Intranasal antifungal treatment in 51 patients with chronic rhinosinusitis

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Background: Chronic rhinosinusitis (CRS) is the most common chronic disease that is frequently refractory to treatment.
Objective: We sought to establish the safety and demonstrate the clinical efficacy of intranasal antifungal drug therapy in patients with CRS in a pilot trial.
Methods: A prospective open-label trial used amphotericin B as a medical treatment in 51 randomly selected patients with CRS. The antifungal agent was applied intranasally as 20 mL of a 100 μg/mL solution twice daily. The outcome was measured by using their symptoms and by using an endoscopic scoring system in all patients. In addition, pretreatment and posttreatment coronal computed tomographic scans of the nose and sinuses were available for evaluation in 13 patients.
Results: By using amphotericin B, improvement of sinusitis symptoms was observed in 38 (75%) of 51 patients. Endoscopically, 18 (35%) of 51 patients became disease free, and an additional 20 (39%) of 51 had improvement of at least one stage ($P < .001$). No effect was seen in 13 (25%) of 51 patients. The available computed tomographic scans before and after treatment demonstrated a significant reduction in the inflammatory mucosa thickening that had occluded the paranasal sinuses ($P < .0001$ in maxillary sinus).
Conclusion: This open-label pilot trial demonstrates that direct mucoadministration of an antifungal drug appears to be both safe and effective in the treatment of patients with CRS. Therefore controlled and blinded trials are indicated to clarify the novel role of intranasal antifungal drugs in the treatment of CRS. (J Allergy Clin Immunol 2002;110:862-6.)

Key words: *Eosinophils, antifungal, chronic sinusitis, nasal polyps, rhinosinusitis*

Chronic rhinosinusitis (CRS) is an inflammatory disease of the nasal and paranasal sinus mucosa that is present for longer than 3 months and is associated with inflammatory changes ranging from polypoid mucosa thickening to gross nasal polyps.[1,2] Approximately 90% of all cases of rhinosinusitis are CRS; it has become the most common chronic disease, with 37 million cases in the United States.[3] In contrast to acute rhinosinusitis, in which a bacterial cause is well established, the cause of CRS is not well understood. Furthermore, even with aggressive medical and surgical therapies, many patients with CRS continue to have persistent or recurrent disease, leading to frequent courses of antibiotics and multiple surgical interventions.

Abbreviations used
CRS: Chronic rhinosinusitis
CT: Computed tomographic
FDA: US Food and Drug Administration

Because treatment efficacy has never been demonstrated in a controlled clinical trial, the US Food and Drug Administration (FDA) has not approved any drugs or treatments for CRS. Antibiotics are important to treat acute and subacute rhinosinusitis and to treat acute bacterial exacerbations in patients with CRS but do not seem to alter the long-term outcome.[4] Indeed, despite improved antibiotics in recent years, the prevalence of CRS is increasing.[3] Antiallergic medications, such as antihistamines, do not benefit patients with CRS. Glucocorticoids are a rational treatment because they reduce the production of inflammatory mediators and cytokines and inhibit the production, migration, and activation of eosinophils.[5] Unfortunately, long-term systemic glucocorticoid use has many adverse side effects, and its utility is somewhat limited.[6,7]

Histologically, intense eosinophilic infiltration into the nasal and sinus mucosa characterizes CRS.[1,8,9] Eosinophilic inflammation is seen in patients both with and without nasal polyposis, and it seems to be independent of the presence of atopy.[1,8,9] Eosinophil granule proteins, such as eosinophil major basic protein, are known to be toxic to airway epithelial cells and are localized to the area of epithelial damage in CRS[10,11]; therefore eosinophils might play an important role in the pathophysiology of CRS. We have confirmed sinus tissue eosinophilia in the majority (96%) of patients with CRS by means of histologic analysis of 101 consecutive patients.[8] In the same study we also found fungal organisms, as examined on the basis of culture (96% of patients) and histology (81%), in the sinus mucus of patients with CRS,[8] suggesting that these organisms might be involved in the disease process of CRS. However, to our surprise, fungal organisms were also detected in the nasal mucus of the majority of healthy control subjects.[8] Thus sinus airway histology of patients with CRS is characterized by eosinophilia and the presence of fungi; however, the presence of fungi alone does not seem to explain chronic inflammation in patients with CRS.

Recently, we found that PBMCs from patients with CRS, but not those from normal individuals, produce large quantities of IL-5 and IL-13 when they are exposed

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TABLE I. Demographics of the participating patients with CRS (n = 51) receiving intranasal amphotericin B treatment

	Mean	Range	Incidence
Age	49.2 y	11-75 y	—
Sex	—	—	26 F/25 M
Previous sinus surgery	—	—	88% (45/51)
No. of previous sinus surgeries	3.2	0-23	—
Duration of disease	15.6 y	2-52 y	—
Incidence of asthma	—	—	71% (36/51)
Incidence of aspirin sensitivity	—	—	39% (20/51)
Total IgE level	250 KU/L	5-2906 KU/L	—
Incidence of increased total IgE level (>128 KU/L)	—	—	53% (27/51)
Positive skin test response to at least one fungal antigen	—	—	45% (23/51)
Positive RAST test result to at least one fungal antigen	—	—	18% (4/22)
Taking topical and systemic corticosteroids	—	—	37% (19/51)
Taking topical corticosteroids only	—	—	29% (15/51)
Positive fungal culture from mucus	—	—	96% (49/51)

to certain fungal antigens in vitro (S.-H. Shin and H. Kita, manuscript in preparation). Therefore in CRS the fungi in the nasal and sinus mucus might activate the sensitized patients' immune systems and induce production of cytokines, which might drive eosinophilic inflammation in the tissues. Therefore we hypothesized that the reduction of fungus in sinus and nasal cavities by means of antifungal treatment might reduce both the immune and inflammatory responses to the organisms and might clinically benefit patients with CRS. Because of potentially serious side effects of systemic treatment with antifungal drugs, we directly administered the antifungal agents into the nasal and sinus cavities. We now report the results of a pilot open-label trial investigating the safety and potential efficacy of intranasal amphotericin B treatment of CRS and provide the rationale for a larger blinded and placebo-controlled study.

METHODS

Patients

After approval by the Institutional Review Board of the Mayo Clinic, 51 randomly selected patients given a diagnosis of CRS refractory to other therapies agreed to participate in the study. Although 45 of these patients had been previously treated surgically (Table I), none had nasal surgery as part of their current treatment. All of the patients had either primary or recurrent postsurgical CRS. A thorough history, endoscopic examination, and direct coronal computed tomographic (CT) scanning were used to verify the diagnosis of CRS; the diagnostic guidelines and criteria published by the American Academy of Otorhinolaryngology–Head and Neck Surgery were used in the diagnosis.[2]

Briefly, a positive history for CRS was based on both major and minor historical criteria. The major criteria include nasal obstruction or blockage (51/51); nasal discharge, purulence, or discolored postnasal drainage (51/51); facial pain or pressure (30/51); facial congestion or fullness (47/51); and hyposmia or anosmia (30/51). The minor criteria include headache, fever, halitosis, fatigue, dental pain, cough, and ear pain, pressure, or fullness. For diagnosis of CRS, the duration of symptoms was 3 months or longer with either (1) 2 major criteria, (2) 1 major plus 2 minor criteria, or (3) nasal purulence on nasal examination.[2] The endoscopic examination of the nasal cavity confirmed inflamed nasal mucosa, which is compatible with CRS. A 4-grade staging system was used to judge the severity of the disease on endoscopy and ranged from inflamed mucosa thickening to gross nasal polyps (Table II). A CT scan was obtained in all 51 patients before the study, and all of them were found to be consistent with the diagnosis of CRS, showing inflammatory mucosa thickening (>5 mm of thickening in ≥2 sinuses).

TABLE II. Effects of intranasal amphotericin B treatment on endoscopic findings

Endoscopic stage* before treatment	No. of patients: Before	No. of patients: After	Endoscopic stage* after treatment
Stage 3	36	8	Stage 3
		6	Stage 2
		10	Stage 1
		12	Stage 0
Stage 2	10	4	Stage 2
		4	Stage 1
		2	Stage 0
Stage 1	5	1	Stage 1
		4	Stage 0
Total	51	51	

**Stage 0,* No evidence of disease; *stage 1,* polypoid changes seen on the basis of endoscopy only; *stage 2,* polyps in the middle meatus; *stage 3,* polyps filling the nasal cavity.

Study design

Patients were treated for at least 3 months with intranasal administration of an antifungal agent. Amphotericin B was chosen as an antifungal agent because it is available in a water-soluble formulation and because it is poorly absorbed through a mucus membrane, thus minimizing systemic bioavailability. The intravenous preparation of amphotericin B was dissolved in sterile water at 100 μg/mL. A bulb syringe was used for drug delivery to overcome the potential delivery difficulty of intranasal drugs to the nose and paranasal sinuses. As shown in Fig 1, patients applied 20 mL of the antifungal solution in each nostril twice daily with a bulb syringe by gently pointing the tip toward the middle meatus region. Thus each patient used 80 mL/d of a 100 μg/mL solution, and the total daily drug dose was 8 mg. The patients were also instructed to bend their head laterally to the side being irrigated to treat the maxillary sinus.

Three parameters were used to evaluate the efficacy of the treatment. The first outcome measurement was the patient's subjective assessment of symptoms. Patients described whether their symptoms had worsened, were the same, improved, or were completely resolved. The second outcome measurement used the objective changes in endoscopic findings. The same physicians (DAS and EBK) evaluated all patients before and after the treatment to reduce interobserver variability and graded the endoscopic findings using a 4-stage scaling system (Table II). The third outcome measurement was the radiographic quantitation of the coronal CT scan. The pretreatment and posttreatment CT images were scanned into a computer graphics program

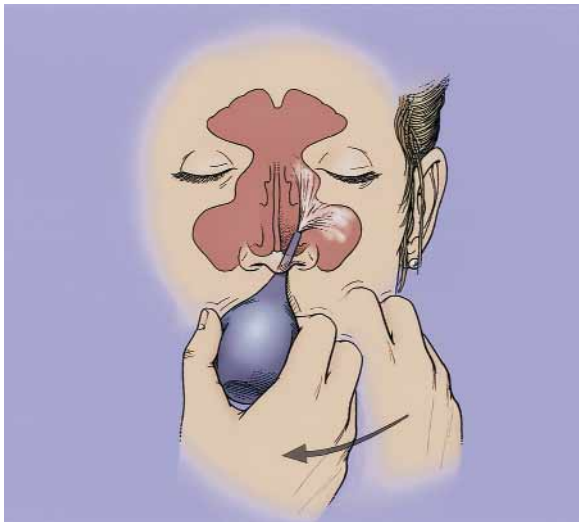

FIG 1. Intranasal application of amphotericin B. Patients apply 20 mL of antifungal solution into each nostril using a bulb syringe. *Arrow* shows hand movement to accomplish successful application of the antifungal drug from medial (ethmoid) to lateral (maxillary) sinuses.

(Adobe Photoshop 5.0; Adobe Systems Inc, Mountain View, Calif). The graphics program converted the area of inflammatory mucosa thickening represented on the CT scan into a number of pixels, and the percentage of sinonasal lumen occluded by the inflammatory thickening was calculated. The comparative CT scan slices were evaluated before and after treatment by using distinct anatomic markers.

Statistical analyses

Statistical analyses were performed by using the χ^2 test and the Mann-Whitney test for endoscopic staging and CT scores, respectively. *P* values of less than .05 were considered significant.

RESULTS

Patient demography

The demographics of the patients are summarized in Table I. Duration of disease was approximately 2 to 52 years (mean, 15.6 years), and 71% and 39% of patients had physician-diagnosed bronchial asthma and aspirin sensitivity, respectively. Forty-five percent of patients had positive skin prick test responses to one or more species of fungi among a panel of 23 fungal species. For economic reasons, only some (22/51 [43%]) of the patients were randomly selected for RAST evaluations. Serum IgE antibodies to at least one species of fungi were found in 18% of patients by means of RAST to 18 fungal species. Before the study, 19 (37%) of 51 patients were using intranasal, as well as systemic, glucocorticoids, and 15 (29%) of 51 patients were using intranasal glucocorticoids alone. Because this was a pilot trial and we were unable to predict the therapeutic effect of the antifungal agent, we believed it would be unethical and potentially harmful to the patients to actively wean them off their current steroid regimen, specifically those receiving systemic glucocorticoids for their coexisting asthma.

Length of study and safety

All the patients were treated and followed for at least 3 months (range, approximately 3-17 months; mean, 11.3 months); 88% (45/51) of patients were treated and followed for more than 6 months. Ten (20%) patients reported a burning sensation on application of the solution, which was probably due to the low osmolarity of the sterile water diluent. The patients reported no other side effects, and no patient withdrew from the study because of side effects. After 3 months of daily intranasal application, amphotericin B was not detected in the sera of the 3 patients tested.

Effects on symptoms

Approximately 75% of patients reported improvement in both nasal obstruction and nasal discharge. No patient reported a worsening of any symptom; 13 (25%) of 51 patients noted no improvement. Another 13 (25%) of 51 patients indicated that their overall symptoms had significantly improved but were not completely resolved. About half of the patients (25/51 [49%]) reported that their symptoms had completely resolved. Patients generally reported improvement in their symptoms 1 month after the initiation of the treatment. Overall, symptomatic improvement was observed in approximately 75% of the patients with CRS. Furthermore, among the 38 patients whose symptoms improved, 16 of them were using systemic glucocorticoids before the study. Among these 16 patients, 8 (50%) of them completely discontinued their systemic glucocorticoid use, and 5 (31%) of them reduced their glucocorticoid dosage.

Effects on endoscopic findings

All 51 patients were evaluated endoscopically by using a staging system before and after treatment. As shown in Table II, the number of patients with stage 3 disease (ie, polyps filling the nasal cavity) decreased from 36 to 8. Thirty-eight (75%) patients improved by at least one stage; among them, 18 patients became endoscopically disease free. On the other hand, no improvement in endoscopic findings was observed in 13 (25%) patients. Overall, improvement in endoscopic findings was highly statistically significant ($P < .001$).

Effects on CT scores

Although all patients had undergone a pretreatment CT examination to establish and confirm the diagnosis of CRS, a CT scan was not routinely performed after treatment because of its cost and because of the unjustified radiation exposure. Thus CT scans from only 13 patients were available for pretreament and posttreatment comparison. Fig 2 shows representative coronal CT scans from a patient before and after intranasal treatment with amphotericin B. Fig 3 summarizes radiographic findings for the 13 patients. Sixty-five percent of the maxillary sinus space was occluded by inflammatory thickening of the mucosa before the treatment, and the occlusion was reduced significantly to 23% after the treatment ($P < .0001$). Furthermore, 12 of 13 patients showed substantial improvement in the maxillary sinus CT findings. In addition, 3 of 13 patients had frontal disease present on CT scans before the treatment. All 3 had substantial improvement after the treatment; however, the results did

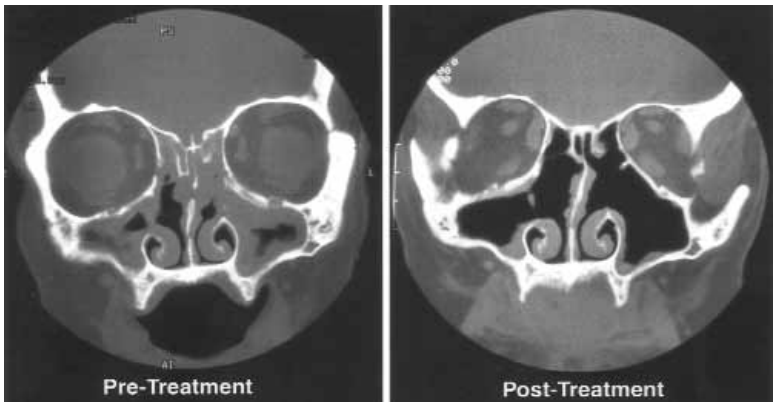

FIG 2. Coronal CT scan of patient with CRS demonstrates inflammatory mucosal thickening partially occluding the nasal cavity and paranasal sinuses before *(left)* and after *(right)* 4 months of intranasal treatment with amphotericin B.

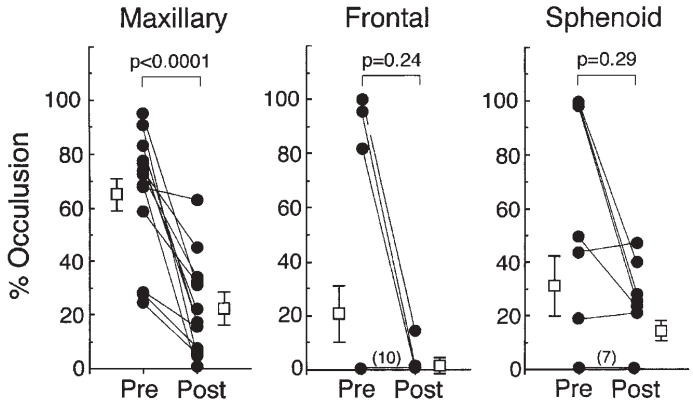

FIG 3. Summary of CT scans before and after intranasal amphotericin B treatment (n = 13). The figures show the percentage of inflammatory mucosal thickening occluding the nasal cavities and paranasal sinuses in patients with CRS before and after amphotericin B treatment. Each dot represents 1 patient, and the results of the same patients are connected by straight lines. Alternatively, the numbers of patients are indicated in parentheses above the straight lines. Open squares and error bars show means ± SEMs from 13 patients (3 patients had frontal and 6 patients had sphenoid disease).

not reach statistical significance because of the small sample size. Similarly, 4 of 6 patients with sphenoid disease experienced substantial improvement, but this failed to demonstrate significance. No worsening of the CT findings was observed in any of the patients.

DISCUSSION

This pilot study suggests that intranasal amphotericin B is a safe and effective treatment in patients with CRS that was refractory to other therapies. This conclusion is based on several observations. First, there were no local or systemic side effects observed, except for a tolerable burning sensation in 20% of the patients, and all patients were able to complete the study. Second, nasal administration of amphotericin B reduced patients' symptoms, decreased the use of oral glucocorticoids, and significantly improved their endoscopic findings and CT scores ($P < .001$ and $P < .0001$, respectively). No benefit was seen in approximately 25% of the patients; this could be explained by one or more of the following factors: poor compliance, heterogeneous mechanisms for CRS, or different sensitivity of the organisms to the amphotericin B. Overall, approximately 75% of the patients with CRS benefited from the treatment. Thus the intranasal antifungal treatment seems to be promising, and a double-blind placebo-controlled trial is highly indicated to formally establish the efficacy of this treatment.

The cause of CRS is unknown, but the observations in this study provide some insight. We previously found fungi in the sinus mucus of essentially all patients with CRS.[8] However, the role for fungi in CRS is unlikely infectious because they are found only in mucus histologically and do not invade the tissues.[8] Furthermore, because IgE antibodies to fungal organisms were detectable in less than 50% of the patients (Table I), a type I hypersensitivity reaction to fungi does not completely explain the disease process. Therefore CRS might be caused by an immunologic response, but not necessarily by the type I hypersensitivity response, to fungi in sinus and nasal cavities of patients with CRS. Indeed, PBMCs from patients with CRS, but not those from normal individuals, produced large quantities of IL-5 and IL-13 when they were exposed to fungal extracts in vitro (S.-H. Shin and H. Kita, manuscript in preparation), suggesting an

immunoreactivity of patients' immune cells to certain fungi. Therefore in this study it is conceivable that intranasal amphotericin B treatment reduced the antigen burden from fungi in patients' sinus and nasal cavities, resulting in reduced inflammatory responses to these organisms. Similarly, in certain patients with late-onset asthma accompanied by the dermatophyte fungus *Trichophyton* species infection, systemic treatment with itraconazole improved patients' symptoms and airway functions.[12] Therefore extramucosal or extracutaneous fungi might play more important roles in the upper and lower airway diseases than one would have appreciated previously.

In the past, systemic administration of antifungal agents has been studied in several patients with allergic fungal sinusitis,[13] which is characterized by sinus mucus eosinophilia and the presence of fungi. However, these studies did not show efficacy. Indeed, systemically administered antifungal agents need to be absorbed, to pass the liver, and to be secreted through the epithelium into the lumen of the sinonasal airways in sufficient concentrations to have an effect on the fungal organisms. Thus the daily dosage of an antifungal drug used systemically tends to be high, and this raises concerns regarding side effects and safety of the treatment. Amphotericin B is associated with especially serious systemic side effects, including renal, liver, and cardiac toxicity. Therefore direct intranasal administration of antifungal drugs to the mucus might have an advantage in that it likely achieves high concentrations in the mucus without systemic side effects. Indeed, in oral candidiasis the suspension of amphotericin B is FDA approved at a concentration of 100 mg/mL, with a daily dosage of 400 mg, resulting in an average serum concentration of 0.05 μg/mL (range, <0.01-0.15 μg/mL). Our study used a daily drug dosage of 8 mg, which is well below the safety levels in other FDA-sanctioned amphotericin B uses.

During this open-label antifungal drug trial, lengthy treatment (4-12 weeks) was required before a therapeutic response was noted. Discontinuation of the daily application of intranasal antifungal drug typically results in disease recurrence, usually within weeks or months. Reinstitution of antifungal therapy usually produced symptomatic and endoscopic improvement.

The potential weakness of this pilot study is that it did not use a placebo group, and therefore the results might need to be interpreted carefully. For example, one must consider whether an irrigative effect of the applied solution could explain the improvement in patient symptoms. Nasal irrigation with hypertonic saline solution seems to reduce sinonasal symptoms.[14,15] However, radiographic findings were not improved in these studies,[15] suggesting that the improvement of CT scores in our pilot study is more than would be expected from the irrigation effects. Also, 34 (67%) of 51 patients were using hypertonic saline irrigation at baseline, and their symptoms improved after starting the antifungal therapy.

We also studied the efficacy of intranasal administration of itraconazole, an antifungal agent with a different mechanism of action than amphotericin B, in 10 patients with CRS. The patients' symptom scores improved by 48% with itraconazole (data not shown), suggesting that the therapeutic effect is not limited to amphotericin B but is shared by other antifungal medications. Finally, in this pilot study we did not analyze the effects of amphotericin B treatment on inflammatory parameters in sinus mucus, such as the levels of eosinophil major basic protein and IL-5. Therefore the efficacy of the antifungal agent could be due to its unknown effects on sinus mucosa or airway inflammation but not due to the reduction in antigenic stimulation.

In conclusion, although there are some limitations, this open-label pilot study strongly suggests that intranasal antifungal therapy is safe and effective for the majority of patients with CRS. Therefore double-blind placebo-controlled trials, combined with the measurement of inflammatory parameters, are needed to establish firmly the efficacy and optimal dosing of the treatment and to provide further evidence for the role of fungal organisms in the cause and pathophysiology of CRS. The success of such studies will have a tremendous effect on the treatment and understanding of this difficult disease.

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