Use of an amphotericin B lipid complex for treatment of blastomycosis in dogs

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Objective—To evaluate efficacy and nephrotoxicity of amphotericin B lipid complex used for treatment of dogs with naturally developing blastomycosis.

Design—Prospective clinical trial.

Animals—11 dogs with blastomycosis.

Procedure—All dogs were treated with an amphotericin B lipid complex. Two dogs received a cumulative dose of 8 mg/kg of body weight, 1 received a cumulative dose of 10 mg/kg, and 8 received a cumulative dose of 12 mg/kg.

Results—The 2 dogs that received a cumulative dose of 8 mg/kg and 1 of the dogs that received a cumulative dose of 12 mg/kg had a relapse of blastomycosis within 30 days after treatment. Seven of the remaining 8 dogs were clinically free of blastomycosis 6 months after treatment. One dog died of an unrelated cause 5.5 months after treatment, but did not have clinical signs of blastomycosis at the time of death. There were not any adverse clinical effects attributable to drug administration in any of the dogs in this study, and none of the dogs developed clinical signs of renal disease or failure.

Clinical Implications—Amphotericin B lipid complex was a safe and effective treatment for blastomycosis in these dogs. (*J Am Vet Med Assoc* 1996; 209:2073–2075)

Amphotericin B has been the standard treatment for systemic fungal infections in dogs and people.[1,2] Other pharmaceuticals (azoles) have been developed and are used; however, amphotericin B remains the drug of choice in individuals who require rapid amelioration of signs or have progressive disease.[2]

Unfortunately, amphotericin B is nephrotoxic,[1,2] and although several protocols have been developed to reduce the severity of nephrotoxicosis associated with administration of amphotericin B, none has been totally successful. For example, IV treatment with saline solution prior to administration of amphotericin B is the primary means to reduce the risk of amphotericin B-induced nephrotoxicosis[3,4]; however, most dogs will still develop some degree of acute renal failure.

Experimental and clinical studies in human beings[5-7] have shown that liposomal or lipid-complexed preparations of amphotericin B are less toxic than the conventional deoxycholate-solubilized formulation.[a] Amphotericin B lipid complex[b] is a suspension of amphotericin B complexed with the lipids L-α-dimyristoylphosphatidylcholine and L-α-dimyristoylphosphatidylglycerol. In a single-dose, acute toxicology study in rodents, amphotericin B lipid complex was found to be a 10th to a 20th less toxic than amphotericin B.[9] In a repeated-dose study in Beagles,[8] nephrotoxic effects of amphotericin B lipid complex (judged on the basis of serum urea nitrogen and creatinine concentrations and severity of histologic lesions in the kidneys) were an eighth to a 10th less severe than those seen with amphotericin B.[8]

In various studies of experimentally induced fungal infections for which amphotericin B was effective,[9-11] the dose of amphotericin B lipid complex (in terms of amphotericin B content) necessary to produce comparable results was found to be similar to or slightly greater than the dose of amphotericin B. However, because amphotericin B lipid complex is a 10th as toxic as amphotericin B, higher doses of amphotericin B lipid complex can be given safely. In some instances in which mice with experimentally induced blastomycosis were not cured after receiving the maximum tolerated dose of amphotericin B, it was possible to achieve a cure using amphotericin B lipid complex, because higher doses could be given safely.[12]

To our knowledge, amphotericin B lipid complex has not been used previously to treat dogs with naturally occurring fungal infections. The purpose of the study reported here was to evaluate the efficacy and nephrotoxicity of amphotericin B lipid complex in the treatment of dogs with naturally occurring blastomycosis.

Materials and Methods

Dogs—Twelve dogs with naturally occurring blastomycosis were included in the study. Mean age at the time of admission to the study was 3.7 years (range, 10 months to 7 years). Four dogs were sexually intact males, 4 were castrated males, 2 were sexually intact females, and 2 were spayed females.

Treatment regimen—For all dogs, a complete physical examination, thoracic radiography, arterial blood gas analysis, CBC, and serum biochemical analyses (creatinine, urea nitrogen, albumin, calcium, phosphorus, sodium, potassium, chloride, glucose, total bilirubin, and cholesterol concentrations, and alkaline phosphatase, alanine transaminase, and γ-glutamyltransferase activities) were performed before any treatment was given. **Glomerular filtration rate (GFR)** was determined by means of nuclear scintigraphy. The severity of pulmonary changes on thoracic radiographs was evaluated subjectively according to the scale used by Legendre et al[2]

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(0, normal; 1, mild or localized lung disease; 2, moderate disease; 3, moderate-to-severe disease; 4, severe disease).

Dogs were then treated with amphotericin B lipid complex, according to manufacturer's recommendations at a dose of 1 mg/kg of body weight, IV, every Monday, Wednesday, and Friday. The amphotericin B lipid complex was diluted in a 5% dextrose solution to a final concentration of 1 mg/ml immediately before administration. To do this, the vial containing the stock solution of amphotericin B lipid complex was shaken for 30 seconds to provide a homogenous suspension, and the calculated dose was drawn into a syringe using an 18-gauge needle. The needle was removed from the syringe, and the amphotericin B lipid complex was injected through an 18-gauge filter needle[e] into the diluent and gently mixed for 15 seconds. Diluted amphotericin B lipid complex was administered at a rate of 4 mg/kg/h, using an infusion pump. Dogs were continuously monitored for adverse effects during the infusion procedure and for 4 hours afterward. Rectal temperature, pulse rate, respiratory rate, and body weight were measured daily while dogs were receiving amphotericin B lipid complex. Serum urea nitrogen, creatinine, albumin, potassium, calcium, total protein, sodium, and phosphorus concentrations were measured and urinalysis was performed before each dose of amphotericin B lipid complex was administered. Glomerular filtration rate was determined after cumulative doses of 4 mg/kg, 8 mg/kg, and 12 mg/kg had been given. Thoracic radiography was performed after the last dose of amphotericin B lipid complex was given, and a pulmonary disease score was determined. Dogs were reevaluated 1 month and 6 months after initiation of treatment. Evaluation 1 month after treatment included obtaining a complete history and performing a physical examination, serum biochemical analyses, urinalysis, and thoracic radiography if there was an original history of respiratory problems. Evaluation 6 months after treatment included obtaining a complete history and performing a physical examination. Dogs were considered cured if they no longer had clinical signs or lesions consistent with blastomycosis. For all lesions found during the 1-month and 6-month evaluations, aspiration or excision biopsy specimens were examined for evidence of blastomycosis.

Statistical analysis—Student's *t*-test was used to determine whether values obtained prior to treatment were significantly different from values obtained after treatment. One-way ANOVA for repeated measures, followed by Dunnett's multiple comparison method, was used to determine whether serum urea nitrogen and serum creatinine concentrations, as well as GFR, differed over the course of treatment. For all analyses, a value of $P < 0.05$ was considered significant.

Results

One dog died of acute respiratory failure 24 hours after receiving the first dose of amphotericin B lipid complex, and data from this dog were, therefore, not included in the analyses. This dog had developed pneumothorax, presumably secondary to respiratory distress, before the first dose of amphotericin B lipid complex had been administered. The remaining 11 dogs all completed treatment with amphotericin B lipid complex. Two dogs received a cumulative dose of 8 mg/kg, 1 received a cumulative dose of 10 mg/kg, and 8 received a cumulative dose of 12 mg/kg.

Pretreatment evaluation—Prior to treatment, all 11 dogs had radiographic evidence of pulmonary disease; mean ± SD radiographic pulmonary disease score was 2.59 ± 0.86. Seven of the 11 dogs had ocular lesions, 6 had skin lesions, 4 had bone lesions, 2 had lymph node lesions, and 2 had testicular lesions. Blood gas analyses were performed in 9 dogs prior to treatment. Mean ± SD difference between alveolar and arterial partial pressures of oxygen ($\Delta P_{A_{O_2}}$-Pa_{O_2}) was 27.7 ± 14.34 mm of Hg. A CBC was performed in all 11 dogs prior to treatment. Mean WBC, segmented neutrophil, band neutrophil, and monocyte counts were high. Seven dogs had leukocytosis (WBC count > 17,000 cells/μl), 5 dogs had neutrophilia (neutrophil fraction > 77%), 2 had left shift (band neutrophil fraction > 3%), and 2 had monocytosis (monocyte fraction > 10%). Serum biochemical analyses were also performed in all 11 dogs.

Short-term outcome—During the course of treatment, 2 male dogs were neutered because of testicular infection. An enucleation was performed in 3 dogs because of ocular complications of blastomycosis. In 7 of the 11 dogs, pulmonary disease score after the last dose of amphotericin B lipid complex was given was lower than the score before treatment. For all 11 dogs, mean pulmonary disease score after the last dose of amphotericin B lipid complex (mean ± SD, 2.14 ± 0.86) was significantly less than the mean pretreatment score. For the 9 dogs in which blood gas analyses had been performed prior to treatment, mean $\Delta P_{A_{O_2}}$-Pa_{O_2} after the last dose of amphotericin B lipid complex was given (mean ± SD, 12.6 ± 5.16 mm of Hg) was significantly decreased and mean Pa_{CO_2} (mean ± SD, 33.9 ± 3.6 mm of Hg) was significantly increased, compared with pretreatment values. We did not detect significant differences between pre- and posttreatment mean values of pH, Pa_{O_2}, bicarbonate concentration, or base excess for these 9 dogs.

For all 11 dogs, mean WBC count and segmented neutrophil, band neutrophil, and monocyte fractions after the last dose of amphotericin B lipid complex was given were within reference ranges. However, 3 dogs still had leukocytosis, and 3 had monocytosis. Mean posttreatment WBC count and neutrophil and band neutrophil fractions were significantly less than pretreatment values.

Long-term outcome—The 2 dogs that received a cumulative dose of 8 mg/kg had a relapse of blastomycosis within 30 days after the last dose was given. In both dogs, the relapse was apparently a recrudescence of old lesions. One dog that received a cumulative dose of 12 mg/kg had a relapse of blastomycosis within 30 days after the last dose was given. Of the remaining 8 dogs, 5 were clinically normal 1 month after treatment. The other 3 dogs had healing skin and eye lesions, but did not have clinical evidence of active blastomycosis and, therefore, were considered cured.

One of the 8 dogs considered cured 1 month after treatment died of an unrelated problem 5.5 months after treatment. The remaining 7 dogs were clinically normal and free of blastomycosis lesions when examined 6 months after treatment. For these 7 dogs, mean body weight 6 months after treatment (mean ± SD, 32.4 ± 13.4 kg) was significantly greater than mean pretreatment body weight (mean ± SD, 24 ± 9.3 kg).

Adverse effects—There were no adverse clinical effects attributable to drug administration in any of the dogs in this study, and none of the dogs developed clinical signs of renal disease or failure. Glomerular filtration rates were measured in 10 dogs prior to treatment and again after 4 and 8 doses of amphotericin B lipid complex. Mean GFR was within the reference range at all time periods, but mean GFR after 4 doses (mean ± SD, 4.6 ± 1.8 ml/min/kg) and after 8 doses (mean ± SD, 4.1 ± 1.2 ml/min/kg) were significantly decreased, compared with pretreatment mean GFR (mean ± SD, 6.4 ± 1.6 ml/min/kg). Only 1 of the 10 dogs had a GFR less than the lower limit of the reference range. Serum creatinine and urea nitrogen concentrations were measured in dogs prior to treatment and after 8 doses of amphotericin B lipid complex. Mean values remained within reference ranges, but values recorded after 8 doses were significantly increased, compared with pretreatment values.

Discussion

In a previous study,[2] 15 of 23 (65%) dogs with blastomycosis treated with amphotericin B (total cumulative dose, 8 to 9 mg/kg over 2 to 3 weeks) and 11 of 18 (61%) dogs treated with a combination of amphotericin B (cumulative dose, 6 mg/kg) and ketoconazole (10 mg/kg, PO, q 24 h, for 60 days)[2] were cured. All dogs in that study were followed up for at least 1 year after treatment. In the present study, 8 of 11 dogs were considered cured 1 month after treatment, and 7 dogs were still considered cured 6 months after treatment.

The initial 2 dogs in this study received a cumulative dose of 8 mg/kg. When it was observed that these dogs both relapsed within 30 days, the cumulative dose was increased to 12 mg/kg.

Lung disease is a major cause of morbidity and mortality in dogs with blastomycosis.[1] Arterial blood gas partial pressures may be affected by excitement, stress, and fear, as well as by changes in the relationship between ventilation and perfusion. Calculation of the $\Delta P_{A_{O_2}}$-$P_{a_{O_2}}$ is 1 method of adjusting for the effect of alveolar ventilation on $P_{a_{O_2}}$. In these dogs, there was a significant decrease in mean $\Delta P_{A_{O_2}}$-$P_{a_{O_2}}$, compared with the pretreatment values, suggesting that there was an immediate improvement in gas exchange after treatment. Clinically, all dogs were judged to have responded appropriately to the treatment and to be in early remission when treatment was completed.

One dog relapsed 1 month after receiving a cumulative dose of 12 mg/kg. On reexamination at the time of relapse, the accessory lung lobe was found radiographically to be consolidated. In our experience, dogs with similar lesions have responded positively to antifungal treatment coupled with surgical lobectomy. However, the owner of this dog refused further treatment, and the dog was euthanatized.

Glomerular filtration rate was within the reference range for all dogs throughout the study except in 1 dog in which GFR decreased from 4.98 ml/min/kg to 1.62 ml/min/kg after 4 doses of amphotericin B lipid complex. This was the only dog that had received conventional amphotericin B treatment prior to entering this study. The dog's GFR was 1.62 ml/min/kg after 8 doses of amphotericin B lipid complex; therefore, the dog's renal function did not appear to be further compromised by administration of additional doses of amphotericin B lipid complex. Similarly, mean GFR in the 10 dogs tested decreased after 4 doses of amphotericin B lipid complex but did not decrease further after 8 additional doses.

The percentage decrease in GFR was small, compared with decreases reported after treatment with amphotericin B.[3] Administration of amphotericin B at a cumulative dose of 6 mg/kg has been reported to induce acute renal failure in young, clinically healthy dogs.[3] In that study, GFR decreased between 67 and 86%, compared with baseline values. Dogs in the present study received cumulative doses of amphotericin B lipid complex as high as 12 mg/kg, and none developed renal failure. Thus, even though higher cumulative doses of amphotericin B lipid complex are needed to achieve efficacy equal to or greater than that of amphotericin B, our results suggest that these higher doses can be given safely.

[a]Fungizone, ER Squibb & Sons, Princeton, NJ.
[b]Abelcet, The Liposome Co Inc, Princeton, NJ.
[c]Monoject 305 filter needle, Sherwood Medical Industries Inc, St Louis, Mo.

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