single dose of 15, 30, or 60 mg/kg to cats, and 5, 10 or 20 mg/kg to dogs. In cats, adult flea emergence was intensively prevented for 30 days by dosing of 15 mg/kg of lufenuron and 40 days by 30 or 60 mg/kg. The average egg hatch rate in 15 mg/kg group was, however, significantly higher than those in 30 mg/kg or more, suggesting necessity to dose 30 mg/kg to cats to prevent development of cat fleas effectively. In dogs, a lower dose of the drug, 10 mg/kg seemed to be sufficient for the complete prevention of flea development.—**KEY WORDS:** cat flea, *Ctenocephalides felis*, flea disinfestation, lufenuron.

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Cat fleas, *Ctenocephalides felis*, are most commonly found on dogs and cats in Japan (Imai *et al.*, personal communication). Adult cat fleas are ectoparasites on cats for life and produce a large number of eggs for weeks [4] and the eggs fall into the environment where the immature stages of fleas develop to adult to be the source of reinfestation.

For environmental flea control, many chemicals with adulticidal effect, such as organophosphates, carbamates, and pyrethroids are available, but practical uses of these drugs are sometimes restricted by emergence of drug resistance [6, 8], toxicity to mammalian hosts [1], less sensitivity of immature stages [8, 16], and poor residual activity [1, 14].

Insect growth regulators (IGRs) such as juvenile hormone analogues and chitin synthesis inhibitors have numerous advantages over the other insecticides because of their low toxicity [1, 2, 9, 18] and long lasting activity [3, 13, 14]. Exposing to IGR, development of flea eggs and/or larvae to adult are prevented, though the adult longevity and egg production are not affected [5, 7, 11, 12, 15]. Recently, it was reported that flea eggs from adult which was parasitic on cats dosed with lufenuron (CGA-184699; N-[2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)-phenylaminocarbonyl]-2,6-difluorobenzamide), a chitin synthesis inhibitor, could not develop to adult [10, 19].

In this study, flea-free cats and dogs purchased for experiments were infested with cat fleas from a colony kept in our laboratory and dosed lufenuron orally to test its disinfestative effect. The present paper describes dose effect relationships and effective periods of the drug.

MATERIALS AND METHODS

Cats: Mixed bred SPF cats for experiment (ICO Cat, Iffa Credo), 10 males and 10 females, aged from 7 to 8 months, were used. The cats were kept in the individual

metal cages (44×61×51 cm) in a room kept at 25°C and lightened for 12 hr a day. They were given adequate amount of cat food (FELINE GROWTH®, Hill's Pet Nutrition, Inc.) a day and tap water *ad libitum*. Before the experiment, they were examined for presences of fleas and flea feces in their hair coat to confirm the flea-free state. The cats were divided into 3 treatment and 1 untreated groups of 5 cats based on sex and body weight; at this time body weight of the cats ranged from 2.4 to 5.4 kg, with a mean of 4.2 kg. On Day 0, the cats in 3 treatment groups were given orally lufenuron (Program liquid®, Ciba-Geigy Ltd.), at 15, 30 or 60 mg/kg of body weight. The other 5 cats served as control.

Dogs: A total of 20 beagle dogs bred in semi-barriered quarter (Nihon Nosan Kogyo K. K.), both sexes, 10 males and 10 females aged from 5 to 6 months, was used. The dogs were kept in individual metal cages (53×75×62 cm) in a room with the same conditions as for the cats. They were given adequate amount of dog food (ONEMORE®, Nippon Formula Feed Mfg. Co., Ltd.) a day, and tap water *ad libitum*. The result of flea examination showed that they were all flea-free before the experiment. The dogs were divided into 4 groups of 5 dogs based on sex and body weight; at this time body weight of the dogs ranged from 6.1 to 10.9 kg, with a mean of 8.2 kg. The dogs in the treatment groups were given orally lufenuron (Program tablet®, Ciba-Geigy Ltd.) at 5, 10 or 20 mg/kg of body weight (Day 0).

Cat fleas: Cat flea, *Ctenocephalides felis*, was used from a colony, kept by serial passage of fleas collected from a naturally infested domestic cat in our laboratory since 1985. In the present study, adult fleas (female and male ratio 2:1), less than 72 hr after emergence, were used. On Day -10 (10 days before animal grouping and drug administration), each cat was infested with 50 fleas, and each dog with 100 ones. Every 10 days the animals were reinfested with the same numbers of fleas as on Day -10,

to make up decreasing in numbers of adult fleas on host animals by grooming and eating of them [4, 17].

Collection of flea eggs from the animals: On Days -5 and 0 (day of drug administration), and every 10 days afterward, flea eggs were collected from each animal. The animals were transferred into individual wire floored cages (30×42×35 cm for cats, 44×61×51 cm for dogs) which were specially designed for flea egg collection. Flea eggs were collected from a tray under the cage at 6 hr after the animals were placed in the cages.

Cultivation of eggs: The eggs collected from each animal were sieved with a mesh with 710 μ m opening to remove debris, and 50 eggs collected them were placed into a plastic Petri dish (90 mm in diameter) containing 2 g of larval rearing medium, which consisted of dried bovine blood (99%) and yeast (1%). The Petri dishes were placed in an incubator conditioned at 25°C and about 90% relative humidity; at this condition, hatching of eggs and emergence of adult fleas could complete by 3 and 30 days after beginning of cultivation, respectively. The number of larvae in each Petri dish was counted at 3 days after beginning of the incubation. The numbers of cocoons and emerged adults were also counted at 30 days after beginning of the incubation.

Statistical analysis: The data on the hatch, cocoon formation, and adult emergence rates were analyzed by chi-square method at a probability of 0.05.

RESULTS

Cats: Time courses of hatch rates in groups are shown in Fig. 1. Hatch rates in dosed and control groups were around 80% during the premedication period, and those in lufenuron administered groups were strongly inhibited

Fig. 1. Time courses of average hatch rates of eggs collected from lufenuron administered cats. Eggs were collected from cats every 5 or 10 days before and after single drug administration of 0 (○), 15 (●), 30 (□), and 60 mg/kg (■).

for 40 days after medication. The inhibition in 15 mg/kg group was significantly lower than the other dosed groups. No complete inhibition of hatch was observed even in 60 mg/kg group.

Total numbers of cocoons developed from larvae and adult fleas developed from cocoons in 4 experimental groups are presented in Table 1. Although the complete inhibition of adult emergences were hardly obtained, a strong preventive effect of lufenuron was evidently observed.

Dogs: As shown in Fig. 2, the complete inhibitions of egg hatch was observed for 20 days in 10 mg/kg and for 40 days in 20 mg/kg groups. Table 2 shows the complete inhibition of adult emergence for more than a month in groups received 10 mg/kg or more.

Table 1. Total numbers of cocoons and of adult fleas developed from 250 eggs from lufenuron treated 5 cats

Treatment (mg/kg)		Numbers of cocoons and of adults at the Days of					
		-5	0	10	20	30	40
0	Cocoon	182	181	187	150	156	166
	Adult	175	181	187	123	156	136
15	Cocoon	199	204	0	4	2	27
	Adult	185	204	0	3	2	27
30	Cocoon	209	188	1	4	0	3
	Adult	208	181	1	4	0	0
60	Cocoon	211	207	1	3	3	1
	Adult	203	186	1	3	1	1

Each cat was infested with 50 cat fleas every 10 days, and given single oral lufenuron on the Day 0.

Fig. 2. Time courses of average hatch rates of eggs collected from lufenuron administered dogs. Eggs were collected from dogs every 5 or 10 days before and after single drug administration of 0 (○), 5 (●), 10 (□), and 20 mg/kg (■).

Table 2. Total numbers of cocoons and of adult fleas developed from 250 eggs from lufenuron treated 5 dogs

Treatment (mg/kg)		Numbers of cocoons and of adults at the Days of					
		-5	0	10	20	30	40
0	Cocoon	168	149	151	156	163	160
	Adult	163	140	140	137	158	151
5	Cocoon	159	143	0	0	12	2
	Adult	138	139	0	0	12	2
10	Cocoon	146	161	0	0	0	10
	Adult	145	152	0	0	0	10
20	Cocoon	158	166	0	0	0	0
	Adult	147	149	0	0	0	0

Each dog was infested with 100 cat fleas every 10 days, and given single oral lufenuron on the Day 0.

DISCUSSION

According to the manufacturer's explanation, orally administered lufenuron to cats causes a long lasting low concentration of the drug in blood. Eggs from fleas fed on the drug containing blood can not develop to adult by the insect growth inhibitory action of the drug which is transferred from the female flea to eggs, or to larvae by feeding on flea feces [19].

This explanation is supported by the following studies. Hink *et al.* [10] administered lufenuron 20 or 50 mg/kg orally to 4 cats, on the shaved skin of which the cages containing 30 newly hatched fleas were attached for 3 days every week. Fleas from the cages were placed in a new cage to produce eggs, which placed on Petri dishes with flea feces to evaluate the egg hatchability and growth of larvae which fed on flea feces. They concluded that single oral dose of lufenuron virtually eliminate the next generation of adult fleas for a period of 44 days. By the same experimental design, Zakson *et al.* [19] determined concentrations of lufenuron to find strong correlations between the concentrations of drug in cat's blood and in flea eggs, between concentration in egg and rate of egg hatch and between concentration in flea feces and mortality to larvae feeding upon feces.

In this study, we used the standard methods for experimental chemotherapeutics, *i.e.* SPF or SPF-like animals to get assurance of an ectoparasite free state and a method of infestation resemble to naturally occurring flea infestation. We also made dogs a target animal, since the fleas on dogs were reported to be exclusively cat fleas in Japan (Imai *et al.*, personal communication).

Under these conditions, lufenuron was found to be effective to prevent not only egg hatch but also larva growth. In cats, the prevention of adult emergence was virtually complete, but average hatch rate in 15 mg/kg group was significantly higher than those in 30 mg/kg or more, suggesting necessity to dose 30 mg/kg to cats to prevent development of cat fleas effectively. In dogs, a lower dose of the drug, 10 mg/kg seemed to be sufficient

for the complete prevention of flea development.

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