The use of liposomal amphotericin B in the management of *Xylohypha bantiana* mycosis in a dog

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ABSTRACT

Xylohypha bantiana is a rare neurotropic fungal infection reported in humans, dogs and cats. In dogs it has only been identified on *post mortem* examination and thus no successful treatments have previously been reported. Amphotericin B is a potent antifungal drug with a low therapeutic index because of its nephrotoxicity. Liposomal encapsulation of the drug has resulted in much safer use in humans. This article reports a case of *Xylohypha bantiana* infection in a dog that was diagnosed antemortally and managed with liposomal amphotericin B, which resulted in the prolongation of quality of life for an infection that invariably results in rapid death.

Key words: liposomal amphotericin B, systemic mycosis, *Xylohypha bantiana*.

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INTRODUCTION

Xylohypha bantiana is an opportunistic fungus that rarely causes disease in humans and animals. Infections are usually neurotropic, resulting in cerebral phaeohyphomycosis (a fungal infection caused by a pigmented (usually dark brown) fungus, characterised morphologically by septate hyphae and yeast-like cells). Although humans with no recognised underlying immune defects may be affected[11], most reported cases of phaeohyphomycosis are associated with an underlying acquired immunosuppression resulting in secondary opportunistic fungal infection[6,11,21,25]. Predisposing immunosuppressive factors that should be considered in dogs include chronic debilitating diseases, such as neoplasia, endocrine disease, canine distemper, canine ehrlichiosis and prolonged antibiotic, glucocorticoid or cytotoxic chemotherapy[6].

Natural sources of this saprophytic fungus are plant debris, wood and soil[18]. The portal of entry is uncertain, although wound contamination[20], and the eye and the respiratory system are all possible sites[9]. Histopathological evidence of vascular involvement as well as the random multifocal distribution of lesions lends support to a haematogenous spread of the infection[9].

Antemortal diagnosis of *X. bantiana* in animals is rare[9,25] and in only 1 other canine infection has the diagnosis been made and treatment attempted before death[23]. In humans the diagnosis is usually made after surgical intervention for a cerebral mass lesion[11,25,26]. Urine, blood and cerebrospinal fluid culture have been attempted in dogs but have not been sensitive. Fine-needle aspiration of radiologically affected disc spaces has been found to be both a sensitive and specific means of diagnosis of mycotic discospondylitis[2,8].

Various anti-fungal therapies have been attempted for this infection in humans and there are conflicting reports on efficacy[11,26]. It should, however, be noted that non-surgical treatment has rarely been initiated in dogs because the course of the disease is rapid and fatal and in the 1 dog where it was tried, it failed[25]. In humans, surgical resection and drainage is usually attempted together with the administration of amphotericin B and flucytosine[7]. In 1 human case the surviving patient had a focal abscess that could easily be resected, and adjunctive anti-fungal therapy was not associated with improved survival[7]. Ketoconazole and itraconazole have both been tried but found to be ineffective[9,11,25]. To date, no effective drug treatments have been reported for humans or animals thus cerebral phaeohyphomycotic infection carries a grave prognosis. Even with surgical intervention, human patients generally do not survive longer than a year[7]. No animal case has to date been reported to survive or have benefited from any chemotherapy.

CASE HISTORY

A 2-year-old, 31 kg, sterilised female German shepherd dog was referred to the Onderstepoort Veterinary Academic Hospital (OVAH) with a complaint of depression, a right-sided head tilt, and non-specific pain that made rising and climbing stairs difficult.

There was a previous history from the referring veterinarian of an episode of disorientation and vomiting 2 months earlier, which was diagnosed as a right-sided peripheral vestibular syndrome (only a right-sided head tilt was present on examination). Prednisolone (Lenisolone, Lennon Ltd) and enrofloxacin (Baytril, Bayer, Isando, South Africa) treatment was unsuccessful.

On admission to OVAH, the dog was depressed, pyrexic, showed a distinct right-sided head tilt and was painful on spinal and pelvic examination. She was tense on abdominal palpation. Neurological examination was normal except for mild atrophy of the muscles of the neck, and there was pain on manipulation of the neck and spine. Otoscopic examination was unremarkable. Survey spinal radiographs demonstrated discospondylitis in the C5–6, T10–11 and L1–6 disc space, with the cervical space showing the most advanced lesion. Bulla radiographs were unremarkable. Cisternal cerebrospinal fluid tap and analysis was within normal limits for protein concentration and cytological assessment. Abdominal ultra-sound examination was unremarkable. Haematological and biochemical evaluations were normal. Serum immunoglobulin determination showed an elevation in IgG (9400mg/dℓ, n = 1000–2000 mg/dℓ), normal IgA (48 mg/dℓ, n = 40–160 mg/dℓ) and slight decrease in IgM (68 mg/dℓ, n = 100–200 mg/dℓ) concentrations. No abnormalities

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were detected on urine or faecal analysis, and urine culture for bacteria and fungi was negative.

A fluoroscopically-guided fine-needle aspirate of disc C5–6 was performed under general anaesthesia. Cytological examination of the fine-needle aspirate showed fungal elements in some scant pyogranulomatous inflammation. Aerobic and anaerobic bacterial cultures were negative. Mycological culture was positive and while specific identification of the fungus was pending, antimicrobial testing showed sensitivity to amphotericin B, flucytocine, nystatin, enilconazole and miconazole. Resistance was seen to ketoconazole and fluconazole. Serum indirect fluorescent antibody testing for canine distemper virus and *Toxoplasma gondii* was negative.

The dog was discharged on meloxicam (0.1 mg/kg for 24 h, Mobic, Ingelheim Pharmaceuticals, Randburg, South Africa) and itraconazole (5 mg/kg for 24 h, Sporanox, Janssen-Cilag, Halfway House, South Africa) before specific fungal identification results were available. An 8-day follow-up examination showed no improvement and it was decided to change anti-fungal treatment to colloidal amphotericin B (Fungizone, Bristol-Meyers Squibb, Bedfordview, South Africa). Treatment was initiated according to a standard protocol[27]. This treatment had to be discontinued within days of initiation due to severe nephrotoxicity (based on rising serum urea and creatinine concentrations), which gradually recovered over the ensuing week following withdrawal of the drug.

In the interim, microbiological identification of the fungus proceeded as follows: cultures were incubated at 22, 37 and 42 °C on Sabouraud's dextrose and Mycobiotic agars (Difco) and cultured after 4 days. The colony had the morphological and microscopic characteristics of the neurotropic fungus *X. bantiana.* These included the production of brown pigment and septate, branched hyphae of an average of 2.5 μm in diameter, and conidiophores bearing long, elliptical chains of conidia. It was also gelatinase negative and grew at 42 °C, which distinguished it from the morphologically similar fungus *Cladosporium caronii.*

Because of the severe side-effects of the colloidal amphotericin B, a course of the liposomal form of the drug (AmBisome, NeXstar Pharmaceuticals, Dublin, Ireland) was initiated. The drug was administered intravenously at a starting test dose of 0.5 mg/kg and then later at 1 mg/kg in a 5 % dextrose solution. The dog was premedicated with pentoxiphyllin (Trental, Hoechst Marion Roussel, Midrand, South Africa) at 10 mg/kg and furosemide (Lasix, Hoechst Roussel Vet, Midrand, South Africa) at 4 mg/kg in an attempt to limit nephrotoxicity. Following the infusion of the agent, Ringer's Lactate fluid infusion was continued for 12 h at 100 mℓ/kg/24 h. In total, 11 AmBisome treatments were given to reach a cumulative dose of just under 11 mg/kg. Treatments were given as close together as urea and creatinine levels would allow. Typically it was possible to administer the drug every 2 or 3 days. Evidence of azotaemia was treated with fluid diuresis while monitoring urine production and sediment analysis. The disease was radiographically static at the end of the treatments and the patient was discharged with meloxicam for use as needed to control pain.

A 1-month follow-up examination confirmed static disease in most disc spaces with a slight worsening of radiographic signs in a lumbar disc. Further treatments would have been instituted at this point but the drug was no longer available. Four months after the last treatment the dog returned suffering from severe spinal pain. She was euthanased and a *post mortem* performed.

At necropsy, there was a generalised peripheral, cranial mediastinal and sublumbar lymph node enlargement. The spleen had multifocal, firm, cream-coloured, raised nodules, some demonstrating necrotic centres. The intervertebral discs spaces between T13 and L1, L1 and L2 were eroded with the disc material showing a decreased consistency. Adjacent vertebral bodies showed focal lysis. The overlying spinal cord was markedly congested. The histopathology of the lymph nodes and the spleen showed multiple discrete, variably sized, well-demarcated fungal granulomas. These were characterised by macrophages, lymphocytes and foreign body giant cells. Periodic-Acid-Schiff staining revealed intra-lesional clusters of extracellular and intracellular fungi. Spinal cord segments from L1 and L2, and T13 and L1, showed a status spongiosis and swollen axonal cylinders. The cerebral ventricular region revealed a markedly diffuse accumulation of Gitter cells and scatterings of glial and giant cells. Adjacent white matter showed status spongiosis. Acridine orange fluorescent staining of lymph node and spleen tissue highlighted the fungus. The *post mortem* diagnosis was severe granulomatous multifocal fungal lymphadenitis, splenitis with associated segmental spinal oedema, localised periventricular encephalitis and multifocal discospondylitis.

Fungal cultures of the lung, kidney, a mediastinal lymph node, cerebrum and spleen yielded heavy growths of the fungus *X. bantiana.*

DISCUSSION

The cause of the initial vestibular syndrome seen in this dog is uncertain. It is unclear if this was ever related to the fungal infection as no evidence of vestibular involvement was found on *post mortem* examination. Antibiotic and steroidal anti-inflammatory treatment failed to improve signs. Haematology in this case was consistently unremarkable whereas in other cases of *X. bantiana* infection[16,25] a thrombocytopaenia and either leukopaenia or leukocytosis has been reported. Lymphopaenia has also been reported[15] and may be a predisposing cause, but this was not evident in this case. Clinical chemistry was unremarkable besides the recurring azotaemia, which occurred following amphotericin B administration. Its occurrence was used to assess how frequently treatments could be administered. Because of previous reports of humoral immunodeficiency in German shepherd dogs with systemic and disc space mycotic infections, immunoglobulin concentrations were measured in this patient[4,22]. Immunoglobulin concentrations in 2 dogs with *X. bantiana* infection have previously been reported[15]. In 1 case, IgG, IgM and IgA concentrations were found to be low. In the other only IgM was found to be low on 1 of 2 occasions on which it was measured. Antibody response in this disease in these 2 cases and the case reported here were too varied to draw conclusions about the competency of the humoral immune system.

In the case reported here and in 1 other[18], no underlying immunosuppressive factors could be found. It is, however, unlikely that this disease would occur without some initiating immunocompromising factor. German shepherd dogs have been described with humoral (specifically IgE)[4] and cellular immunodeficiencies predisposing them to a unique deep pyoderma syndrome[3,5]. This breed appears to be over-represented in reports of systemic aspergillosis[2]. In 2 cases of aspergillus discospondylitis in German shepherd dogs, compromised humoral (undetectable IgA concentrations) and reduced lymphocyte blast transformation were reported[2]. Healthy German shepherd dogs have also been found to have low IgA concentrations (although not the case in this dog), which may predispose them to deficient mucosal immunity and hence a predisposition for systemic fungal infections[4]. Immunological competence has been evaluated in German shepherds with German shep-

herd dog deep pyoderma syndrome, and T lymphocyte deficiencies seem to play a distinct role in the pathogenesis of this disease and may influence immune competency of this breed in general[3].

A diagnosis of discospondylitis was made in this case based on survey spinal radiographs. Identifying the specific aetiology in cases of discospondylitis relies on disc material collected surgically or via fluoroscopically-guided fine-needle aspirate of the disc space. The latter method is substantially quicker, and is effective for the collection of sufficient material for culture and cytological examination[2,8]. It is specifically useful in cases where blood and urine cultures are negative. The disadvantage of this method of diagnosis is the need for fluoroscopy that limits its use to institutions with access to this facility.

Evaluation of cerebrospinal fluid has previously been reported as unrewarding[9,21,25]. No significant abnormalities were seen in the CSF of the case reported here. Although the vestibular syndrome was one of the remarkable clinical signs, no evidence of vestibular involvement could be found post-mortally. Despite this, there was clearly central nervous system pathology as evidenced on the histopathology of the periventricular tissue of the brain and the spinal cord overlying the affected disc spaces. It is unclear why this dog never showed more obvious clinical signs of cerebral infection. The organism was also successfully cultured from the cerebrum on tissues collected at *post mortem*. Previous cases of *X. bantiana* infection in the dog have shown pathology more akin to that which has been described in humans, namely CNS abscessation or granuloma formation[11,26]. It is possible that evidence of nervous tissue infection was obscured by the treatment that was applied. Previously reported canine cases were examined postmortally without any exposure to amphotericin B.

There are no reports of successful treatment of this infection in the dog and very few in humans. It would appear that surgical debridement offers the best outcome in humans and that systemic antifungal therapy does not alter outcome[7,11].

Colloidal amphotericin may cause intolerable nephrotoxicity. Recently a liposomal form of this drug has been used which allows for a greatly reduced nephrotoxicity at higher doses[1,12,13,17,19,24]. Liposomal encapsulation of drugs has received some attention recently as it offers several benefits to drug delivery of especially toxic compounds[1]. It allows delivery of drugs to specific body sites, precise control of drug release rates and altered routes of drug elimination[12]. Efficacy against cerebral *Candida* infection in rabbits was found to be both concentration- and time-dependent and hence bioavailability of the active compound at the site of infection is essential[10]. Unless inflammation of the CNS is sufficiently severe to compromise blood–brain barrier function and allow easy access to brain tissue, the ability of an agent to cross this barrier dictates its efficacy at this unique site. The liposomal formulation of amphotericin allows drug penetration of the brain across the blood–brain barrier in humans and rabbits (and thus most probably in dogs as well), making this an ideal agent for the treatment of fungal infections of the CNS[10,14]. The principal site of elimination of liposomes from the blood is the mononuclear phagocytic system. Active drug is only released into circulation after the liposome has been disrupted. Hence the mechanism of action of liposome-encapsulated drugs is related to gradual release of the active drug and the ability of the liposome to preferentially accumulate in diseased tissues (a passive form of targeted drug delivery)[12,13,17]. Liposomes circulate according to the degree of vascularity of an organ or tissue site and hence organs or sites of particularly high vascularity have relatively more encapsulated drug present (e.g. sites of inflammation)[23,24]. The volume of distribution of liposome-encapsulated drugs is usually substantially reduced because the drug is held in the vascular compartment or tissues with high vascular permeability[1]. The reduction of drug concentration in normal tissues thus alters the toxicity profile of liposome-encapsulated drug relative to free drug[1]. Thus, liposomes are capable of increasing the therapeutic index of amphotericin B by decreasing the free drug concentration delivered to the kidney, increasing the drug concentration at the intended site of action and altering the mechanism of drug clearance[1]. Peak plasma concentrations are also reduced and are kept consistent for longer. In addition, fungi show an increased selective uptake of the lipid-based amphotericin formulation because of their ergosterol-containing cell wall that has a higher affinity for the liposome than the cholesterol-containing host cell membranes[23].

The benefits of the liposomal form of the drug in this case were clear. The colloidal form of amphotericin resulted in the rapid development of azotaemia. Although azotaemia still developed with the liposomal form, the degree was far less and it resolved quickly, allowing for many treatments in quick succession. It is the authors contention that had the drug been readily available the dog's life may have been further prolonged, or a cure effected.

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