Assessment of clotrimazole gels for in vitro stability and in vivo retention in the frontal sinus of dogs

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Objective—To evaluate the stability and retention of viscous formulations of the antifungal drug clotrimazole in vitro and to evaluate retention times, absorption, and histologic response to these compounds when placed in the frontal sinus of dogs.

Animals—6 male Beagles.

Procedures—1% clotrimazole gels were formulated with hydroxypropyl cellulose, poloxamer, and carboxymethylcellulose sodium bases. Commercially available 1% clotrimazole creams were also evaluated. Each compound was incubated at 37°C in a funnel. Volume retained and clotrimazole stability were evaluated for 4 weeks. Six compounds were then chosen for in vivo evaluation. The frontal sinuses of 6 dogs were filled with 1 of the 6 compounds. Computed tomographic evaluation was performed weekly for up to 4 weeks to evaluate gel retention. Blood samples were collected to evaluate clotrimazole absorption. Following euthanasia, sinuses were examined histologically.

Results—Commercially available clotrimazole creams were not retained in funnels in vitro. In vivo, hydroxypropyl cellulose– and carboxymethylcellulose-based gels resulted in the most severe inflammatory response and were retained the longest. Poloxamer-based gels had a shorter retention time and were associated with less inflammation. Clotrimazole was minimally absorbed. Despite a marked inflammatory response to several of the clotrimazole-containing gels, no notable adverse clinical responses were observed.

Conclusions and Clinical Relevance—Poloxamer gels had the most promise for improving drug contact within the frontal sinus of dogs, while limiting the inflammatory response. Poloxamer gels have the additional benefit of improved handling as a result of reverse gelation (ie, they gel when warmed to 37°C).(*Am J Vet Res* 2009:70:640–647)

Nasal aspergillosis is a common condition in dogs that results in massive turbinate destruction, pain, and if untreated, death or euthanasia from lysis of surrounding skeletal structures such as the cribriform plate.[1–4] Oral administration of antifungal drugs is often ineffective (40% to 70% success rate), requires prolonged expensive treatment, and is associated with numerous adverse effects.[1–4] Currently, one of the most effective treatments for nasal aspergillosis in dogs involves a 1-hour intranasal infusion of 1% clotrimazole liquid[a] while the patient is anesthetized.[1–3] Success with this technique has been achieved in two thirds of affected dogs with a single treatment. If nasal discharge has not resolved within 2 weeks, repeat treatment is recommended. In most instances, topical treatment with clotrimazole has been associated with only minor complications.[2] Dogs with fungal granulomas within the frontal sinuses are often refractory to treatment and may require debridement followed by topical application of clotrimazole during the same anesthetic episode. Despite debridement and topical treatment, dogs may require repeat treatments.[1]

The 1% liquid formulation of clotrimazole most commonly used is in a polyethylene glycol base that is not viscous enough to be retained in sinuses. The drug in this formulation flows out of the nasal cavity prior to recovery from anesthesia. Increasing the viscosity of the drug via formulation into a gel could result in retention of the medication at the infected site. This could result in decreased anesthesia time and, most importantly, could prolong drug contact with residual fungus and increase the likelihood of a successful outcome.[5] Clotrimazole is fungicidal at high concentra-

Abbreviations

CBMC	Carboxymethylcellulose
CT	Computed tomographic
HPLC	High-performance liquid chromatography
USP	United States Pharmacopeia

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tions but acts as a fungistatic drug at lower concentrations through the inhibition of membrane synthesis.[6,7] Therefore, long exposure times are theoretically needed for maximum result, which is not always accomplished with current treatment regimes.

Clotrimazole is poorly absorbed across the vaginal mucosa, but its absorption across the nasal mucosa has not been evaluated to our knowledge.[8] It is possible that prolonged clotrimazole retention time within the frontal sinus may result in increased transmucosal absorption of the drug or increased likelihood that the drug is swallowed. It is unclear if prolonged contact time would have detrimental local effects.

The objectives of the study reported here were to evaluate the stability and retention of viscous formulations (gels and creams) of the antifungal drug clotrimazole in vitro and to evaluate retention times, absorption, and histologic response to these compounds when placed in the frontal sinus of dogs. Our hypotheses were that a 1% clotrimazole gel or cream formulated with a variety of vehicles would remain chemically stable and that these compounds would be retained within the frontal sinus of dogs for at least 2 weeks, be nonirritating, be minimally absorbed, and result in no adverse effects.

Materials and Methods

Preparation of gels—Stock 1% clotrimazole gel preparations were prepared by means of standard pharmacy protocols. Specifically, 10-mL batches of 1% clotrimazole gel were formulated in hydroxypropyl cellulose[b] (10%, 20%, and 30%), poloxamer[c] (25%, 30%, and 35%), and CBMC[d] (3%, 4%, and 5%), all in an aqueous base. After being weighed, 100 mg of clotrimazole powder was transferred to the barrel of a sterile 12-mL polypropylene luer lock syringe attached to the luer lock syringe adapter. The plunger was carefully replaced, pushing all powder toward the hub of the syringe. Another sterile 12-mL luer lock syringe containing 2 mL of propylene glycol USP was locked on to the other port of the luer lock syringe adapter. Gentle pressure was applied to introduce the propylene glycol into the syringe containing the clotrimazole. Clotrimazole and propylene glycol were transferred from syringe to syringe until dissolution was visibly achieved. The empty syringe (formerly containing propylene glycol) was removed and replaced by a 12-mL syringe containing 7 mL of each of the respective gels. The gels were transferred into the clotrimazole-containing syringes in a quantity sufficient to make a 10-mL final volume. The remainder of the vehicle gel (if any) was ejected from the syringe and the syringe reattached to the syringe adapter. By use of firm pressure, the contents were transferred from syringe to syringe via the adapter until a total of 50 depressions had been made. Syringes were then capped with sterile tip caps and stored protected from light in brown opaque ziplock plastic bags.

Formulation of hydroxypropyl cellulose gel—One hundred milligrams of clotrimazole powder was solubilized in approximately 0.6 mL of ethoxydiglycol (to achieve visible dissolution) plus a quantity of water sufficient to make a 1-mL total volume. Ten-milliliter batches of 1% clotrimazole gel were formulated by mixing 1 mL of clotrimazole (100 mg/mL in ethoxydiglycol) dissolved with 9 mL of hydroxypropyl cellulose (10%, 20%, and 30%).

Formulation of poloxamer gel—Poloxamer gel vehicles were formulated into 25%, 30%, and 35% concentrations. An appropriate amount of poloxamer powder was weighed on an electronic scale. This powder was placed in the barrel of a 12-mL luer lock syringe and pressed to the end of the syringe by replacing the syringe plunger, as described. As solutions of poloxamer are liquid at cold temperatures and solid at warmer temperatures, care was taken to ensure that components were cold during formulation. Ice-cold sterile water was placed in another 12-mL luer lock syringe and connected to a syringe connector, as described. The ice-cold sterile water was added to the poloxamer powder in a quantity sufficient to make a final volume of 10 mL. Remaining sterile water (if any) was ejected from the syringe and the syringe reattached to the syringe connector. Resulting gel was transferred syringe to syringe via the connector until a total of 50 depressions had been made. Poloxamer gels were then placed in the refrigerator to achieve liquid state until ready for suspending clotrimazole.

Formulation of sodium CBMC gels—Sodium CBMC gels were formulated into 2%, 3%, and 4% concentrations. An appropriate amount of CBMC was weighed on an electronic scale. This powder was placed in the barrel of a 12-mL luer lock syringe and pressed to the end of the syringe by replacing the syringe plunger, as already described. Two milliliters of propylene glycol were placed in another 12-mL luer lock syringe and connected to the syringe containing CBMC powder via a syringe connector. Propylene glycol was transferred into the CBMC-containing syringe and mixed thoroughly via syringe to syringe transfer. The empty syringe was removed from the connector and filled with 10 mL of sterile water. The syringe was then reconnected to the connector and added to the CBMC-containing syringe in a quantity sufficient to make a final volume of 10 mL. Remaining sterile water (if any) was ejected from the syringe and the syringe reattached to the syringe connector. Resulting gel was transferred syringe to syringe via the connector until a total of 50 depressions had been made.

In vitro study methods—Twenty-five milliliters of each gel (n = 9) and commercially available 1% clotrimazole creams (from the United States[e] [n = 1] and Great Britain/Canada[f] [1]) were placed in separate borosilicate glass funnels with a 1-cm-diameter neck and incubated at 37°C to simulate the frontal sinus of dogs. Funnels were covered with plastic to prevent evaporation and suspended vertically on a graduated cylinder. Volume retained was assessed daily for 1 month. One milliliter of each gel and a commercially available 1% clotrimazole cream (United States) was submitted for HPLC evaluation to determine clotrimazole stability at 2 and 4 weeks. The clinical pharmacology laboratory at North Carolina State University used USP standards for HPLC methods.[9] For this assay, we followed the assay provided by the USP-National Formulary 2008[10] and a

pure analytic reference standard purchased from USP.[g] The assay was performed in replicates of 3 for each sample. On the basis of the results of the in vitro portion of the study, 6 compounds were chosen that would have a reasonable chance of being retained for 2 to 4 weeks in the frontal sinus of dogs.

In vivo study methods—Six male Beagles weighing 7.5 to 10 kg (mean, 8.9 kg) were used for this portion of the study. The study protocol was reviewed and approved by the North Carolina State University Institutional Animal Care and Use Committee. Dogs were individually housed in runs and fed ad libitum. A CBC, serum biochemical analysis, and urinalysis were performed for each dog. Each dog was premedicated (acepromazine, 0.025 mg/kg, IV, and hydromorphone, 0.05 mg/kg, IV) and then anesthetized (thiopental, 10 to 15 mg/kg, IV, followed by intubation and maintenance with isoflurane at 1% to 3% and oxygen). A single dose of carprofen (2 mg/kg, SC) was given at induction. The hair over the frontal sinuses was clipped and the site aseptically prepared for surgery. A 2-cm skin incision was made over each lateral frontal sinus followed by trephination of the frontal sinus with a 6-mm intramedullary pin, bilaterally. Each hole was enlarged with rongeurs to form a 1-cm^2 opening so that the entire sinus was visible. Dogs were randomly assigned so that each individual received 2 gel formulations (1/side). Each lateral frontal sinus was filled, to the level of the osteotomy, with 1 of the 6 compounds that were hand injected through a 14-gauge needle attached to a syringe, and the volume injected was recorded. All gels were sufficiently viscous to initially prevent them from entering the nasal cavity. Two simple interrupted buried sutures (3-0 polydioxanone) were placed in the subcuticular layer, followed by 2 simple interrupted cruciate sutures (3-0 nylon) in the skin, bilaterally. Skin incisions were blocked with 0.5% bupivacaine. Computed tomographic evaluation of sinuses (1-mm-thick contiguous slices) was performed immediately after surgery, and each dog was recovered from general anesthesia.

Dogs were examined a minimum of twice daily for 10 days and then once daily thereafter. At each check, food and water consumption was evaluated, as was activity level and overall physical appearance. Incisions were inspected and palpated for evidence of swelling, discharge, or discomfort. Buprenorphine (0.05 mg/kg, IM) was given prophylactically every 8 hours for the first 24 hours after surgery. Skin sutures were removed 10 days after surgery.

Computed tomographic evaluation of sinuses to evaluate drug retention was repeated weekly (under sedation with hydromorphone [0.05 mg/kg, IV] and medetomidine [10 µg/kg, IV]) for up to 4 weeks as long as soft tissue opacity consistent with drug was still present. Blood sample collection to evaluate clotrimazole absorption and perform serum biochemical analysis and CBC determination was done at weeks 2 and 4 or sooner if the gel was no longer present on the basis of CT evaluation. Urinalyses were performed at the same time. Determination of plasma clotrimazole concentration was performed by means of a modified method of HPLC for other azole antifungal agents in our laboratory.[11,12] At the end of 4 weeks, or when the drug was no longer present on the basis of CT evaluation, each dog was euthanatized by IV administration of a combination of phenytoin and pentobarbital[h] (1 mL/5 kg).

The frontal sinuses were removed en bloc and fixed in neutral-buffered 10% formalin. These bony specimens were decalcified in 10% formic acid and sectioned transversely into 4 numbered and nearly equal-sized blocks starting at the sinus ostia (ethmoturbinates) and ending at the caudal recess. Four sinuses from 2 clinically normal Beagles, euthanatized as part of a different study, provided tissue sections for histologic comparison and were processed following the same methods. Blocks were routinely processed and embedded in paraffin, and 5- to 7-µm-thick tissue sections were stained with H&E. The pathologist was blinded to the treatment status for each sinus.

On histologic evaluation, tissue sections were scored for inflammation, tissue injury, and presence of treatment material; a standard severity scheme (0 = absent, 1 = minimal, 2 = mild, 3 = moderate, and 4 = severe) was used. Each variable (ie, inflammation, tissue injury, and presence of treatment material) was scored separately for each of the 4 tissue sections from each sinus. Overall inflammation intensity was scored and included luminal and tissue infiltrates of inflammatory cells. The presence of granulomas and inflammatory cell types was scored including macrophages, giant cells, neutrophils, eosinophils, lymphocytes, and plasma cells. Tissue injury scores included epithelial ulceration, epithelial hyperplasia, active necrosis of epithelium or submucosa, and fibrosis. Reaction of the surrounding bone was scored, which took into account periosteal hyperplasia and proliferative new bone formation. The presence of foreign treatment material (clotrimazole-containing gel) in the submucosa and sinus lumen was included in a single score. Only descriptive statistics were reported as a result of the small sample size.

Results

In vitro study results—On the basis of HPLC results, clotrimazole did not degrade in either the 2- or 4-week samples (no degradation peaks were present). Some shrinkage and solidification of each compound were evident during sample collection by 4 weeks. The mean concentration of clotrimazole at week 4 had increased overall as a result of evaporation of the vehicle. Commercially available 1% clotrimazole cream (United States) contained 2.5% clotrimazole on analysis by HPLC at week 4. In addition, results of HPLC analysis revealed that 10%, 20%, and 30% hydroxypropyl cellulose contained 11%, 23%, and 10% clotrimazole, respectively; 25%, 30%, and 35% poloxamer contained 62%, 32%, and 42% clotrimazole, respectively; and 3%, 4%, and 5% CBMC contained 2.4%, 1.0%, and 1.1% clotrimazole, respectively. The other commercially available 1% clotrimazole cream (Great Britain/Canada) was not analyzed by use of HPLC.

The commercially available 1% clotrimazole creams and the 30% hydroxypropyl cellulose had run out of their respective funnels within 24 hours. The 35% po-

loxamer had solidified by 2 weeks, and the 5% CBMC had a firm rubbery consistency by 4 weeks. These compounds were thus considered unsuitable for in vivo evaluation. Ten milliliters of 3% CBMC had migrated out of the funnel within 24 hours with no change after that. All remaining compounds stayed within their respective funnels for the entire 4-week period. On the basis of these findings, 6 compounds were chosen that were considered as having a reasonable chance of being retained for 2 to 4 weeks in the frontal sinus of dogs. These included gels of hydroxypropyl cellulose (10% and 20%), poloxamer (25% and 30%), and CBMC (3% and 4%).

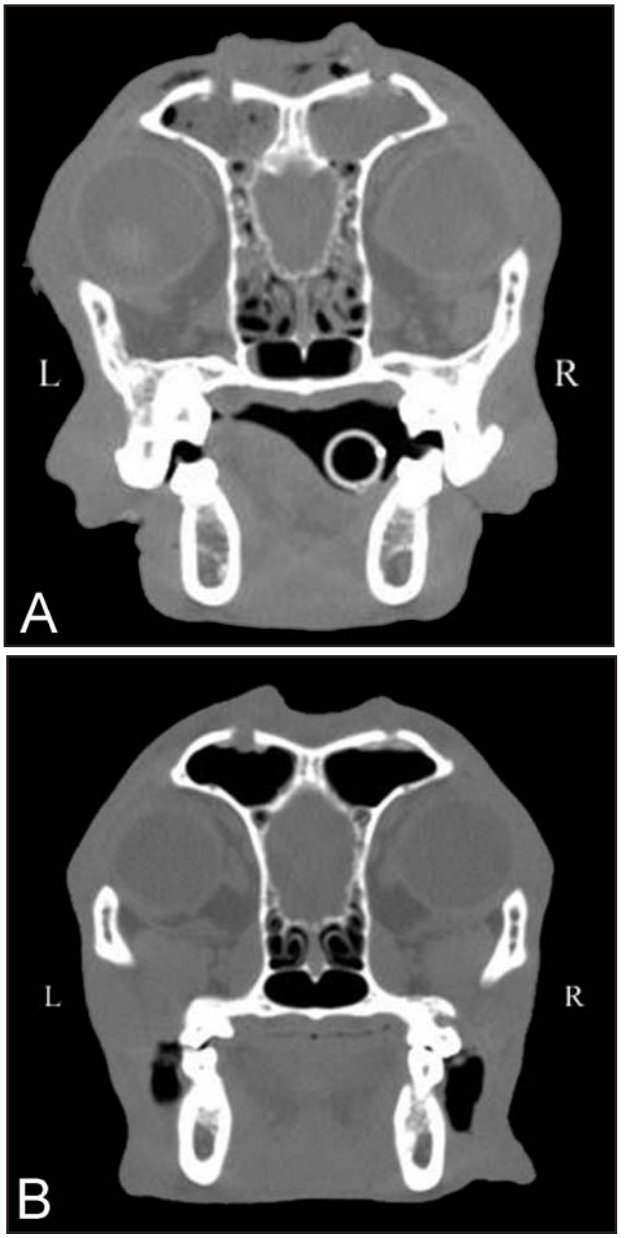

Figure 1—Computed tomographic image of the frontal sinus of a dog. Transverse CT slices were made through the center of the frontal sinuses immediately after surgery (A) and 7 days after surgery (B). Notice that the left (L; 3% CBMC) and right (R; 30% poloxamer) sinuses were completely devoid of their respective compounds on day 7 after surgery.

In vivo study results—The volume of drug placed in sinuses varied from 1 to 3 mL (mean, 1.7 mL). No substantial alterations were found in results of serum biochemical analysis, CBC, or urinalysis for dogs at any period. Adverse effects were minimal. Postoperative analgesics were not needed after 24 hours. All dogs had temporary (3 to 7 days) SC emphysema associated with the sinusotomy and developed minimal clear nasal discharge (the inner surface of the nares was moist), which often lasted throughout the study. All incisions healed without incident. One dog vomited once on the day after surgery, and another had inappetence for 1 day. Both of these dogs were treated with a single dose of an antiemetic (dolasetron, 0.6 mg/kg, IV) and SC administration of fluids and recovered without incident. No other gastrointestinal tract problems were observed. A soft intermittent sneeze was observed in 2 dogs, lasting 1 to 4 days.

CT evaluations—One dog was euthanized on day 7 because of a lack of visible drug within either frontal sinus on CT evaluation (**Figure 1**). All other dogs went on to complete the study. The presence of a soft tissue opacity consistent with retained drug within the frontal sinus was variable with 0% to 100% of the sinus still full after 28 days, depending on the vehicle used (**Table 1**; **Figure 2**).

Drug analysis—Some systemic absorption of clotrimazole occurred in each dog. Mean plasma concentration of clotrimazole was 0.031 µg/mL (median, 0.035 µg/mL; range, 0.013 to 0.044 µg/mL) on day 14 and 0.021 µg/mL (median, 0.020 µg/mL; range, 0.016 to 0.029 µg/mL) on day 28.

Pathologic findings—The most common abnormal finding on gross examination of the frontal sinuses was the presence of a soft to firm tan mass often adjacent to the sinusotomy site. These were present in sinuses

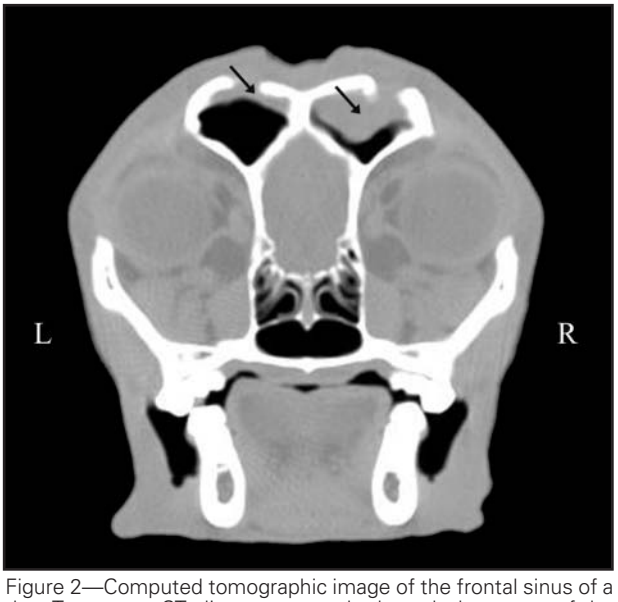

Figure 2—Computed tomographic image of the frontal sinus of a dog. Transverse CT slices were made through the center of the frontal sinuses at 28 days after surgery. Notice the soft tissue opacity consistent with retention of drug dorsally (arrows) in the region of the sinusotomy. L = Left. R = Right.

containing 10% and 20% hydroxypropyl cellulose and 4% CBMC. Histologically, inflammation developed in all sinuses to a variable extent depending on the vehicle and was predominately located in the submucosa. Exudates were generally not observed in the lumen. On histologic evaluation, inflammation scores were higher in the 2 tissue sections of the caudal portion of the sinus and in dorsal areas adjacent to the osteotomy sites. Inflammation scores were lowest in the rostral-most tissue section. Inflammation intensity generally paralleled the location and intensity of retained drug (presumed) material (**Figure 3**).

Overall inflammation scores were highest in response to hydroxypropyl cellulose (10%, mean score of 2.25; 20%, mean score of 2.625) followed by CBMC (3%, mean score of 1.875; 4%, mean score of 2.125); inflammation for both was dominated by macrophage infiltrates that were most intense around visibly retained material. Overall inflammation scores had mean values of 1.0 and 1.5 in response to 25% and 30% poloxamer, respectively, and inflammation was characterized by minimal to mild infiltrates of lymphocytes and plasma cells. Multinucleated giant cells were observed in response to 4% CBMC but not 3% CBMC. Multinucleated giant cells were rarely seen in response to 10% or 20% hydroxypropyl cellulose; multinucleated giant cells were observed in only 1 tissue section in response to 30% poloxamer and were not observed in tissue sections in response to 25% poloxamer. Organized granulomas were not a histologic feature of any treatment. Eosinophils were not consistently associated with a drug treatment and were observed as minimal to mild infiltrates in both sinuses from 1 dog; neutrophils paralleled eosinophils in this dog where neutrophil infiltrates were mild in all sections for both sinuses, except for 1 tissue section with minimal neutrophil infiltrates. Neutrophils were absent or minimal in all other tissue sections of other sinuses regardless of treatment. Only 1 sinus of another dog, which received 20% hydroxypropyl cellulose, had an eosinophil infiltrate, which was considered minimal or mild and observed in only 2 tissue sections. In untreated clinically normal dogs, lymphocytes and plasma cells were minimal in all tissue sections and other inflammatory cell types were not a histologic feature.

On histologic evaluation, retained drug material scored highest for hydroxypropyl cellulose followed by CBMC; both compounds were retained either in the caudal portion of the sinus or around the osteotomy site, ranging from minimal to severe. Minimal drug material was observed in only 1 tissue section with 30% poloxamer and was not seen with 25% poloxamer. Drug material within the lumen was uncommon; drug material was often within the submucosal stroma and in association with inflammatory cells, consistent with previous or concurrent ulceration. Retained drug material was pale gray and amorphous, and it was present extracellularly and in the cytoplasm of macrophages, the latter of which was more prominent for hydroxypropyl cellulose and was associated with prominent cytoplasmic vacuolation.

Fibrosis and reactive bony changes, along the supporting surfaces of adjacent bone, were observed in all treatment groups. In sinuses receiving hydroxypropyl cellulose or CBMC, these changes paralleled the locations of inflammation and retention of drug material. However, both fibrosis and reactive bony changes were of a similar or higher score for both formulations of poloxamer for which histologic retention of drug material was absent, except in 1 tissue section with a score of minimal for 30% poloxamer. Some tissue sections of the caudal portion of the sinus for each drug had moderate or severe fibrosis.

Epithelial ulceration was quite variable, was generally focal or focally extensive, and was only seen in areas of high inflammation, fibrosis, or bony reaction scores, usually near drug material, and not in other areas of sinuses. Ulceration was not a histologic feature of the rostral-most tissue section. Epithelial hyperplasia was absent or focal and minimal in most tissue sections where it was observed. Active necrosis was not a prominent histologic feature and was generally focal to multifocal and minimal if present. Fibrosis, bony reactive changes, epithelial ulceration, epithelial hyperplasia, or active necrosis was not observed in tissue sections of sinuses from untreated clinically normal dogs.

Table 1—Subjective assessment of the percentage of frontal sinuses filled with gel on the basis of evaluation of transverse 1-mm CT slices in 6 dogs.

	Percentage gel retention (location)				
Vehicle	**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
10% Hydroxypropyl cellulose	85	50 (R)	30 (R, D)	25 (D)	20 (D)
	100	15 (L, D)	15 (L, D)	15 (L, D)	15(L,D,M)
20% Hydroxypropyl cellulose	100	60 (C)	30 (C)	25 (C, D)	20 (D)
	100	100	100	100	100
25% Poloxamer	50*	25 (L)	25 (L, D)	25 (L, D)	20 (L, D)
	100	90 (L)	75 (L)	75 (L)	20 (L)
30% Poloxamer	95*	0	NA	NA	NA
	80*	5 (D)	5 (D)	0	0
3% CBMC	95*	0	NA	NA	NA
	100	10 (D)	10 (D)	5 (D)	5 (D)
4% CBMC	100	85 (C)	70 (C, D)	70 (C, D)	60 (C, D)
	100	100	80 (C, D)	80 (C, D)	75 (C, V, D)

*Sinus not completely full dorsally.
R = Rostrally. D = Dorsally. L = Laterally. M = Medially. C = Caudally. NA = Not applicable. V = Ventrally.

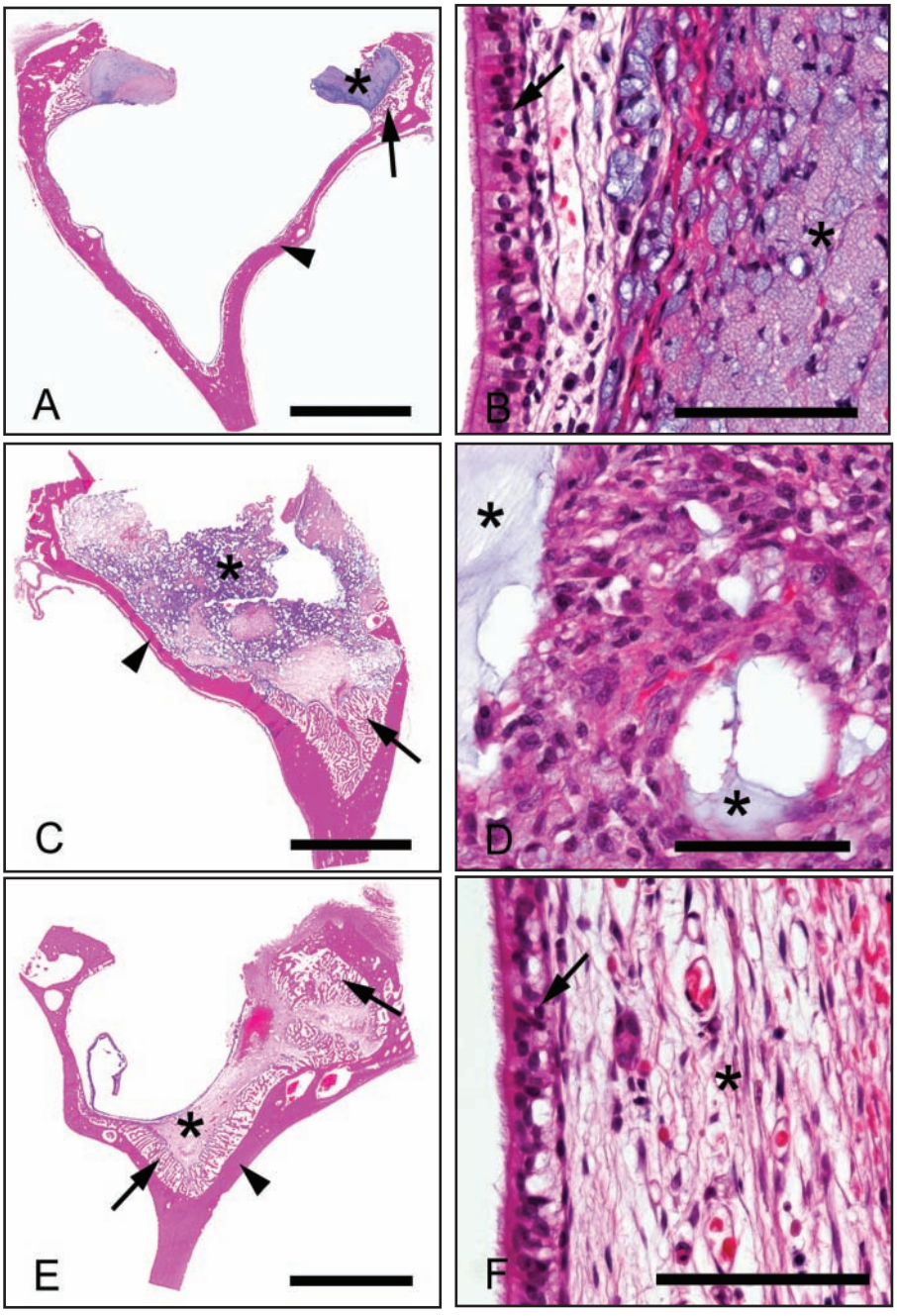

Figure 3—Paired (A and B; C and D; E and F) photomicrographs of mid cross sections of frontal sinuses of dogs obtained at low magnification (1X, A, C, and E) and high magnification (400X, B, D, and F). A—In a dog treated with 10% hydroxypropyl cellulose, notice the histiocytic inflammation (asterisk) near the osteotomy site that is supported by trabecular new bone formation (arrow) arising from the bony sinus wall (arrowhead). B—At higher magnification, hydroxypropyl cellulose is seen as finely vacuolated material in the cytoplasm of numerous macrophages (asterisk) below the respiratory epithelium (arrow). C—In a dog treated with 4% CBMC, notice the severe histiocytic inflammation nearly filling the sinus lumen (asterisk), which is supported by more trabecular new bone formation (arrow) over the bony sinus wall (arrowhead). D—At higher magnification, CBMC is present predominately as large extracellular lakes of material (asterisks) and less prominently as fine vacuolation in macrophages; respiratory epithelium is not seen. E—In a dog treated with 25% poloxamer, notice the fibrosis (asterisk) associated with 25% poloxamer and abundant trabecular new bone (arrows) along the sinus wall (arrowhead), filling a substantial portion of the sinus lumen. F—At higher magnification, fibrosis (asterisk) is seen below the respiratory epithelium (arrow), but inflammation is minimal. H&E; panels A, C, and E, bar = 5.0 mm; panels B, D, and F, bar = 75 µm).

Discussion

There is little transmucosal absorption of clotrimazole when administered intravaginally.[8] It is not known if this holds true for absorption across normal or inflamed nasal mucosa. When delivered into a frontal sinus it is likely that a clotrimazole gel eventually drains into the nasal cavity and that some of it is eventually swallowed. Because clotrimazole is not formulated for systemic use, adverse effects from exposure to high doses administered orally in veterinary patients are rarely reported.[13] Clotrimazole has been used in people for the treatment of a variety of conditions, including the topical treatment of oropharyngeal or esophageal candidiasis.[14–17] The LD_{50} for orally administered clotrimazole in rodents and rabbits ranges from 700 to 2,000 mg/kg, but the toxic dose has not been determined in dogs because such high doses induce emesis.[18] The fact that few dogs in this study had signs of gastrointestinal tract problems, and that most had unilateral or bilateral clear nasal discharge for some time after gel instillation, would suggest that in these dogs, much of the compound was eliminated via the nares or, if swallowed, the amount ingested was not enough to be of clinical concern. The mean total volume of 1% clotrimazole administered to each dog in this study was 3.4 mL (34 mg/dog), which if completely ingested would have resulted in a mean total dose of 3.8 mg/kg. A mean oral dose of 35 mg/kg/d given to healthy human volunteers over 28 days, or 150 mg/kg/d given to dogs for 1 year, resulted in reversible increases in serum liver enzyme activities but few other adverse effects other than emesis.[16,18] Therefore, the potential ingested dose in this study would not be expected to cause clinically important adverse effects, and increases in liver enzymes were not seen. Analysis of plasma samples in this study by use of HPLC indicated that a small amount of the clotrimazole was absorbed either directly or after ingestion. A single small peak associated with the parent compound was detected in plasma samples of each dog near the lower limit of quantification. Other peaks rep-

resenting degradation products were not detected but may have been present and too small to detect. Mean plasma clotrimazole concentration when evaluated at 2 weeks (0.031 μg/mL) and 4 weeks (0.021 μg/mL) was similar to that previously reported for human volunteers (0.03 μg/mL) at 1 to 3 days after vaginal application of a 100-mg tablet (1.4 mg/kg for a volunteer with a body weight of 70 kg).[8,19] When given orally, clotrimazole is rapidly absorbed with peak serum concentrations (approx 30× higher than if the same dose was administered vaginally) occurring 3 hours after administration.[19] It is unclear if the low plasma concentrations measured in the study reported here were the result of transmucosal absorption, prolonged ingestion of small volumes of clotrimazole as it drained from the nasal cavity, or both. It is also not known if larger volumes administered to larger-breed dogs would yield different results. Additionally, it is not known if the presence of fungus with associated turbinate lysis and inflammation would affect the absorption of clotrimazole in clinically affected dogs. Although the volume needed to fill the frontal sinuses of dogs in this study was small, it was proportional to the size of the dog. Given that each dog had 2 different viscosities of clotrimazole gel or cream present (1/side), correlations between drug absorption and viscosity could not be determined.

In the United States, clotrimazole[e] is available as a 1% cream with a complex base composed of sorbitan monostearate, polysorbate 80, and cetyl and benzyl alcohol for vaginal application. Another brand of clotrimazole[f] is available in Great Britain and Canada, which has a base composed of sorbitan monostearate, polysorbate 60, 2-octyl dodecanol, cetostearyl and benzyl alcohols, and cetyl esters wax. The instillation of the 1% clotrimazole cream from Great Britain into the frontal sinus of dogs has recently been reported.[5] In that report, 12 of 14 dogs with nasal aspergillosis responded favorably to sinus lavage with saline (0.9% NaCl) solution and clotrimazole solution followed by the deposition of the cream into the frontal sinuses. The severity of disease was not reported, and retention of the drug was not evaluated. We did not initially intend to evaluate the 1% clotrimazole cream from Great Britain; however, the recent publication by Sissener et al[5] prompted its inclusion into the in vitro portion of the study. In the present study, neither of the commercially available creams (ie, from the United States or Great Britain) was retained in vitro; thus, they were not studied in vivo. The in vitro method used here has several potential drawbacks. The orientation and diameter of the frontal sinus opening (ostium) varies between breeds and with varying degrees of turbinate lysis or obstruction associated with fungal rhinitis and sinusitis. This method was only used as a means of limiting the number of vehicles to be studied in vivo. Additionally, the ideal retention time of the drug is not known. It is possible that short retention times could be efficacious. This likely depends on extent of disease, but further in vivo work evaluating retention times and inflammatory response to these creams is needed. The use of a commercially available cream would be convenient, as the other vehicles studied in this report would require compounding by a pharmacist.

The hydroxypropyl cellulose gel[b] used in this study contains a hydrophilic gel that is used as a vehicle for a variety of drugs used to treat dermatopathies and is without adverse effects, but nasal use has not been previously reported. The hydroxypropyl cellulose gel contains polyacrylamide, paraffin, laureth-7, xanthan gum, and hydroxypropyl cellulose. Given the degree of inflammation associated with this vehicle as used in this study, it cannot be recommended for use in the treatment of nasal aspergillosis in dogs.

Carboxymethylcellulose sodium[d] used in this study is widely used in oral and topical pharmaceutical formulations, primarily for its viscosity-increasing properties.[20] Carboxymethylcellulose is one of the main ingredients of self-adhesive ostomy, wound care, and dermatologic patches, where it is used as a mucoadhesive. This mucoadhesive property is nonirritating and nonreactive and is used in products designed to prevent tissue adhesions after surgery.[21] Carboxymethylcellulose has also been used to localize and modify the release kinetics of active ingredients applied to mucous membranes.[22,23] As used in this study, CBMC resulted in more inflammation than the poloxamer vehicle and was observed histologically at the end of the study. When administered intranasally twice daily to rabbits, it has been shown to result in mild to moderate inflammation with decreased nasal epithelial ciliary beat frequency.[24]

Poloxamer gels are unique polymers (copolymer of polyoxyethylene and polyoxypropylene) in that they undergo reverse gelation with an increase in temperature. The poloxamer gel[c] used in this study is in its liquid phase at colder temperatures, but when warmed to 37°C, the polymer chains form a gel (ie, is an in situ gelling liquid).[25] This characteristic would be of benefit when treating dogs with nasal mycoses in that it would be easy to inject into the frontal sinuses through catheters or small sinusotomy incisions. After contact with the tissues, it would then gel, resulting in prolonged antifungal retention. Poloxamer gels have been previously evaluated as a vehicle for a variety of drugs, including antibiotics, and antifungals in a variety of settings, including ophthalmologic application, topical and parenteral administration, and instillation within the pleural cavity.[26–28] As used in this study, there was no gross and little microscopic evidence of retention of 25% or 30% poloxamer in the frontal sinuses. Computed tomography results also indicated that this vehicle was the most rapidly cleared from the frontal sinuses. Inflammatory scores were lower for poloxamer than the other vehicles. It is unclear if this is because the vehicle incites a less robust inflammatory reaction or if it is simply the result of decreased retention times.

Instillation of a clotrimazole-containing gel into the frontal sinuses of dogs with nasal aspergillosis may be beneficial in that it may reduce the anesthetic time associated with current topical drug protocols and has the potential to increase the efficacy of topical treatment by prolonging drug contact with the fungus.[5] In this study, all compounds resulted in some degree of inflammation in the frontal sinuses. This may be a result of the vehicle, the antifungal, or both. The small sample size in the present study precluded statistical comparisons among treatments. Although the effect of

trephination alone was not evaluated in this study, the presence of retained vehicle surrounded by inflammatory tissue suggests that tissue reaction to medications or vehicles within the frontal sinuses requires further examination. Results of an in vitro study[29] revealed that nasal epithelial ciliary beat frequency was diminished by the topical application of a variety of antifungals, including 10% to 50% clotrimazole. The inflammatory and functional response to 1% clotrimazole placed in the nasal cavity without a vehicle is not known. Interestingly, gels containing poloxamer and clotrimazole have been shown to be less cytotoxic to human cervical epithelial cells in vitro, compared with clotrimazole in a polyethylene glycol base similar to that used to treat nasal aspergillosis in dogs (ie, the polymer had a protective effect).[30] Also, the addition of the polymer to clotrimazole significantly increased the antifungal duration of action in rats with *Candida albicans*–induced vaginitis.[30] Further study is required to determine the most appropriate clotrimazole-containing vehicle, one that will result in minimal local inflammation, while maximizing response to treatment. Results of this study indicate that clotrimazole-containing poloxamer gels should be included in these investigations.

In conclusion, there was minimal absorption of clotrimazole when applied to the frontal sinus of dogs. Of the vehicles evaluated here, poloxamer gels appear to hold the most promise for administration of 1% clotrimazole into the frontal sinus of dogs because it resulted in the least amount of inflammation and because of its unique handling characteristics (reverse gelation). The ideal retention time for treating nasal mycoses in dogs is not known. Given the inflammatory response to all of the vehicles studied, similar evaluation of any topical medications to be used in this manner is warranted.

a. Lotrimin solution, TEVA Pharmaceuticals, Sellersville, Pa.
b. KRISgel 100, Professional Compounding Centers of America, Houston, Tex.
c. Pluronic 127 NF, Professional Compounding Centers of America, Houston, Tex.
d. Carboxymethylcellulose sodium, Spectrum Chemical Manufacturing Corp, Gardena, Calif.
e. Lotrimin cream, Schering-Plough Healthcare Products Inc, Memphis, Tenn.
f. Canesten, Bayer Inc, Toronto, ON, Canada.
g. USP Reference Standard for Clotrimazole, United States Pharmacopeial Convention 2008, Rockville, Md.
h. Beuthanasia-D Special, Schering-Plough Animal Health Corp, Union, NJ.

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