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Reduced susceptibility to fluconazole in a cat with histoplasmosis

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Abstract

Case summary An 11-year-old neutered male domestic longhair cat was diagnosed with histoplasmosis from fine-needle aspirates of an abdominal lymph node. Lymph node size initially decreased with fluconazole therapy (11.8 mg/kg PO q12h); however, after 13 months of continuous fluconazole therapy, lymphadenomegaly worsened and samples were collected for culture and antifungal susceptibility. The *Histoplasma capsulatum* isolate had a very high fluconazole minimum inhibitory concentration (MIC) of 64 µg/ml and an itraconazole MIC of 0.06 µg/ml. The owner declined a change to itraconazole and, ultimately, the cat developed neurologic signs and was euthanized. Owing to the initial response to fluconazole followed by treatment failure and high MIC value, acquired fluconazole resistance was suspected. Clinical breakpoints for fluconazole for the dimorphic fungi are not available to define true antifungal resistance.

Relevance and novel information This is the first published report of reduced susceptibility to fluconazole in a cat being treated for histoplasmosis. Fluconazole failure and increases in MIC between pretreatment and long-term treatment isolates are known to occur in humans with histoplasmosis. Practitioners should be aware of this possibility when treating cats with fluconazole (particularly in cases with long-term [>1 year] fluconazole therapy or in cases with disease recrudescence).

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Case description

An 11-year-old neutered male domestic longhair cat was presented for examination after escaping from the house for 2 weeks. Upon returning home, the cat did not show signs of illness except apparent weight loss. On physical examination, a large (approximately 2 cm × 5 cm), firm mass was found on abdominal palpation. The remainder of the physical examination was considered unremarkable. Serum biochemistry analysis and complete blood count revealed a mild, non-regenerative anemia (hematocrit 26%; reference interval [RI] 29–48%). Abdominal ultrasonography showed an enlarged, hypoechoic and irregular mesenteric lymph node that measured 4.5 cm × 1.6 cm. The remainder of the abdominal ultrasonographic examination was considered normal. Ultrasound-guided, percutaneous fine-needle aspiration (FNA) and cytopathology of the mesenteric lymph node showed mild histiocytic inflammation with sample hemodilution. No *Histoplasma capsulatum* organisms were found. The following day, repeat ultrasound-guided, percutaneous FNA and cytopathology of the lymph node showed the same findings (no yeasts seen). Urine and serum *Histoplasma* antigen tests (HcAg; MVista *Histoplasma* antigen quantitative enzyme immunoassay; MiraVista

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Diagnostics) were both negative (none detected). The *H capsulatum* IgG antibody test by enzyme immunoassay revealed a high IgG result (>80 enzyme units [EU]; RI 0–9.9 EU; MVista *Histoplasma* feline antibody IgG enzyme immunoassay [MiraVista Diagnostics]). Owing to the high IgG and a strong clinical suspicion for histoplasmosis, a third FNA and cytopathology of the lymph node were performed and revealed mild histiocytic inflammation with intracellular yeasts consistent with *H capsulatum*. Fluconazole was prescribed at 11.8 mg/kg PO q24h and increased to 11.8 mg/kg PO q12h after 1 month of treatment (Glenmark Pharmaceuticals).

The cat was examined at repeat hospital visits four times over the following 6 months. The cat remained clinically normal and gained 0.4 kg body weight over that time. At each visit an abdominal ultrasonographic examination was performed and lymph node size had subjectively decreased but remained abnormal in size and appearance. The lymph node was approximately 2 cm × 2 cm at a recheck examination 6 months after initial diagnosis. Owing to the failure of the lymph node to return to normal size, a change in antifungal therapy was discussed but declined by the cat's owner on the basis of financial constraints.

The cat was not examined again for 7 months, approximately 13 months after initial diagnosis. Fluconazole therapy had been consistent during that time. At that examination, the cat was clinically normal. Repeat abdominal ultrasonographic examination revealed an enlarged, hypoechoic, irregular mesenteric lymph node, which was larger than the prior examination (4.4 cm × 2.7 cm). A second intra-abdominal lymph node (2.0 cm × 0.7 cm) was also seen immediately adjacent to the other enlarged lymph node. Repeat urine and serum HcAg tests were negative. FNA samples were collected of the enlarged mesenteric lymph node for fungal culture and susceptibility. Fungal culture grew *H capsulatum*, which was confirmed by DNA sequence analysis of the 28S (large subunit) ribosomal DNA (Oklahoma Animal Disease Diagnostic Laboratory, Stillwater, OK, USA). Antifungal susceptibility testing (performed according to the Clinical and Laboratory Standards Institute M38-A2 protocol)[1] showed a very high minimum inhibitory concentration (MIC) for fluconazole (64 µg/ml) and low MIC for itraconazole (0.06 µg/ml; University of Texas Health Science Center, San Antonio, TX, USA [Table 1]). The fluconazole MIC was much higher than the expected feline blood fluconazole concentration based on a previous pharmacokinetic study (mean ± SD 26.4 ± 3.6 µg/ml mean maximum plasma concentration after receiving 100 mg fluconazole orally).[2] Clinical breakpoints for antifungal susceptibility are not available for *H capsulatum*; however, therapeutic failure is more likely in humans with isolates with fluconazole MICs ⩾5 µg/ml.[3] Based on low susceptibility of the isolate to fluconazole and the apparent treatment failure in this cat, a drug change to itraconazole was recommended. The cat was again lost to follow-up for 5 months, and fluconazole therapy was continued by the cat owner (despite the recommendation for itraconazole). The cat presented again with signs of neurologic disease, including an altered mentation and proprioceptive ataxia in all limbs. Owing to progressive neurologic disease, the cat was euthanized. Necropsy showed pyogranulomatous inflammation and *H capsulatum* organisms in the mesenteric lymph node, leptomeninges, subdural space of the spinal cord, kidneys, liver and lung.

Table 1 Antifungal susceptibility testing results for *Histoplasma capsulatum* (performed according to the Clinical and Laboratory Standards Institute M38-A2 protocol) from the mesenteric lymph node of a cat with histoplasmosis while being treated with fluconazole

Drug	Minimum inhibitory concentration (µg/ml)
Amphotericin B	⩽0.03
Fluconazole	64
Itraconazole	0.06
Posaconazole	0.125
Voriconazole	0.125
Terbinafine	0.215

Discussion

Azole antifungals are the treatment of choice for histoplasmosis, and successful treatment outcomes have been observed with both itraconazole and fluconazole.[4,5] Itraconazole is recommended for treating histoplasmosis in the 2013 European Advisory Board on Cat Diseases guidelines;[6] however, it might be cost prohibitive in some cases. A commercially available generic suspension is lacking and products compounded from bulk powder may have poor bioavailability, necessitating use of either the innovator-formulated oral suspension or resized generic capsules.[7] Additionally, adverse effects with itraconazole are common and include inappetence, gastrointestinal distress and hepatotoxicity.[8] For these reasons, fluconazole has often been used in cats as a lower-cost, better-tolerated alternative. In one study of 30 cats with histoplasmosis, treatment with fluconazole (19.3 mg/kg/day) led to similar survival and recrudescence rates as itraconazole (10.0 mg/kg/day), suggesting that fluconazole is a viable therapy for feline histoplasmosis.[5]

In humans, itraconazole is known to be more effective than fluconazole for the treatment of histoplasmosis, as 30.5% of patients on maintenance therapy with fluconazole relapsed vs <5% on itraconazole.[9,10] Reduced susceptibility to fluconazole has been documented during therapy, with one older study showing at least a

four-fold increase in MIC in the isolates from more than half of patients with paired pretreatment and relapse/failure samples.[3] The mechanism of this increase in MIC is reduced inhibition of ergosterol synthesis due to be decreased sensitivity of cytochrome P450-dependent enzymes 14α-demethylase (CYP51p; encoded by *ERG11*) and 3-ketosteroid reductase to fluconazole.[11] One mechanism for this was demonstrated in a later study, as sequencing of a post-treatment isolate with reduced susceptibility to fluconazole revealed a single amino-acid substitution (Y136F) in CYP51Ap that was absent in a baseline pretreatment isolate.[12] Alignment of the CYP51Ap from *H capsulatum* with CYP51 from *Candida albicans* showed that Y136 is analogous to Y132 of *C albicans*, and Y132 substitution can interfere with fluconazole binding.[12,13] Owing to the potential for reduction in susceptibility to fluconazole during treatment, that drug is no longer commonly used to treat histoplasmosis in humans. An evaluation of the newer triazoles in human patients with histoplasmosis who failed fluconazole showed that 41% also had reduced susceptibility to voriconazole (but not posaconazole or ravuconazole).[12] Reduced susceptibility to itraconazole has not been described for *H capsulatum.*

Resistance to azole antifungal agents among other fungi is emerging as a global health problem. Fluconazole is one of the most commonly prescribed antifungal drugs for *Candida* species infections.[14] In the USA, *C albicans* has a low incidence of fluconazole resistance; however, *Candida glabrata* exhibits up to 13% resistance and the emerging yeast *Candida auris* may show up to 93% resistance.[15,16] Many molecular mechanisms of fluconazole resistance have been described for *Candida* species, including increased drug targets (eg, overexpression of *ERG11*), alterations in drug targets (eg, point mutations in the coding region of *ERG11* leading to alteration of protein structure and decreased azole binding), alterations in sterol biosynthesis (eg, development of bypass pathways within sterol byiosynthesis), increased drug efflux (eg, overexpression of transport proteins resulting in a failure of drug to accumulate intracellularly) and aneuploidy or other chromosomal abnormalities.[17] Recently, fluconazole resistance in a cat with *Cryptococcus gattii* was demonstrated, and the resistant isolate showed overexpression of *ERG11* and the efflux pump *PDR11* compared with the pretreatment isolate.[18]

The present case report describes reduced susceptibility to fluconazole in a cat being treated for histoplasmosis. Given the initial response to fluconazole, followed by long-term worsening of disease and dissemination to the central nervous system, this case most likely represents acquired drug resistance. Unfortunately, no pretreatment isolate was available to compare the MIC values with those of the post-treatment isolate. The MIC for itraconazole (0.06 µg/ml) was low; therefore, itraconazole therapy would likely have been an effective treatment (although it was never attempted).

Conclusions

Reduced susceptibility to fluconazole has been documented in humans with histoplasmosis, and fluconazole is known to be less effective than itraconazole for the treatment of histoplasmosis in humans. This report presented a case of reduced susceptibility over time in a cat with histoplasmosis. Veterinarians should be aware of the possibility of low susceptibility in animals being treated for histoplasmosis. Although fluconazole may be efficacious in many cases, this drug should likely be avoided as a long-term (>1 year) or maintenance therapy, or in cases that show recrudescence.

Conflict of interest JSR and LJW are employees of MiraVista Diagnostics, the diagnostic laboratory that performed the antigen and antibody testing for this cat.

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