Two Cases of Feline Orbital Aspergillosis Due to *Aspergillus udagawae* and *A. viridinutans*

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(Received 16 March 2012/Accepted 1 August 2012/Published online in J-STAGE 10 August 2012)

ABSTRACT: *Aspergillus udagawae* and *A. viridinutans* are members of the section Fumigati; both cause invasive aspergillosis in humans. These two *Aspergillus* species are discriminated from *A. fumigatus* by molecular methods. Herein, we report two cases of feline orbital aspergillosis, one caused by *A. udagawae* and the other by *A. viridinutans*. To our knowledge, Case 1 represents the first reported case of treatment with a high dosage of itraconazole, and Case 2 represents the first reported case of treatment with a high dosage of itraconazole and micafungin. To our knowledge, Case 1 represents the first reported case of treatment with AMB, itraconazole (ITZ), or micafungin. Aspergillus strains isolated from human aspergillosis showed high minimum inhibitory concentrations (MICs) of amphotericin B (AMB) and azoles [1]. Previously, we reported the first case of treatment of *A. udagawae* with a high dosage of itraconazole, and Case 2 represents the first reported case of treatment with *A. viridinutans* infection associated with sarcoma. Identification of the etiologic agents of these cases was confirmed by comparative analyses of the sequences of β-tubulin-encoding genes. With the spread of non-*fumigatus* aspergillosis, increasing emphasis should be placed on molecular identification of the infecting *Aspergillus* species and the use of *in vitro* drug susceptibility tests to ensure the selection of appropriate antibiotics.

KEY WORDS: feline, *in vitro* susceptibility test, molecular identification, orbital aspergillosis.


Reports of feline cases of orbital aspergillosis are on the rise, and the infection is considered an emerging disease [3, 5, 7, 12]. *Aspergillus fumigatus* has been the most frequently reported etiologic agent of sino-orbital aspergillosis in cats [5]. Two other *Aspergillus* species, *A. udagawae* and *A. viridinutans*, are also members of the section Fumigati and cause invasive aspergillosis in humans [1, 2]. These two species are discriminated from *A. fumigatus* by molecular methods using sequences of the β-tubulin-encoding (*benA*) genes [1, 11]. *Aspergillus* strains isolated from human aspergillosis showed high minimum inhibitory concentrations (MICs) of amphotericin B (AMB) and azoles [1]. Previously, we reported the first case of *A. udagawae* infection in a cat, and noted that the infection did not respond to treatment with AMB, itraconazole (ITZ), or micafungin [7]. In this study, we report two other feline cases of orbital aspergillosis, one caused by *A. udagawae* and the other by *A. viridinutans*. To the best of our knowledge, Case 1 represents the first reported case of treatment with a high dosage of ITZ for feline orbital aspergillosis due to *A. udagawae*, and Case 2 represents the first reported case of feline *A. viridinutans* infection.

MATERIALS AND METHODS

**Case 1**: A spayed female domestic short-hair cat (10 years old; weight, 4 kg) was referred to the Animal Medical Center, Gifu, University of Gifu, Japan, in April 2011 with progressive protrusion of the left third eyelid and eyeball (Fig. 1). The case had been treated with clindamycin for the previous 2 months. Hematology and serum biochemistry showed no abnormal findings. The cat was negative both for plasma p27 antigen of feline leukemia virus (FeLV) and for antibodies against feline immunodeficiency virus (FIV). CT scan revealed a soft-tissue mass within the orbit of the left eye (Fig. 2). Histopathologic examination of biopsy samples from this mass revealed granulomatous inflammation containing many branching hyphal filaments (Fig. 3). Samples from this mass were inoculated on Sabouraud’s dextrose agar (SDA) and grown at 28°C. Velvety, grayish-white colonies developed within a week. Based on gross and microscopic characteristics, isolates were identified as *Aspergillus* species, and the case was diagnosed as orbital aspergillosis.

**Case 2**: A spayed female domestic short-hair cat (10 years and 3 months old; weight, 3.7 kg) was referred to the Veterinary Medical Center, The University of Tokyo, Tokyo, Japan, in July 2011 with progressive protrusion of the left third eyelid and serous ocular and nasal discharge for the previous 2 months. Hematology and serum biochemistry showed no abnormal findings. Tests for plasma p27 antigen of FeLV and for antibody against FIV were negative. CT scan revealed a soft-tissue mass within the orbit of...
the left eye. Histopathologic examination of biopsy samples from this mass revealed a poorly differentiated sarcoma in the greater part of the mass and granulomatous inflammation containing many branching hyphal filaments in the peripheral area of the granulomatous lesions (Fig. 4). Following inoculation of SDA and growth at 28°C, velvety, grayish-white colonies developed within a week. Based on gross and microscopic characteristics, isolates were identified as *Aspergillus*. The case was diagnosed as secondary aspergillosis associated with sarcoma.

**Molecular identification of fungal species:** Molecular analysis of the *Aspergillus* isolates was performed as follows. Genomic DNA was extracted with a DNeasy Tissue Kit (QIAGEN, Vallencia, CA, U.S.A.), and a segment of the β-tubulin-encoding gene was amplified using the universal fungal primers benA-F (5′ AAT TGG TGC CGC TTT CTG G) and benA-R (5′ AGT TGT CGG GAC GGA ATA G) [2]. The PCR amplification and sequence analyses were performed as described previously [2].

The *in vitro* susceptibilities of both isolates to the antifungal drugs AMB, ITZ, ketoconazole (KTZ), and voriconazole (VRZ) were assessed by the E-test method [10].

**RESULTS**

Results of comparative sequence analyses by nucleotide BLAST analysis on the National Center for Biotechnology Information (NCBI) website showed that the sequence amplified for the isolate from Case 1 was 100% identical to the β-tubulin gene of *A. udagawae* (GenBank accession no. DQ058392 and AB248303).

Thus, molecular analysis of Case 1 confirmed the diagnosis of aspergillosis due to *A. udagawae*. High-dose ITZ (50 mg/dose) was administered orally twice a day for 3 months. After 4 months of treatment, the mass was no longer detected by gross (visual) inspection. However, 2 months after
the end of the treatment, a swelling mass was again seen by gross inspection in the left orbit. However, the CT scan did not perform again because of the owner declined. After a further month of treatment with ITZ, the mass again shrank and was no longer detected by visual inspection. No further clinical signs were seen at a follow-up by treatment with ITZ (50 mg/dose) on twice weekly.

Unfortunately, the condition of the case 2 continued to deteriorate; the owner declined the use of antifungal therapy, and the cat died of aspergillosis and sarcoma 10 days after referral.

Subsequent drug susceptibility testing of this *A. udagawae* isolate revealed MICs by E-test of $>32 \mu g/ml$ for AMB, $>32 \mu g/ml$ for KTZ, 0.19 $\mu g/ml$ for ITZ, and 0.64 $\mu g/ml$ for VRZ.

Comparative sequence analysis of the amplified region of the isolate from case 2 revealed 99% identity to the corresponding segment of the $\beta$-tubulin gene of *A. viridinutans* (DDBJ accession no. AB248299 and HE578084) and <93% identical to *Neosartorya spinosa* NRRL 32569 (GenBank accession no. EF669816).

Drug susceptibility testing of this *A. viridinutans* isolate by E-test revealed MICs of 0.064 $\mu g/ml$ for AMB, $>32 \mu g/ml$ for KTZ, 2 $\mu g/ml$ for ITZ, 0.38 $\mu g/ml$ for VRZ, and 0.064 $\mu g/ml$ for posaconazole (POS).

DISCUSSION

To the best of our knowledge, Case 1 represents the first reported case of treatment with a high dosage of ITZ for feline orbital aspergillosis due to *A. udagawae*, and Case 2 represents the first reported case of feline *A. viridinutans* infection. These cases were diagnosis by histopathologic examination and mycological identification. The CT scan is a very powerful tool, but is not sufficient to differential diagnosis of orbital aspergillosis or tumors like this Case 2.

Barrs *et al.* reported that there was no evidence in previous reports (including one of our previous studies) of an association between retrovirus infection and upper respiratory tract aspergillosis [4]. Consistent with that paper, retroviral infection was not detected in either of the cases described in the present report (data not shown). Therefore, retrovirus infection may not be a predisposing factor for orbital aspergillosis.

Hematological and serum biochemical findings for feline sinonasal and sino-orbital aspergillosis indicated that hyperglobulinemia is the most common biochemical abnormality among infected animals [4]. However, hyperglobulinemia was not seen in either of the cases described in the present report. Therefore, hyperglobulinemia is not a useful marker for such infection.

In summarizing 22 cases of feline sinonasal aspergillosis, Barrs *et al.* reported that the fungal pathogen was *A. fumigates* (n=4), *Neosartorya fischeri* or *A. lentulus* (n=1), or a non-specified *Neosartorya* spp. (n=1) [4]. Moreover, in all cases of sino-orbital aspergillosis (n=17), the fungal pathogen was identified as a *Neosartorya* species [4]. Therefore, non-*fumigatus* Aspergilli should be considered as potential fungal pathogens in feline sino-orbital aspergillosis.

*A. udagawae* has been isolated from soil in the past, and has recently also been isolated from invasive infections in human and cat. Previously, we reported the first case of *A. udagawae* infection in a cat, and noted that the infection did not respond to treatment with ITZ at 20 mg/kg orally once a day and AMB 0.2 mg/kg, 3 days per week for 4 weeks, or micafungin (1 mg/kg, 3 days per week for 5 weeks) [7]. The isolate of *A. udagawae* from feline sino-orbital aspergillosis has low susceptibilities to AMB [7]. The drug susceptibility testing of *A. udagawae* from Case 1 also revealed low susceptibilities to AMB and KTZ.

However, AMB is frequently selected for therapy of canine and feline aspergillosis, including feline orbital aspergillosis [6, 8, 9]. Therefore, it may now be important for therapeutic decision-making to examine the MICs of other antifungal drugs against feline aspergillosis.

Clinical isolates of *A. viridinutans* from human invasive infections exhibit high MICs to ITZ compared to isolates of *A. fumigatus* [1]. Consistent with this observation, the clinical isolate of *A. viridinutans* from Case 2 also had higher MICs to ITZ and KTZ than typically are seen in *A. fumigatus*. These azoles are often chosen to treat canine and feline aspergillosis, including orbital aspergillosis [8]. The low susceptibility to azoles seen in these non-*fumigatus* Aspergilli indicates that molecular identification of *Aspergillus* species and *in vitro* susceptibility testing are needed for the treatment of aspergillosis. Further studies are required to determine whether the distinct resistance profiles of infecting non-*fumigatus* Aspergilli are a major determinant of treatment outcome.

ACKNOWLEDGMENTS. This study was supported by grants from the Academic Frontier Project of the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) and Kariya Animal Hospital Inc.

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