Successful management of an intracranial phaeohyphomycotic fungal granuloma in a dog

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A 12-month-old castrated male Boxer was examined at the Foster Hospital for Small Animals at Tufts University with a 3-day history of progressive neurologic abnormalities and inappetence. The dog was also failing to gain weight. Prior to examination, there had been continued deterioration despite penicillin G, amoxicillin, cefazolin sodium, carprofen, and IV fluid treatment by the referring veterinarian.

Physical examination revealed a temperature of 37.7°C (99.9°F), grade II systolic heart murmur, heart rate of 100 beats/min, and respiratory rate of 20 breaths/min.

Neurologic examination revealed a dull responsive patient. Intermittent opisthotonus was noted, during which the dog staggered to the left. There was mild generalized ataxia and occasional dragging of the left thoracic limb. The dog showed a right head turn and a left head tilt. Cranial nerve examination revealed positional ventral strabismus of the right eye and positional, horizontal nystagmus (30 beats/min), with the fast phase to the left. The left median nasal mucosa was analgesic. Hopping reactions and paw replacement were delayed to the left. The left medial nasal mucosa was analgesic. Ventral strabismus of the right eye and positional, horizontal head tilt. Cranial nerve examination revealed positional ventral strabismus of the right eye and positional, horizontal head tilt.

Lesion localization was multifocal, apparently including the right prosencephalon and central vestibular system. Either focal forebrain disease with secondary herniation or multifocal disease was suspected. Differential diagnoses that were considered consisted of anomalous disease (eg, congenital hydrocephalus or intra-arachnoid diverticula), focal granuloma or abscess formation (bacterial or fungal), neoplasia (eg, lymphoma or glioma), multifocal infection (viral, bacterial, protozoal, or fungal), and metabolic disease (eg, hepatic encephalopathy).

Results of hematologic testing, serum biochemical analysis, urinalysis, and measurement of baseline and postprandial serum bile acids concentrations were within reference limits. Magnetic resonance imaging revealed a 2-cm mass in the right parietotemporal cerebrum (Figure 1). The center of the mass was hyperintense, compared with the surrounding gray matter, on T2-weighted images, and the mass had a 2-mm-thick hypointense rim. On T2-weighted images, hyperintensity was also noted within the surrounding white matter tracts. The center of the mass was hypointense, compared with the surrounding gray matter, on T1-weighted images and the rim was isointense. Following IV administration of gadolinium, there was marked homogenous enhancement of the rim of the mass, with...
no detectable central enhancement. There was mild enhancement of the surrounding meninges. The mass caused a midline shift to the left, right lateral ventricle compression, and mild caudal transtentorial and severe caudal cerebellar herniation. When these herniations were identified on the initial MRI series (parasagittal T2-weighted images), mannitol (0.5 mg/kg [0.23 mg/lb], IV, once) was administered.

Following MRI, primary differential diagnoses were abscess formation (bacterial or fungal) or cavitated neoplasia. Cerebrospinal fluid analysis revealed an inflammatory pattern with pleocytosis (TNC, 34 cells/μL; reference range, 0 to 5 cells/μL) and high total protein concentration (51 mg/dL; reference range, 0 to 25 mg/dL), but RBC count was within reference limits (1 cell/μL; reference range, 0 to 5 cells/μL). The WBC differential count was 43% mononuclear cells, 26% neutrophils, 23% lymphocytes, and 6% eosinophils. No infectious organisms were observed.

Ranked differential diagnoses at this time were fungal abscess formation, bacterial abscess formation, and, of a lesser consideration, neoplasia. Surgery was recommended to alleviate clinical signs, alleviate the risk of fatal elevations in intracranial pressure, permit definitive diagnosis, allow microbial culture, and excise any avascular areas to which antimicrobial or cytotoxic drug delivery would be potentially inadequate.

A routine approach was made to the lateral aspect of the skull, and a rectangular rostroventricular craniectomy was performed. After a rostrocaudal durotomy was performed, cerebral cortex herniated through the durotomy site (Figure 2). The cerebral cortex was bluntly incised, and purulent fluid leaked from the underlying cavity. A sample of this fluid was submitted for cytologic examination and microbial culture and susceptibility testing. Purulent fluid and a surrounding capsule were removed by use of debridement, lavage, and suction, and the tissue was submitted for histologic examination. Distinction between the capsular structure and normal cerebrum was difficult, but grossly abnormal tissue was removed layer by layer until the remaining brain tissue appeared macroscopically normal. Closure was routine.

Postoperative management included fluconazole (2.3 mg/kg [1 mg/lb], PO, q 12 h), cefotaxime (22 mg/kg [10 mg/lb], IV, q 8 h), enrofloxacin (10 mg/kg [4.5 mg/lb],...
IV, q 24 h), metronidazole (7.5 mg/kg [3.4 mg/lb], IV, q 12 h), dexamethasone sodium phosphate (2 mg/kg [0.9 mg/lb], IV, once), famotidine (0.5 mg/kg, IV, q 12 h), lantanol (1.5 μg/kg/h [0.7 μg/lb/h] as a continuous rate infusion), and an isotonic crystalloid fluid (2.5 mL/kg/h [1.1 mg/lb/h]). A single loading dose of phenobarbital (8 mg/kg [3.6 mg/lb], IV) was administered, followed by continuous administration (2.2 mg/kg [1 mg/lb], IV, q 12 h). The patient's head was elevated to 30°, and a urinary catheter was placed. Body temperature, heart rate, respiratory rate, oxygenation (as measured by pulse oximetry), ECG, systolic and diastolic blood pressure (measured directly via an arterial catheter), and urine output were monitored.

Cytologic analysis of the fluid sample obtained from the intracranial mass revealed degenerate neutrophils, vacuolated macrophages, and lymphocytes. Multiple 100- to 150-μm-long, 3- to 5-μm-wide hyphal elements were seen. Hyphae were branching, septate, and pigmented. Histologic examination of the surgical biopsy specimen revealed severe pyogranulomatous and necrotizing meningoencephalitis with intralesional pigmented hyphae (Figure 3).

Fungal culture was performed at 34.4°C (95°F) in oxygen on an inhibitory mold agar plate and yielded Cladophialophora organisms. These were identified on the basis of gross (ie, greenish-brown to black colonies that grew within 7 days) and microscopic (ie, dark, septate hyphae; dark, branched conidiophores of various lengths; and brown, oval conidia in branching chains) features. Routine aerobic and anaerobic bacterial cultures did not yield any growth; however, a Cladophialophora sp was isolated from thioglycolate broth.

Three days after surgery, the patient was discharged. The owners were instructed to treat the dog with enrofloxacin (12.3 mg/kg [5.6 mg/lb], PO, q 24 h for an additional 2 weeks), fluconazole (2.3 mg/kg [1 mg/lb], PO, q 12 h), and phenobarbital (2.5 mg/kg [1.1 mg/lb], PO, q 12 h).

During a follow-up examination at the Foster Hospital 16 days after surgery, the patient remained mildly paretic. Serum phenobarbital concentration was 16.5 μg/mL. One month after surgery, left medial nasal mucosa analgesia was the only neurologic abnormality.

At 4 months after surgery, results of a neurologic examination were normal. The phenobarbital dosage was being tapered by 10% every 2 weeks. Administration of fluconazole was discontinued, and treatment with voriconazole* was initiated (3.4 mg/kg [1.5 mg/lb], PO, q 12 h). The decision was made to treat the dog with voriconazole to increase the likelihood of definitively eradicating the fungal brain infection.

The patient was examined 10 more times at the Foster Hospital during the next 13 months, and during this time, it remained neurologically normal. No clinically important abnormalities were detected on hematologic testing performed 3 times during this period or on serum biochemical testing performed 5 times during this period.

Eight months after surgery, MRI revealed complete resolution of the granuloma (Figure 4). A loss of cerebral parenchyma was seen as a midline shift toward the cranectomy site; the previously compressed right lateral ventricle was now enlarged. A thin line of contrast enhancement was noted in the meninges at the cranectomy site. Analysis of CSF obtained from the cerebellomedullary cistern revealed a TNC of 3 cells/μL, protein concentration of 15 mg/dL, and RBC count of 4 RBCs/μL. The WBC differential count was 63% lymphocytes, 33% monocytes, 3% neutrophils, and a single eosinophil (1%). No infectious organisms were noted.

After 14 months, a CSF sample was again obtained from the patient, and analysis revealed a TNC of 2 cells/μL.
and protein concentration of 11 mg/dL (both within reference limits). The WBC differential count included 11% neutrophils and 2% eosinophils, which were attributed to blood contamination (RBC count, 108 RBCs/μL). Hematologic testing of a peripheral blood sample included 2% eosinophils. Voriconazole treatment was discontinued. Nine weeks after voriconazole administration was discontinued, the patient underwent follow-up MRI; no recurrence of the granuloma was evident (Figure 5). Focal meningeal enhancement was noted but was slightly decreased in intensity, compared with previous images. Analysis of a cisternal CSF sample revealed a TNC of 0 cells/μL, protein concentration of 12 mg/dL, and RBC count of 3 RBCs/μL. Forty-one months after surgery and 27 months after voriconazole administration was discontinued, the patient was apparently healthy.

Discussion

Phaeohyphomycotic organisms, also known as melanized or dermatiaceous fungi, are neurotropic opportunistic pathogens.1–3 Cladophialophora bantiana is the most common organism, causing 48% of human cases of CNS phaeohyphomycosis.1 Synonyms include Cladosporium bantianum, Cladosporium trichoides, and Xylohypha bantiana.1,4,5 Cladophialophora spp are saprophytes and have been cultured from the conjunctiva and skin of healthy dogs.6,7 Intracranial phaeohyphomycotic granulomas in people are well reported. Primary cerebral infections are in fact predominated by phaeohyphomycosis, although this neortropism is not yet well understood.2 Most human patients with intracranial phaeohyphomycosis are not immunosuppressed, and nearly all are examined because of brain abscesses or intracranial granulomas.1 Fungal CNS infections in small animals are rare. Cryptococcosis is the most common; other fungal infections in small animals include systemic blastomycosis or coccidiomycosis and nasal aspergillosis.8,9
Phaeohyphomycosis in dogs can cause disease localized to the CNS or systemic disease with intracranial involvement.\textsuperscript{4,10-14} Cladophialophora spp is the most common cause of intracranial phaeohyphomycosis in dogs.\textsuperscript{4,10,11,13,14}

Seven dogs with CNS phaeohyphomycosis have been described previously.\textsuperscript{4,10-14} Seven cats with intracranial infection have also been reported.\textsuperscript{11,15-16} Often, a single granuloma with associated meningitis was found, although multiple pyogranulomas with diffuse meningoencephalitis have also been described. In 10 of these 14 animals, the infection was limited to the brain,\textsuperscript{11,12,13,15-18} whereas in the others, there was variable additional involvement of abdominal and thoracic organs\textsuperscript{4,10,13,14} and, in 1 dog, the vertebral column.\textsuperscript{10} All 14 died as a result of the infection, although 1 dog temporarily improved after computed tomography-guided aspiration to debulk the intracranial infection and fluconazole administration.\textsuperscript{10} The only antifungal medications administered in these animals were fluconazole and ketoconazole, and to our knowledge, there are no previously published reports of small animal patients with intracranial phaeohyphomycosis that have been successfully treated. Additionally, although most reports have included dogs with a normal immune system, intracranial phaeohyphomycosis has also been reported in association with dieterem infection,\textsuperscript{11} ehrlichiosis,\textsuperscript{4} and leukopenia.\textsuperscript{12}

The choice of antifungal medication may be an important factor contributing to a positive outcome in dogs and people with CNS phaeohyphomycosis. Treatment of intracranial phaeohyphomycotic granulomas is often unsuccessful in people. Surgery is thought to reduce the burden of infected tissue and remove avascular, purulent, or necrotic material to which drug delivery is poor, and outcome has been reported to be associated with the extent of excision.\textsuperscript{1,2,3} The mortality rate with medical treatment alone in human patients approaches 100%,\textsuperscript{1,19} whereas for patients treated with a combined surgical and medical approach, mortality rates of 62% to 75% has been reported.\textsuperscript{1,18} These reports are based on treatment protocols involving amphotericin B,\textsuperscript{1,2,19} flucytosine,\textsuperscript{1,19} itraconazole,\textsuperscript{1,2} and fluconazole.\textsuperscript{1,2,19} Not only have these medications produced complications. Of the triazole medications, itraconazole and voriconazole have the most consistent activity against melanized fungi,\textsuperscript{3} but itraconazole does not penetrate the blood-brain barrier effectively, reaching a CSF concentration < 1% of the plasma concentration.\textsuperscript{20} However, brain concentrations of itraconazole are higher during fungal infection,\textsuperscript{21} and this may help to explain why it is comparable to fluconazole for the treatment of cryptococcal meningitis in humans.\textsuperscript{24} The bioavailability of itraconazole is affected by variations in diet and gastric pH,\textsuperscript{23} and the drug has been associated with various adverse effects including anorexia (≤ 15% of dogs),\textsuperscript{26} gastrointestinal disturbance,\textsuperscript{23} and rare cases of hepatotoxicosis.\textsuperscript{23,27} Fluconazole has been suggested as the treatment of choice for various CNS fungal infections in dogs.\textsuperscript{28} However, voriconazole has a broader spectrum than fluconazole and itraconazole and is reported to be 50 to 100 times as potent against certain fungal species and also to have excellent bioavailability and ability in penetrating the brain and CSF.\textsuperscript{1,8,23,26,29} No adverse effect was noted with doses of 3 to 12 mg/kg (1.4 to 5.5 mg/lb) in healthy dogs in a laboratory setting, other than autoinduction of its own metabolism and increased liver cytochrome P450 concentrations.\textsuperscript{29} Such pharmacokinetic and pharmacodynamic data suggest voriconazole may be a first-choice drug for treatment of cerebral phaeohyphomycosis in dogs, although further study is required.

In the patient described in the present report, the combination of fluconazole administration followed by voriconazole treatment was associated with complete eradication of the fungal agent without any apparent adverse effects. It is impossible to speculate whether fluconazole treatment alone would have been equally successful. In 1 report\textsuperscript{10} of phaeohyphomycosis in a dog, the patient initially improved after treatment with fluconazole alone but later deteriorated and was euthanatized, and to our knowledge, there are no other reports of fluconazole being curative in affected dogs. Early data regarding the use of voriconazole in human patients\textsuperscript{1-3,10,12} and in animals with experimentally induced phaeohyphomycosis\textsuperscript{21} suggest that complete eradication may be more common with voriconazole than with fluconazole. Voriconazole is currently much more expensive than fluconazole (> 60 times the cost, at current market prices), which may limit its use in veterinary patients at present. The voriconazole was donated for treatment of the patient in this report. Our treatment of the patient in the present report might have been further guided by results of antifungal susceptibility testing. However, given the cost of alternatives to fluconazole (such as voriconazole or liposomal amphotericin B), we did not pursue this at the time of initial culture, and it was only later that voriconazole was donated. The voriconazole dose (3.4 mg/kg; 75 mg for a 22.2-kg patient) used in this report was based on a study of voriconazole pharmacokinetics in dogs.\textsuperscript{29} The recommended maintenance dose for human patients weighing < 40 kg (88 lb) is 100 mg.\textsuperscript{30}

\textbf{References}