CASE REPORT

Feline Phaeohyphomycosis: Treatment with Ketaconazole and 5-Fluorocytosine

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Summary

Two cats with phaeohyphomycosis, one infected with *Phialophora verrucosa* and the other with *Exophiala jeanselmei*, were treated with ketaconazole alone and in combination with 5-fluorocytosine after recurrence of the infections following surgical excision. The drugs were given orally at various doses and for various lengths of time, but were ineffective. Hepatocellular damage occurred in one cat.

Key words: Domestic cat, phaeohyphomycosis, ketaconazole, 5-fluorocytosine, *Phialophora verrucosa, Exophiala jeanselmei.*

Résumé

Traitement de la phaeohyphomycose féline, avec de la kétaconazole et de la 5-fluorocytosine

Cet article rapporte deux cas de phaeohyphomycose féline, dont l'un était dû à *Phialophora verrucosa* et l'autre, à *Exophiala jeanselmei.* Les auteurs décidèrent de traiter les récidives ultérieures à l'excision chirurgicale des lésions, avec de la kétaconazole, seule ou en association avec de la 5-fluorocytosine. Ils en administrèrent diverses doses, par la voie buccale et pendant divers laps de temps, mais sans succès. Les hépatocytes d'un des deux chats subirent aussi des dommages.

Mots clés: chat domestique, phaeohyphomycose, kétaconazole, 5-fluorocytosine, *Phialophora verrucosa, Exophiala jeanselmei.*

Introduction

The name phaeohyphomycosis refers to those subcutaneous and systemic infections which are caused by various dark colored molds which grow in the tissues in the form of brown-walled hyphae or budding cells. The lesions are usually dermal or subcutaneous and are slow growing.

Since reporting a case of feline phaeohyphomycosis caused by *Phialophora verrucosa* (1), three additional cases of this type of mycosis have been seen. All three cats had slowly spreading subcutaneous growths on the face or legs and the causative fungi were isolated from two of them and identified as *P. verrucosa* in one and as *Exophiala jeanselmei* in the other. The growths were surgically excised but reappeared within six months in both cats: there has been no recurrence of growth in the third cat after eighteen months.

Recent reports of feline phaeohyphomycosis due to various dematiaceous fungi (2,3,4,5,6) indicate that this type of mycosis is not uncommon and a routine method of treatment would be of value to the practitioner. Excision of the growth is the primary treatment but in many cases it is difficult to ensure complete removal of all fungal cells and an appropriate course of chemotherapy following surgery could be beneficial.

The management of mycotic infections is hampered by the fact that fungi are eukaryotic organisms and drugs which are toxic to the fungal cells are often toxic to the similarly eukaryotic host cells. Two antibiotics, ketaconazole and 5-fluorocytosine (5-FC), have been used with varying success in the treatment of human phaeohyphomycosis (7,8,9) and since they can be administered orally, it was decided to treat two of the cats with these drugs. Ketaconazole, an imidazole derivative, is a synthetic broad spectrum antifungal agent which has been shown to impair the synthesis of ergosterol, a major component of fungal cell walls, and cause morphological alterations to the cell membranes. It has been used extensively on an experimental basis for the treatment of human mycotic diseases (10) but has had only limited trials in veterinary medicine (11,12). Therapeutic dose levels are not established at the present time, although it has been suggested that 10-30 mg/kg once or twice daily may be used (13).

The synthetic pyrimidine derivative 5-FC interferes with fungal nucleic acid synthesis. It has been found to be of limited value, being mainly employed against some of the pathogenic yeasts, but it has been reported to be an effective treatment for chromohyphomycosis (8). Recent studies have shown that it has an enhanced *in vitro* activity against selected fungal pathogens, including *E. jeanselmei,* when combined with ketaconazole (14). Published data are not available on the use of 5-FC in the cat and neither drug has been approved for use in dogs or cats.

Materials and Methods

Ketaconazole ("Nizoral", Janssen Pharmaceutica Inc., Mississauga, Ontario) was supplied by the makers in 200 mg tablet form. 5-fluorocytosine ("Ancotil 500", Hoffmann-La Roche Ltd., Vaudreuil, Quebec) was

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bought as 500 mg capsules. Both drugs were divided into appropriate doses and 5-FC was mixed with the diet. The cats remained with the owners who gave the prescribed medications, except for short stays in the clinic for testing and observation. Laboratory tests included complete blood counts, urinalysis, serum glutamic pyruvic-transaminase (SGPT), serum alkaline phosphatase (SAP), creatinine and blood urea nitrogen (BUN).

Fragments of biopsy tissue were mounted directly in 10% KOH for microscopic examination and placed in formalin for histopathological examination. The remaining tissue was plated onto Modified Sabouraud Dextrose agar (MSD) containing 40 mg/L chloramphenicol and Mycosel agar (BBL, Div. of Becton, Dickinson Co. Ltd., Mississauga, Ontario). All cultures were incubated in duplicate at 25° C and 37° C for two weeks.

History and Treatment
Case #1

A seven year old ovariohysterectomized Abyssinian cat weighing about 2 kg was admitted with a lump on the medial aspect of the left paw (Figure 1). The cat was not lame, the lesion was not painful, and other abnormalities were not found. The growth was removed and submitted in 10% formalin for histopathological examination. A diagnosis of mycotic granulomatous dermatitis was made and a chest radiograph was taken but no lesions were discerned. Four months later the cat was admitted again. The growth had reappeared and the cat was lame on the affected leg. The mass was excised and fresh tissue taken for mycological examination. *Exophiala jeanselmei* was isolated and treatment with ketaconazole was initiated. Details of the course of therapy are given in Tables I and II.

After three months of ketaconazole therapy the mass had become progressively softer with less distinct borders until it had almost disappeared. The cat was healthy but because of the lowered white blood cell count it was considered advisable to reduce the dose. Within three weeks the mass had enlarged again and in spite of restoring the dose of ketaconazole to the original level the mass continued to spread until it involved the leg from stifle to

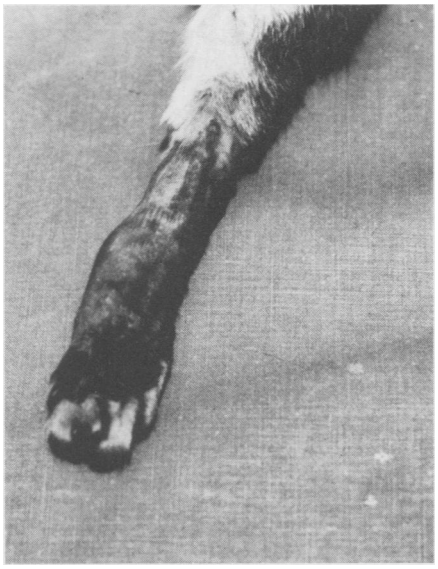

FIGURE 1. Subcutaneous nodule caused by *Exophiala jeanselmei.*

metatarsals. Following a further increase in dose the cat refused to eat and became lethargic, ataxic, and uncoordinated. The mass regressed slightly, but because of the severely emaciated condition of the cat, ketaconazole therapy was discontinued after eight months.

After two weeks the cat had regained most of its original weight and was alert, active, and eating well. Combined therapy using ketaconazole (25 mg/kg once daily) with 5-FC (40 mg/kg once daily) was initiated. A month later there was no noticeable change in the size of the mass and because of elevated SGPT levels the chemotherapy was discontinued and plans were made to remove the growth surgically.

Mycology

The lesion was a typical granuloma with many giant cells containing fungal cells (Figure 2). Examination of a KOH mount of fresh tissue from the regrown mass showed that only hyaline, budding yeast cells were present; no hyphal forms were seen. Many cells

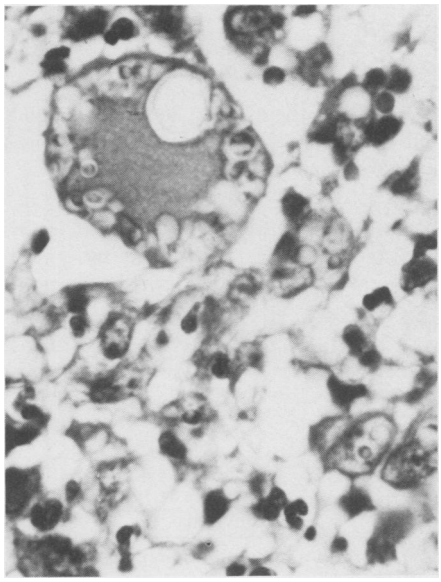

FIGURE 2. Cells of *E. jeanselmei* inside a giant cell. H & E. X250.

TABLE I
LABORATORY DATA FOR CAT WITH *E. JEANSELMEI* INFECTION TREATED WITH KETACONAZOLE AND 5-FC

	Day 244	Day 260	Day 281	Day 337	Day 399	Day 413	Day 484
PCV	38	30	32	35	35	32	37
S.P.	7.2	6.5	6.7	7.2	7.0	6.7	>7.2
WBXC	6,400	8,600	5,400	3,600	3,100	3,900	2,200
Seg. N.	37	81	84	86	88	92	53
Band.	1	-	-	1	2	3	-
Lymph.	53	14	8	6	6	3	39
Mono.	2	1	3	1	1	1	3
Eds.	7	4	5	6	3	1	5
Morph.	Normal	Aniso-cytosis	Normal	Aniso-cytosis	Rapid sed. rate aniso-cytosis crenation	Rapid sed. rate aniso-cytosis crenation	Aniso-cytosis
Alk. Phos. (Bodanski-Units)	0.8	0.6	1.3	1.6	0.5	0.9	1.6
SGPT (R-F units)	74	41	104	58	31	26	>175
Bun (Mg/%)	36	27.5	22.6	25	29.6	29.3	30.7
Creat (Mg/%)	0.9	1.4	1.1	1.1	0.9	1.2	1.7

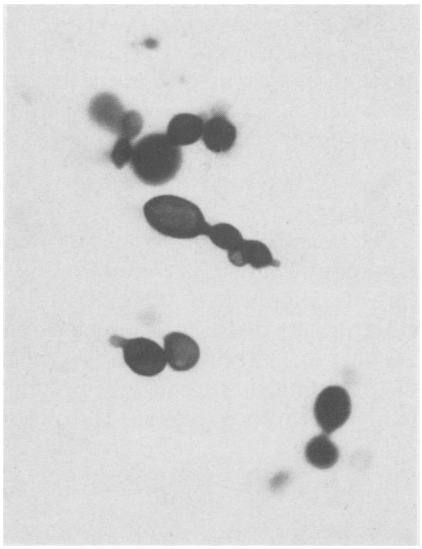

FIGURE 3. Early stage of infection. Budding cells of *E. jeanselmei* in tissue. Cell walls stained black with methenamine silver. X400.

had multiple buds and there were a few chains of cells connected by short necks (Figure 3). At this stage none of the fungal cells showed the brown walls characteristic of agents of phaeohyphomycosis. Growth in culture was visible within two days, the colonies being greenish-black, yeast-like and mucoid on MSD with a tendency to flow down the agar slope. Later a fringe of greyish mycelium developed around the edges and spread across the centre until small, downy, mouse grey colonies were formed. The fungus grew well, although more slowly, at 37°C and was not sensitive to cycloheximide as it grew on Mycosel agar. Large numbers of oval, one-celled annelloconidia were produced in culture and the fungus was identified as *Exophiala jeanselmei.*

Tissue from a second biopsy taken after three months of treatment with ketaconazole contained many brown-walled hyphae and budding cells. Cultures were dry and mycelial from the beginning and did not exhibit the black yeast phase so noticeable in the previous isolations. The last mycological examination was made after the ketaconazole dosage had been increased to 50 mg/kg daily and at this time there were none of the characteristic budding cells and hyphae in the tissues. Occasional large, brown thick-walled cells were interspersed among collapsed and distorted fungal elements. Growth from these tissues was delayed for ten days when a sparse grey mycelium became visible to the naked eye.

Case #2

A four year old ovariohysterectomized domestic short haired cat weighing about 2 kg was referred because of a recurrent growth on the plantar aspect of the left front paw proximal to the metacarpal pad. Six months earlier a growth had been excised and submitted for histopathology. At that time a diagnosis of phaeohyphomycosis was made based on the presence of brown-walled hyphae and budding cells surrounded by a mixed inflammatory cell reaction and the presence of giant cells.

The cat appeared healthy. The mass was well circumscribed, soft and fluctuating, ulcerated and closely-

TABLE II
TREATMENT OF CAT WITH *E. JEANSELMEI* INFECTION WITH KETACONAZOLE AND 5-FC

Day	Condition of Mycotic Granuloma	Treatment	Comments
1	1st surgical excision	Amoxil 50 mg BID	Diagnosis: mycotic granulomatous dermatitis
29		None	Chest radiograph — no discernible lesions
133	2nd surgical excision	None	*Exophiala jeanselmei* isolated
244	Growth 2 x 2 x 0.5 cm Firm, nonfluctuating	Ketaconazole 25 mg/kg/day	Laboratory screen — no preexisting abnormalities
260	Reduced to 1.5 x 1.5 cm soft, and fluctuating	Same	Drop in PCV and lymphopenia
281	Almost invisible, indistinct borders	Ketaconazole reduced 12.5 mg/kg/day	Drop in WBC from 8,600 to 5,400. Cat clinically healthy
308	Rapid increase in size	Ketaconazole 25 mg/kg/day	
337	Extending up leg 1st biopsy	Same	Significant leukopenia and weight loss *E. jeanselmei* cultured
393	No change	Ketaconazole 25 mg/kg/BID	
399	No change	Same	Cat lethargic, ataxic, poor coordination "dull" recovered by day 402
413	2 x 4 x 1 cm firm	Same	Weight loss and poor appetite
420	Decreasing 1.5 x 3 cm, borders indistinct	Multivitamins, antibiotic eye ointment as well as ketaconazole 25 mg/kg/BID	Cat lethargic: upper respiratory infection and ocular discharge
434	No change — 1.5 x 3 cm 2nd biopsy	Ketaconazole discontinued	Severely emaciated. *E. jeanselmei* cultured
449	No change	Ketaconazole reinstated 25 mg/kg/day with 5 FC 40 mg/kg/BID	Cat had regained weight, and was alert and active
484	No change	Therapy discontinued	Drop in WBC to 2,200. SGPT greater than 175 R/F units

TABLE III
TREATMENT OF CAT WITH *P. VERRUCOSA* INFECTION WITH KETACONAZOLE AND 5-FC

Day	Condition of Mycotic Granuloma	Treatment	Comments
1	2 x 3 x 1 cm ulcerated 2nd surgical excision	Amoxil 50 mg BID for 6 days	Laboratory screen — no preexisting abnormalities *Phialophora verrucosa* isolated
35	No growth evident 1st biopsy	None	*P. verrucosa* isolated
44	No growth evident	Ketaconazole 25 mg/kg BID	
64	No growth evident 2nd biopsy	Ketaconazole 25 mg/kg TID 5-FC 40 mg/kg TID	*P. verrucosa* isolated Drop in WBC from 6,200 to 3,200
71	No growth evident	Discontinued	Owner unable to medicate
118	No change	Ketaconazole 50 mg/kg BID for 1 week	Anorexia and vomiting — persist for 2 weeks after therapy discontinued
151	No change	Ketaconazole 25 mg/kg BID	Therapy restarted after 1 month without medication
175	No change	Discontinued	Complete anorexia and severe weight loss
194	Rapid regrowth 2 x 4 x 1 cm, ulcerated 3rd surgical excision	None	Cat regained weight *P. verrucosa* isolated

adherent to the foot pad. Nothing abnormal was found as a result of clinical pathological examinations. The growth was easily excised and was not involved with the subcutaneous tissues. The mass was light brown in color and friable, and after five days *P. verrucosa* was isolated from the tissues. The course of treatment is detailed in Table III.

Mycology

A wet mount in 10% KOH of tissue from the recurred growth revealed the presence of golden brown septate hyphae and budding cells. The fungus was visible in three days in cultures incubated at 25° C and within a week at 37° C. It grew on both media producing slow growing mouse grey, downy colonies. After ten days abundant phialoconidia were produced from rather squat phialides which had the prominant dark collarettes constricted at the base characteristic of *Phialophora verrucosa.*

A month after initiation of therapy with ketaconazole a KOH mount of biopsy tissue showed that even though there was no external sign of regrowth hyphae and budding cells were present and cultures grew vigorously. Cultures were again positive from the large ulcerated growth which was surgically removed after therapy had been discontinued. These tissues were densely packed with dark-walled hyphae and budding cells.

Discussion

Experimental chemotherapy as an alternative to repeated surgery in the two cases of phaeohyphomycosis described was not successful.

The use of ketaconazole in the treatment of the *E. jeanselmei* infection was promising initially as the granuloma regressed until it had almost disappeared after thirty-seven days of treatment. The cat tolerated the drug well and appeared healthy, despite a leukopenia and lymphopenia which persisted throughout therapy. Unfortunately it is not possible to state if the ketaconazole treatment would have been successful had the dosage been maintained at the initial level for a longer period of time. When the daily dose was halved, the granuloma rapidly increased in size indicating that a number of fungal cells had survived the treatment and resumed growth once the inhibitory drug level was lowered. The debilitated condition and reduced viability of the fungus indicated that a dosage of 50 mg/kg daily might well have eliminated the *E. jeanselmei* infection but the adverse effects on the health of the cat precluded continued use of such a high level.

Ketaconazole was not effective when used against the infection caused by *P. verrucosa.* An initial dose of 25 mg/kg twice daily was prescribed since treatment of the first case indicated that long term therapy was likely to result in the development of fungal resistance. After a month there was no outward sign of regrowth but the fungus was alive in a biopsy of the tissues. At a higher dose the cat became anorexic and lost weight but the vigor of the fungus appeared to be undiminished. Depression of the cellular immune response as a result of the reduction of white blood cells during therapy may have been responsible for the rapid regrowth of the granulomas in both cats after treatments were discontinued.

Combined treatment with ketaconazole and 5-FC was also unsuccessful. In case #1 medication was discontinued after one month because of a dramatic fall in the WBC and elevated SGPT levels. The occurrence of an upper respiratory infection during therapy could be attributed to the weakened condition and increased susceptibility of the animal. In case #2 the owners were unable to medicate with 5-FC because of its unpleasant taste and the fractious nature of the cat.

Based on these studies we cannot recommend the combined use of ketaconazole and 5-FC for the treatment of feline mycoses because of evidence of hepatocellular damage and a severe leukopenia. Treatment with ketaconazole alone has proved effective against feline histoplasmosis (12) and may be useful against some fungi which cause phaeohyphomycosis at dosage levels between 25-50 mg/kg daily, but more experimental data are needed to arrive at correct levels and schedules.

Acknowledgments

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LETTER TO THE EDITOR

Tyzzer's Disease in a Puppy

DEAR SIR:

First described in mice in 1917, Tyzzer's disease was recognized in the 60's as the causative agent of a fatal disease in rabbits and subsequently in rats, gerbils, monkeys, cats and horses. There are few reports concerning the occurrence of the disease in dogs (1,2). This letter is to report the identification of Tyzzer's disease in a dog in Ontario.

A seven week old Cocker spaniel puppy died after having suffered chronic diarrhea for a week or more. The puppy had been imported from the USA at five weeks of age by a pet store, and reportedly had been fully vaccinated prior to importation. It was thin and considered a "poor-doer". Fecal examination revealed the presence of coccidia and tapeworms for which the pup underwent treatment.

Autopsy revealed no significant gross lesions except congestion and partial consolidation of the lungs suggesting the presence of pneumonia. Histologically there was follicular necrosis in the spleen, extensive but mild enteritis with dilatation of crypts and necrosis of crypt epithelium, and a focal bronchopneumonia with a more diffuse interstitial reaction. The most striking lesion was widespread focal hepatic necrosis. Warthin-Starry stain revealed large numbers of organisms typical of *Bacillus piliformis* in hepatocytes in the periphery of necrotic foci. These organisms were not demonstrated in tissues other than the liver.

The pet store in which this puppy died keeps not only dogs but also mice, rats, gerbils, rabbits and other more exotic species. As of this date — some two months subsequent to the death of the puppy — no untoward illnesses or deaths suggestive of the spread of Tyzzer's infection have occurred in other puppies or in any of the other various species maintained there.

The source of the infection in this case remains unknown. This instance would suggest a very low degree of contagion. It should however alert clinicians to the necessity of considering Tyzzer's disease as a possible causative factor in puppy deaths following diarrhea and wasting.

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ABSTRACT

PEDERSEN NC, BLACK JW. **Attempted immunization of cats against feline infectious peritonitis, using avirulent live virus or sublethal amounts of virulent virus.** *American Journal of Veterinary Research* 1983; 44: 229-234. (Dep. Med. Sch. Vet. Med. Univ., Davis, California 95616, USA).

Vaccination with modified live feline infectious peritonitis virus or serum from resistant animals not only failed to protect cats against the disease but appeared to make them more susceptible to challenge with virulent virus. The normal 8-12 day incubation period was reduced to 24-72 hours in vaccinated cats. Some kittens could be immunized with sublethal doses of virulent virus, but this was too variable and hazardous for practical application. Fluorescent and virus-neutralizing antibodies were not correlated with disease or immunity, and it was concluded that protection was conferred by cell-mediated, not humoral immunity.

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