Journal of Feline Medicine and Surgery (2009) **11**, 91–95
doi:10.1016/j.jfms.2008.05.006

ELSEVIER

jfms

A lufenuron pre-treatment may enhance the effects of enilconazole or griseofulvin in feline dermatophytosis?

Francesca Mancianti DVM, PhD, Full Professor[1]*, **Sara Dabizzi** DVM, Practitioner[2], **Simona Nardoni** DVM, PhD[1]

[1]*Dipartimento di Patologia Animale, Profilassi ed Igiene degli Alimenti, University of Pisa, Viale delle Piagge 2, I 56124 Pisa, Italy*
[2]*Dipartimento di Patologia Animale, Università di Pisa, Italy*

The effectiveness of enilconazole (4 weekly rinses with a 0.2% solution) or griseofulvin (50 mg/kg twice daily for 40 days) following a pre-treatment with oral lufenuron (100 mg/kg by-weekly for 8 weeks) was tested on 25 (11 + 14) *Microsporum canis* infected cats. Control animals were treated with lufenuron, griseofulvin and enilconazole alone. At day 150 pre-treated animals were culturally negative and clinically cured. While lufenuron alone was found to be ineffective against *M canis* infection, an immunomodulatory effect of the drug can be suggested, as reported in literature. Its use could be reserved to long-lasting infections, unsuccessfully treated with conventional drugs. Further studies are required to clearly establish the possible adjuvant effect of this molecule when used prior to enilconazole or griseofulvin.

Date accepted: 27 May 2008

Microsporum canis represents the most common agent of dermatophytosis in cats. Clinical features of this infection can vary, and cases without clinical signs are often reported.[1] Although *M canis* infection/dermatophytosis may undergo spontaneous resolution in shorthaired cats, pharmacological treatment is strongly recommended as the healing time can vary and it is has a highly contagious spread to other animals and humans, and the environment.

Each case has to be carefully evaluated to decide whether the animal should receive topical only or systemic treatment as well. Several treatment protocols have been employed and exhaustively reviewed by Moriello.[2] Nevertheless, the risk of adverse drug reactions should always be considered, when an infected cat is treated. So, the interest for new therapeutic options with a lower risk of toxicity is always high.

Lufenuron is a benzoylphenylurea drug that interferes with chitin synthesis. It is used in veterinary medicine as a flea prophylactic product, due to its non-specific inhibitory effect on chitin synthesis, probably related to serine protease inhibition.[3] This polysaccharide is also a primary component of the fungal cell wall, not present in mammalian cells, so avoids the risk of toxicity for animals. The drug, administered orally, reaches the subcutis and the corium in canine skin.[4] For these reasons, lufenuron has been proposed as an alternative treatment for dermatophytosis in small animals.

The use of lufenuron as a treatment for pet animals with existing dermatophytosis is reported in literature, with controversial results. The drug has been used following variable protocols, and in very different experimental conditions. A recent work performed by DeBoer et al[5] proposed the possible adjuvant effect of lufenuron, when administered in combination with other antifungal drugs.

The aim of the present paper was to evaluate the efficacy of oral lufenuron as a pre-treatment in naturally *M canis* infected cats, followed by a treatment with other antifungal drugs such as enilconazole or griseofulvin.

Material and methods

Fifty domestic shorthair cats, tested negative for feline immunodeficiency virus, symptomatic and asymptomatic and affected by dermatophytosis due to *M canis*, diagnosed on the basis of fungal culture on day 0, were selected. All the cats, of both genders with ages ranging from 2 months to 10 years, belonged to private owners and were referred to the clinician for dermatological disease or with an anamnesis of cases of human dermatophytosis based on culture. To avoid the risk of considering actively infected cats being only mechanical carriers, Wood's

*Corresponding author. Tel: +39-050-2216952; Fax: +39-050-2216941. E-mail: manciant@vet.unipi.it

1098-612X/08/020091+05 $34.00/0

lamp-positive subjects were selected to be included in the trial and microscopic examination of fluorescent hair was performed. Clinical lesions were visually examined both at time of initial referral and before each treatment. Briefly, the animals showed various combinations of scaling, crusting, focal, multifocal or generalised alopecia, erythema and miliary dermatitis. Fourteen cats were asymptomatic.

An open, uncontrolled study was performed and the animals were assigned to each group on the basis of owner's compliance.

Thirty-eight animals were treated with lufenuron scheduled as follows: 13 subjects were given oral lufenuron (Program oral suspension; Novartis Animal Health, Basel, Switzerland) at 100 mg/kg every 15 days until day 60 (group A). Eleven subjects were treated as the animals of group A, but after the last lufenuron administration they were treated with four weekly rinses of enilconazole (Clinafarm Spray; Janssen–Cilag, Milan, Italy) diluted in water to form a 0.2% solution, as described by Guillot et al[6] (group B). Fourteen subjects (group C) after the last lufenuron administration were given oral griseofulvin (Fulcin; Zeneca, United Kingdom) 50 mg/kg twice daily for 40 days.[7]

The other 12 cats were used as controls. Seven (group D) were administered griseofulvin at the dosing schedule of group C subjects, five (group E) were bathed with enilconazole as the sole treatment, with weekly rinses as the animals of group B.

The course of the infection was assessed by regular direct observation for any change of dermatological conditions such as lesion size and development of satellite lesions, and examination of the haircoats with Wood's lamp. Animals were examined by culture, recording of persistence, partial and/or total remission of cutaneous signs and Wood's lamp on days 15, 30, 45 and 60 for subjects given lufenuron (groups A, B and C), on days 7, 14, 21 and 28 after the start of the weekly rinses for animals treated with enilconazole (groups B and E), and 40 days after the first drug administration for cats given griseofulvin (groups C and D). On day 88 a further control was performed on groups A and B.

Asymptomatic animals were considered cured when both cultural and Wood's lamp examinations were negative. In all animals, clinical and mycological recovery was monitored by means of a last assessment on day 150.

Hair samples were drawn by means of McKenzie brush technique: the brushes were seeded on to Sabouraud agar supplemented with chloramphenicol (0.5 g/l) and cicloheximide (0.5 g/l) on Petri dishes, and incubated at 25°C for at least 4 weeks.

The plates were checked weekly for *M canis* growth. The number of colonies developed on each plate was counted and assessed on a semi-quantitative basis, scoring the infection as heavy (>50 colonies), mild (5–49 colonies) and low (<5 colonies). To standardise the results of mycological follow-up for the administered protocols, only cats from which more than 50 CFU/plate were cultured had been admitted to the study.

At the study's onset, each owner was recommended to house the infected cat in a closed space, and to keep it separated from the other rooms of the house, and from other animals. Environmental decontamination was carried out with vacuuming and washing concrete floors and all non-porous surfaces housing cats with 1:10 bleach solution each week, until the last control (day 150).

The environment was checked for *M canis* contamination at the end of each protocol treatment, on days 60 (groups A), 88 (group B), 100 (group C), 28 (group D) and 40 (group E). A last sampling was executed on day 150 for all the animals. All controls were performed by using contact plates on surfaces, as previously described.[8]

Statistical analysis on mycological results of groups B and C versus controls (groups E and D, respectively) at day 150 was performed using the χ^2 test. Both groups B and C were compared to group A to assess differences in the efficacy of pre-treatment with lufenuron versus the administration of this last drug alone.

Results

No subject showed adverse reactions during and after the treatments. Two subjects from group C died from trauma. Among symptomatic animals, clinical cure at the end of lufenuron administration was observed in 10 subjects from group A, in all cats from group C and in 3/5 animals from group B. Indeed, two of the latter group, cats 17 and 22, showed a complete remission of signs after the first rinse with enilconazole. Clinical recovery (no lesions and Wood's lamp negative) at the end of whole treatments (day 60 for group A, day 88 for group B and day 100 for group C, day 40 for group D and day 28 for group E) occurred in all the animals, except for cat 50 from group E, who was still symptomatic after the last rinse. In group A, 12 cats remained infected and negative culture was obtained in cat 6 on day 60.

Mycological cure (negative culture) within 88 days from the beginning of the treatment was reported from all the animals of group B. Hair samples from cats 17 and 24 did not yield mycotic growth at day 81. In cats from group C *M canis* failed to grow from the 40th day after the start of griseofulvin treatment (day 100), except of cat 31, who scored a low infection. At the end of the treatment (day 40) 2/7 animals from group D were culturally negative. *M canis* was isolated from all the animals from group E at the end of the enilconazole rinses. On day 150 all the animals did not show any clinical signs. At that time mycological cure was demonstrated in all the animals from groups B and C, in 7/13 cats belonging to group A, in 4/7 in group D and in 1/5 the animals from group E.

At the end of each protocol treatment the total number of CFU observed from cultures decreased in all the animals from groups B and C, in 4/13 from group A

and in 4/7 and 4/5 from groups D and E, respectively. Lufenuron allowed the shift from heavy to mild infection in 10 animals, cat 17 on day 15, cats 6, 10, 19, 23 on day 45 and cats 2, 11, 18, 34 and 35 on day 60. Enilconazole after lufenuron induced a decrease of fungal colonies at the second rinse (cats 16, 17, 18, 20, 22, 23, and 24) and at the third rinse (cats 14, 15, 16, 17, 19, 20, 21, and 24). Griseofulvin reduced

Table 1. Signalment, outcome of mycotic charge and culture results in cats treated with lufenuron alone, lufenuron and enilconazole, lufenuron and griseofulvin

Cat number	Gender	Age	Presence/ absence of clinical signs	Lufenuron treatment				Enilconazole/ griseofulvin after lufenuron treatment			Mycological control	
				Day 15	Day 30	Day 45	Day 60	Day 67	Day 74	Day 81	Days 88–100	On day 150
Group A												
1	F	6 ms	+	[h]	[h]	[h]	h				m	Negative
2	F	3 ms	−	h	h	h	m				m	Negative
3	F	3 yrs	+	[h]	[h]	[h]	h				h	m
4	M	6 ms	+	[h]	[h]	[h]	h				l	l
5	F	5 ms	+	[h]	[h]	[h]	h				Negative	Negative
6	M	5 yrs	+	h	h	m	Negative				Negative	Negative
7	F	8 ms	+	[h]	[h]	[h]	(h)				h	m
8	F	6 ms	+	[h]	[h]	[h]	h				h	l
9	M	1 yr	+	[h]	[h]	[h]	h				m	l
10	F	5 yrs	+	h	h	m	m				m	Negative
11	M	3 yrs	+	(h)	h	h	m				m	Negative
12	F	2 yrs	−	h	h	h	h				h	m
13	F	4 yrs	+	[h]	(h)	h	h				h	Negative
Group B												
14	M	3 ms	+	(h)	h	h	h	h	h	m	Negative	Negative
15	F	3 ms	−	h	h	h	h	h	h	l	Negative	Negative
16	F	1 yr	−	h	h	h	h	h	m	l	Negative	Negative
17	F	4 ms	+	[m]	[m]	[m]	[m]	[m]	l	Negative	Negative	Negative
18	M	4 ms	−	h	h	h	m	m	l	l	Negative	Negative
19	M	3 yrs	−	h	h	m	m	m	m	l	Negative	Negative
20	F	2 yrs	+	(h)	(h)	(h)	h	h	m	l	Negative	Negative
21	F	5 yrs	+	[h]	[h]	[h]	h	h	h	m	Negative	Negative
22	F	1 yr	+	[h]	[h]	(h)	(h)	(h)	m	m	Negative	Negative
23	M	7 yrs	−	h	h	m	m	m	l	l	Negative	Negative
24	M	1 yr	−	h	h	h	h	h	l	Negative	Negative	Negative
Group C												
25	M	2 ms	+	h	h	h	h				Negative	Negative
26	M	2 ms	+	h	h	h	h				Negative	Deceased
27	F	5 yrs	+	[h]	(h)	h	h				Negative	Negative
28	M	2 ms	+	[h]	[h]	h	h				Negative	Negative
29	M	2 ms	+	[h]	[h]	h	h				Negative	Negative
30	F	5 ms	+	[h]	(h)	h	h				Negative	Negative
31	F	3 yrs	+	[h]	[h]	(h)	h				l	Negative
32	M	6 ms	+	[h]	[h]	(h)	h				Negative	Negative
33	F	4 yrs	+	(h)	(h)	(h)	h				Negative	Negative
34	M	1 yr	+	h	h	h	m				Negative	Negative
35	M	8 ms	+	(h)	(h)	h	m				Negative	Deceased
36	F	2 yrs	−	h	h	h	h				Negative	Negative
37	M	10 ms	+	h	h	h	h				Negative	Negative
38	F	3 yrs	+	h	h	h	h				Negative	Negative

h = Heavy infection; m = mild infection; l = low infection; M = male, F = female, yrs = years, ms = months. [ ] = Any remission of clinical signs; ( ) = partial remission of clinical signs; No brackets = total remission (clinical cure).

mycotic charge in 4/7 animals when used without lufenuron. On day 150 cats 3, 4, 7, 8, 9 and 12 yielded positive cultures with mild and low infections. Three subjects from group D did not show any reduction in mycological score, compared with the results obtained at the end of griseofulvin administration. A shift from heavy to mild infection was observed in group E in two subjects at the second rinse (cats 48 and 49) in one cat at the third (cat 46) and at fourth (cat 47). At day 150, all infected subjects of this group but one (cat 48) scored mild, cat 50 showed a shift from heavy and remission of clinical signs. More detailed results are reported in Tables 1 and 2.

At the end of the treatments environmental contamination was reported in 11 and 9/13 (groups A and B, respectively), 9/11 (group C), 3/7 (group D) and 2/5 (group E). All the environments appeared negative on day 150.

Differences between lufenuron pre-treated cats and controls were assessed to be significative ($P < 0.01$ for group B versus group E, and $P < 0.05$ for group C versus group D, respectively). Group A was also significantly different from groups B and C ($P < 0.05$).

Discussion

All the administered drugs were well tolerated by all the cats. The lesions disappeared in 27 out of 29 symptomatic animals treated with lufenuron at day 60.

However, all animals given lufenuron alone were still positive at day 60 with the exception of cat 6, confirming that the usefulness of the drug when used as the sole treatment is very limited. Lufenuron did not reduce conspicuously the amount of fungal infection of the cats, based on standardised sampling and culture techniques and, thus, seems to be useless to control infection's spreading.

Furthermore, lufenuron was proven not to prevent dermatophytosis nor modify the course of infection when used as a pre-treatment on experimentally infected cats.[2]

After the treatment with lufenuron followed by enilconazole or griseofulvin, all the animals were culturally negative and clinically healthy, apparently indicating that the treatment was beneficial. This result has to be carefully evaluated, considering that dermatophytosis sometimes acts as a self-limiting infection. In the present case, it is impossible to know if negative culture and Wood's lamp examination post-day 88 represented the results of an effective treatment or of self-curing of cats, even if seven control subjects out of 12 scored still positive on day 150. Guillot et al[6] reported a failure of this association, following different dosing schedules. In their work, resolution of clinical signs and a decrease in fungal colonies numbers were observed over a period of 90 days in 100 naturally infected cats living in two catteries, but didn't cure the animals. Anyway, cats received lufenuron and griseofulvin at low dosages (60 mg/kg on days 0 and 30, and 25 mg/kg bid, respectively) and enilconazole was administered together with the other drugs. The use of topical enilconazole is reported in cats with controversial results. De Jaham et al[9] obtained good response in treating 10 Persian cats experimentally infected with *M canis*, while Hnilica and Medleau[10] report a failure of the product in eradicating

Table 2. Signalment, outcome of mycotic charge and culture results of control groups

Cat number	Gender	Age	Presence/absence of clinical signs	Enilconazole treatment			End of therapy	Mycological results on day 150
				Day 7	Day 14	Day 21		
Group D								
39	M	1 yr	+				h	h
40	M	6 ms	+				h	h
41	F	9 ms	+				h	Negative
42	M	10 yrs	−				l	Negative
43	F	2 yrs	−				m	m
44	F	6 yrs	+				Negative	Negative
45	M	2 yrs	−				Negative	Negative
Group E								
46	F	6 ms	+	[h]	[h]	[m]	m	m
47	F	4 yrs	+	[h]	h	h	m	m
48	M	2 yrs	−	h	m	m	m	Negative
49	F	1 yr	−	h	m	m	m	m
50	M	3 ms	+	[h]	[h]	[h]	[h]	m

h = Heavy infection; m = mild infection; l = low infection; M = male, F = female, yrs = years, ms = months. [ ] = Any remission of clinical signs; no brackets = total remission (clinical cure).

microsporiasis in a chronically infected cattery. The authors suggest that the failure of the treatment was probably due to the heavy environmental contamination demonstrated in the facility, but in our case the same results were observed in animals of private owners, kept in a not heavily contaminated environment.

In our work, the presence of arthrospores in households harbouring infected animals was checked after the treatment with lufenuron, and they were detected in 13/17 cases (76%), demonstrating that the drug was not able to limit environmental spreading of *M canis* elements. This evidence has an important epidemiological value, considering that infected cats are the main source of contamination in the environment.[8]

Previous studies have reported experimental dermatophytic infection carried out using a unique *M canis* strain. In our case, only wild strains responsible for feline microsporiasis were selected. Even if all isolates were Wood positive, a difference in the results obtained within cats belonging to the same treatment group could be ascribed to a variable lufenuron susceptibility of different fungal strains.

Macro- and microscopic features of *M canis* strains obtained in culture from treated cats were always typical. This is in contrast with the observations of Ben-Ziony and Arzi,[11] who describe modified fungal colonies from lufenuron-administered cats.

Finally, the treatment with lufenuron in combination with the other antifungal drugs in the present study seems to have provided good results for the management of dermatophytosis in cats naturally infected with *M canis*. However, the protocols treatment require a long time of administration, so they cannot be recommended as a routine aid to control fungal infection. From the comparison of culture results performed at day 150 between groups B and C with their respective controls (groups D and E), it appears that all lufenuron pre-treated animals were negative versus 4/5 and 3/7 from groups D and E, still positive. Furthermore, topical enilconazole as the sole drug was proven to fail in the treatment of both natural and experimental feline microsporiasis.[10,12] Griseofulvin is an effective antifungal agent for feline microsporiasis, and its use combined with other therapies seems to be successful.[13] Even if lufenuron was found to be ineffective against *M canis* infection, an immunomodulatory effect of the drug is reported as possible.[14] Its use could be reserved to long-lasting infections, unsuccessfully treated with conventional drugs. Further studies are required to clearly establish the possible adjuvant effect of this molecule when used prior to enilconazole or griseofulvin.

References

1. Mignon BR, Coignoul F, Leclipteux T, Ch Focant, Losson J. Humoral and cellular immune response to a crude exo-antigen and purified keratinase of *Microsporum canis* in experimentally infected guinea pigs. *Med Mycol* 1999; **37**(2): 123–9.
2. Moriello KA, DeBoer DJ, Schenker R, Blum J, Volk LM. Efficacy of pre-treatment with lufenuron for the prevention of *Microsporum canis* infection in a feline direct topical challenge model. *Vet Dermatol* 2004; **15**(6): 357–62.
3. Dean SR, Meola RW, Meola SM, Sittertz-Bhatkar H, Schenker R. Mode of action of lufenuron in adult *Ctenocephalides felis* (Siphonaptera: Pulicidae). *J Med Entomol* 1999; **36**(4): 486–92.
4. Hotz RP, Hassler S, Maurer MP. Determination of lufenuron in canine skin layers by radioluminography. *Schweiz Arch Tierheilkd* 2000; **142**(4): 173–6.
5. DeBoer DJ, Moriello KA, Volk LM, Schenker R, Steffan J. Lufenuron does not augment effectiveness of terbinafine for treatment of *Microsporum canis* infections in a feline model. In: Hillier A, Foster AP, Kwochka KW, eds. Advances in Veterinary Dermatology Vol 5: Oxford: Blackwell Science, 2005: 123–9.
6. Guillot J, Malandain E, Jankowski F, Rojzner K, Fournier C, Touati F, Chermette R, Seewald W, Schenker R. Evaluation of the efficacy of oral lufenuron combined with topical enilconazole for the management of dermatophytosis in catteries. *Vet Rec* 2002; **150**(23): 714–8.
7. Foil C. Ringworm update. In: Plumb D, ed. Plumb's Veterinary Drug Handbook. Proceedings: Western Veterinary Conference. 5th edn. Iowa: Blackwell Publishing, 2005: 544.
8. Mancianti F, Nardoni S, Corazza M, D'Achille P, Ponticelli C. Environmental detection of *Microsporum canis* arthrospores in the households of infected cats and dogs. *J Feline Med Surg* 2003; **5**: 323–8.
9. De Jaham C, Page N, Lambert AJ, Paradis M. Enilconazole emulsion in the treatment of dermatophytosis in Persian cats: tolerance and suitability. In: Kwochka KW, Willemse T, Von Tscharner C, eds. Advances in Veterinary Dermatology Vol 3: Oxford: Butterworth Heinemann, 1998: 299–307.
10. Hnilica KA, Medleau L. Evaluation of topically applied enilconazole for the treatment of dermatophytosis in a Persian cattery. *Vet Dermatol* 2002; **13**: 23–8.
11. Ben-Ziony Y, Arzi B. Use of lufenuron for treating fungal infections of dogs and cats: 297 cases. *J Am Vet Med Assoc* 2000; **217**(10): 1510–3.
12. DeBoer DJ, Moriello KA. Inability of topical treatment to influence the course of experimental feline dermatophytosis. *J Am Vet Med Assoc* 1995; **207**: 52–7.
13. Moriello KA. Treatment of dermatophytoses in dogs and cats: review of published studies. *Vet Dermatol* 2004; **15**: 99–107.
14. Zur G, Elad D. In vitro and in vivo effects of lufenuron on dermatophytes isolated from cases of canine and feline dermatophytoses. *J Vet Med B, Infect Dis Vet Public Health* 2006; **53**: 122–5.