

Treatment of chronic rhinosinusitis with intranasal amphotericin B: A randomized, placebo-controlled, double-blind pilot trial

Jens U. Ponikau, MD,^a David A. Sherris, MD,^b Amy Weaver, MS,^c and Hirohito Kita, MD^d

Rochester, Minn, and Buffalo, NY

Background: Chronic rhinosinusitis (CRS) is one of the most common chronic diseases. Its etiology is unknown, and there is a paucity of effective medical treatments.

Objective: We tested the hypothesis that intranasal antifungal treatment improves the objective computed tomography (CT) findings (inflammatory mucosal thickening), nasal endoscopy stages, and symptoms of CRS.

Methods: A randomized, placebo-controlled, double-blind, single-center trial used amphotericin B to treat 30 randomly selected patients with CRS. Patients were instructed to instill 20 mL amphotericin B (250 µg/mL) or placebo to each nostril twice daily for 6 months. The primary outcome was a quantitative reduction in inflammatory mucosal thickening on CT scans of a standardized coronal cut. Secondary outcome measures were endoscopic scores, patient symptom scores, and levels of intranasal inflammatory mediators.

Results: Twenty-four patients completed the 6 months of treatment. Patients receiving amphotericin B achieved a relative reduction in the percentage of mucosal thickening on CT scans ($n = 10$; -8.8%) compared with placebo ($n = 14$; $+2.5\%$; $P = .030$). Likewise, the changes in the endoscopic scores improved in the amphotericin B group compared with placebo ($P = .038$). Between-group comparisons of the changes in the intranasal mucus levels of eosinophil-derived neurotoxin showed a reduction in the amphotericin B group and an increase in the placebo group ($P = .046$); levels of IL-5 showed similar tendencies ($P = .082$).

Conclusion: Intranasal amphotericin B reduced inflammatory mucosal thickening on both CT scan and nasal endoscopy and decreased the levels of intranasal markers for eosinophilic inflammation in patients with CRS. (*J Allergy Clin Immunol* 2005;115:125-31.)

Key words: Eosinophils, antifungal, chronic sinusitis, nasal polyps, chronic rhinosinusitis, amphotericin B

Chronic rhinosinusitis (CRS) is defined as a disease of the nasal and paranasal sinus mucosa present for >3 months with mucosal changes ranging from inflammatory thickening to gross nasal polyps.^{1,2} The term CRS has replaced the previously used term *chronic sinusitis* because “sinusitis is often preceded by rhinitis and rarely occurs without concurrent nasal airway inflammation.”² CRS is one of the most common chronic diseases, with 29.2 million adult patients (14.2% of the population) in the United States.³ Patients with CRS have long-term nasal congestion, thick mucus production, loss of sense of smell, and intermittent acute exacerbations secondary to bacterial infections; they also have quality of life impairment more severe than patients with congestive heart failure.⁴ The etiology and pathophysiology of CRS are poorly understood; thus, there are no targets for therapeutic intervention. Indeed, because of the lack of efficacy, the US Food and Drug Administration has not approved any drug or treatment for CRS.

There have been no reports to show that even the new and improved antibiotics alter the long-term prognosis in CRS, and the prevalence of CRS is not decreasing despite their frequent use. In contrast, antibiotics might have a role in the short-term treatment of acute bacterial exacerbations in patients with CRS. Antiallergic medications, such as antihistamines, likely do not benefit patients with CRS, except perhaps for patients with comorbid allergic rhinitis. Thus, a role for an IgE-mediated allergy in the pathophysiology of CRS is questionable.

Intense heterogenous eosinophilic inflammation characterizes CRS, is independent of both nasal polyposis and atopy, and is absent in healthy controls and in patients with acute bacterial or viral infections.⁵⁻⁹ A key cytokine for eosinophilic inflammation in CRS is IL-5.^{10,11} An eosinophil granule protein (major basic protein) is toxic to sinus epithelium and colocalizes with the epithelial damage

From ^athe Department of Otorhinolaryngology–Head and Neck Surgery, Mayo Clinic Rochester; ^bthe Department of Otorhinolaryngology, University at Buffalo, State University of New York; ^cthe Division of Biostatistics, Department of Health Sciences Research, Mayo Clinic Rochester; and ^dthe Department of Internal Medicine, Division of Allergic Diseases, Mayo Clinic Rochester.

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Reprint requests: Jens U. Ponikau, MD, Assistant Professor of Otorhinolaryngology, Mayo Clinic Rochester, 200 First Street SW, Rochester, MN 55905. E-mail: ponikau.jens@mayo.edu.

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Abbreviations used

CRS: Chronic rhinosinusitis
CT: Computed tomography
EDN: Eosinophil-derived neurotoxin
SNOT-20: Sinus Nasal Outcome Test

found in CRS.^{8,12} Intact eosinophils migrate through the epithelium into the mucus of the nasal and sinus lumen in patients with CRS, where they degenerate within distinctive clusters.^{9,13} Three recent histologic analyses detected fungi within the eosinophilic mucin and within the eosinophil clusters in mucus in 80% to 100% of surgical patients with CRS.^{9,13,14} Moreover, sensitive new culture techniques confirmed the presence of common airborne fungi in the mucus of the nasal cavities and sinuses in nearly every patient with CRS and healthy control.^{9,13}

Although fungi are ubiquitous in everyone's sinonasal cavities, there may be a unique immune response to them in patients with CRS that induces production of cytokines and drives eosinophilic inflammation in CRS. Thus, reducing the potential antigenic stimulus in the sinus and nasal cavities may attenuate the inflammatory responses to these organisms and clinically benefit patients with CRS. Because of serious side effects from systemic treatment with antifungals and the extramucosal, noninfectious nature of the fungi, we directly administered the antifungal agents into the nasal and sinus cavities. This approach was clinically successful in 2 uncontrolled, open-label trials.^{15,16} Patients with CRS with severe obstructive polyposis who were treated for ≤ 8 weeks did not improve.^{16,17} From our trial, we found that the therapeutic effect with amphotericin B occurred after a prolonged time, usually 10 to 12 weeks.¹⁵ Moreover, the signs and symptoms of CRS tended to recur if the intranasal amphotericin B was discontinued¹⁵; we think that the recurrence is a result of continuous exposure to these common airborne fungi.

We now tested the hypothesis that direct mucoadministration of intranasal antifungal treatment improves the objective (inflammatory mucosal thickening) and subjective (disease stage by endoscopy and symptoms) signs of CRS. In addition, we evaluated whether intranasal amphotericin B reduces the fungal antigen load and inflammatory mediators present in the mucus of patients with CRS.

METHODS

Study population

This double-blind, placebo-controlled, randomized, prospective, single-center trial investigated the effectiveness of intranasal amphotericin B or placebo for 6 months in adult patients with CRS. The Institutional Review Board of the Mayo Clinic approved the study; each patient provided written informed consent. The Food and Drug Administration granted an exemption from filing an investigational new drug application in compliance with the Code of Federal Regulations (CFR 312.2 [b]). Patients presenting to the

TABLE I. Exclusion criteria for patients with CRS

Known hypersensitivity to amphotericin B
Female patients who are pregnant or lactating
Patients younger than 18 years of age
Immunocompromised patients (HIV, posttransplant, diabetes)
Acute respiratory illnesses (within the last 7 days)
Acute complication of CRS (ie, abscess)
Acute bacterial exacerbation of CRS (acute pain, acute pressure, fever, pus on discharge)
Orbital or central nervous system complications of CRS
Antibiotic therapy in the last 7 days
Systemic steroid use in the last 3 months
Systemic antifungal use in the last 7 days

Otorhinolaryngology Department, Mayo Clinic Rochester, from May 2001 to March 2003 were recruited (D.A.S.); as an alternative to being enrolled in the study, patients were offered treatment with intranasal antifungal therapy.

The diagnosis of CRS used the criteria published by the American Academy of Otorhinolaryngology.² In addition to CRS symptoms for >3 months, the patients demonstrated mucosal thickening on coronal CT scans >5 mm in 2 or more sinuses (evaluated by the Department of Radiology, Mayo Clinic Rochester) and on nasal endoscopy (D.A.S.). Because the study required symptomatic patients with CRS, their current medical regimen did not control their signs and symptoms, and patients were recruited regardless of other medications (with exclusions listed in Table I) or previous sinus surgery. Women with childbearing potential used adequate birth control methods.

Intervention

Patients applied 20 mL amphotericin B solution (250 μ g/mL) or placebo to each nostril twice a day by using a bulb syringe.¹⁵ They were instructed to point the tip toward the middle meatus region and to bend their heads laterally to the side being irrigated. The amphotericin B was dissolved in sterile water, resulting in a clear, yellow solution. The sterile water for the placebo was colored with a yellow dye (0.4 mL/L FD&C yellow #5; 1% solution). Sterile water was used as a diluent because the intravenous formulation of amphotericin B forms precipitants when diluted in saline. The osmolality of both the placebo and the amphotericin B solutions was below the detection range of the probe. The pH of the placebo was 7.0 if unopened but depended on how long it was exposed to ambient air (all solutions open to air are in equilibrium with atmospheric CO₂). After air exposure, the pH was measured at 5.7; the pH of the amphotericin B solution was 7.5 to 7.6. No difference in the appearance, taste, or smell could be detected. Both groups continued with their current treatment regimen but were instructed to record any change.

Outcomes

The primary outcome measure was the reduction from baseline in the percentage of inflammatory mucosal thickening, which occluded the nasal and paranasal cavities as measured by CT scan, after 6 months. The pretreatment and posttreatment CT scans were digitized (J.U.P.; Photoshop 6.0; Adobe Systems Inc, San Jose, Calif). To standardize the comparison of the pretreatment and posttreatment scans, we selected the image plane showing the ostium of the maxillary sinus supported by distinctive bony landmarks, which were unique for that plane. The graphics program converted the area of inflammatory mucosal thickening, which is represented with a specific grayscale value on the CT scan, into a number of pixels (Fig 1). Thus, the extent of mucosal thickening before treatment (number of pixels

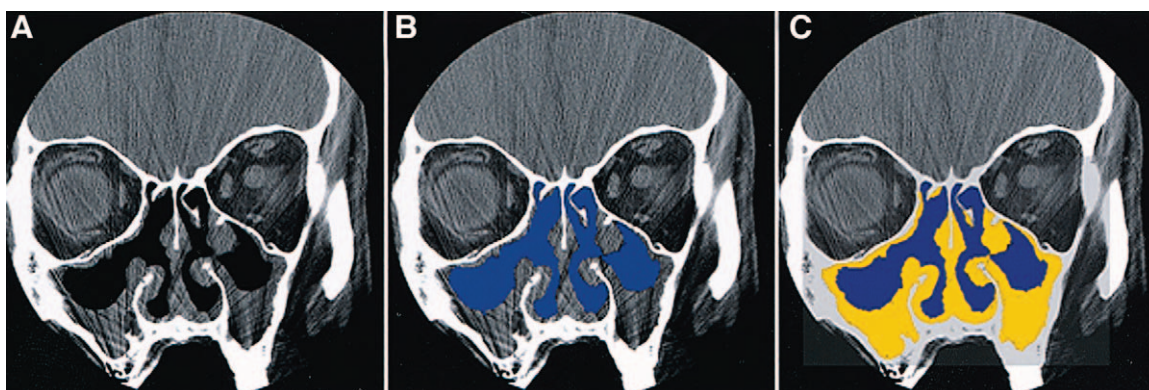


FIG 1. Procedure to obtain objective measurement of inflammatory mucosal thickening from a coronal CT scan. **A**, Standardized cuts through the middle meatus that were digitized for comparison. **B**, Example of the area representing the airspace (blue). **C**, Area representing the inflammatory mucosal thickening (yellow). Once digitized, this area provides an objective quantitation of inflammatory mucosal thickening.

on the pretreatment baseline CT scan) could be compared with the mucosal thickening after treatment (number of pixels on the posttreatment CT scan).¹⁵ A reduction in the percentage of occluded space showed improvement. The reproducibility of this method was independently confirmed by 3 blinded investigators (J.U.P., D.A.S., H.K.). The numbers of pixels returned by the graphics program were less than 1% different after repeated measurements, independent of different observers. Secondary outcomes included the change from baseline of the mucosal thickening by using an endoscopic staging system and the change from baseline by using the Sinus Nasal Outcome Test 20 (SNOT-20) to measure patient symptoms.¹⁸ The levels of inflammatory mediators (IL-5 and eosinophil-derived neurotoxin [EDN]) and intranasal *Alternaria* protein in the nasal mucus, as well as blood eosinophilia, were measured at baseline and 6 months.

To avoid interobserver variability, 1 observer (D.A.S.) performed the endoscopic staging that used the following criteria: no evidence of disease (stage 0), inflammatory mucosal changes confined to the middle meatus superior to the lower edge of the middle turbinate (stage 1), polypoid changes between the lower edge of the middle turbinate and the root of the inferior turbinate (stage 2), polypoid changes between the root of the inferior turbinate and the lower edge of the inferior turbinate (stage 3), and polypoid changes below the lower edge of the inferior turbinate (stage 4). The stages of the 2 sides were added (range, 0-8).

To measure inflammatory mediators and total *Alternaria* protein, nasal secretions were obtained under endoscopic guidance by using a sterile sinus secretion collector (Xomed Surgical Products, Jacksonville, Fla). Each secretion specimen was extracted by adding twice the volume of 0.9% NaCl, vortexing, and centrifugation. The supernatants were stored at -20°C. As a marker of eosinophilic inflammation, EDN was quantitated by radioimmunoassay with a detection limit of 40 ng/mL.¹⁹ The eosinophil-activating cytokine IL-5 was measured by using matched pair antibodies (Pierce-Endogen, Rockford, Ill) with a detection limit of 2.5 pg/mL.²⁰ To monitor changes in fungal antigen burden with treatment, we immunochemically quantitated total *Alternaria* proteins in nasal secretions essentially as described for latex proteins.²¹ Briefly, samples and a standard extract from *Alternaria alternata* (Greer Laboratories, Lenoir, NC) competed with solid-phase *A alternata* proteins for rabbit anti-*Alternaria* IgG antibodies (ALK-Abelló, Round Rock, Tex). Radiolabeled, affinity-purified goat antirabbit IgG was used for detection. The results are expressed as mass protein per milliliter nasal secretion supernatants. The detection limit of the assay was 12 ng/mL for total *Alternaria*

proteins. Sufficient quantities of mucus were not available to perform all of the assays for each patient.

Sample size

The study was designed to enroll 70 patients (35 per group). Because most patients would likely present with a marked occlusion of their nasal passages on CT scan, a small reduction (even less than 10%) of mucosal swelling could double their nasal lumen and yield significant clinical improvement with less obstruction and pressure. In a preliminary, open-label, intranasal antifungal study,¹⁵ the percentage of inflammatory mucosal thickening for the maxillary sinus region showed a mean reduction >30% (SD, 26) after ≥3 months. With $n = 30$ per group, the current trial would have an 80% chance of detecting a 20% difference between the group means (effect size of 0.75) for this endpoint. Assuming a potential 15% noncompliant rate, the sample was increased to 35 per group. This calculation was based on a 2-sample t test with a 2-sided alternative hypothesis assuming equal group variances and a type I error level of 5%.

Randomization

The Division of Biostatistics, Mayo Clinic Rochester (Minn), generated the randomization schedule by using a block randomization scheme (block size of 4). Investigators were unaware of the block size. The pharmacist produced numbered bottles with each patient's study number, containing either amphotericin B or placebo, according to the randomization schedule. Patients were sequentially assigned the next study number and given a 3-month supply of medication. Patients, individuals administering the interventions, and individuals assessing the outcomes were not aware of the treatment assignments.

Statistical analysis

Analyses were based on all patients with available follow-up. As specified a priori in the trial protocol, the outcome measures were each evaluated by using change scores (6 months minus baseline) and were compared between the 2 groups by using a 2-sample t test or an exact Wilcoxon rank-sum test, as appropriate. Although not specified in the protocol, analysis of covariance models were also fit to assess for a treatment effect because this method has been reported to account better for imbalances at baseline because of regression to the mean.²² With this method, the 6-month scores are compared between

TABLE II. Demographics of patients with CRS at enrollment

Characteristic	Placebo (N = 15)	Amphotericin B (N = 15)
Male gender, n (%)	11 (73)	10 (67)
Age, y		
Mean (SD)	49.7 (13.2)	56.9 (16.8)
Median	49	53
Range	33-75	27-85
Asthma, n (%)	9 (60)	9 (60)
Nasal irrigation,* n (%)	4 (27)	7 (47)
Intranasal corticosteroids, n (%)	7 (47)	8 (53)
Antihistamines, n (%)	2 (13)	3 (20)
Decongestants, n (%)	2 (13)	1(7)
Duration of CRS, y		
Mean (SD)	15.2 (10.2)	24.1 (16.2)
Median	14.5	20
Range	2-40	2-57
Previous CRS surgery, n (%)	12 (80)	13 (87)
1-2	6	9
3-4	2	1
5-6	3	1
>6	1	2

*Number of patients using nasal irrigation at the time of enrollment.

Patients who performed the nasal irrigation continued this during the study but were instructed to apply the amphotericin B or placebo after irrigation and not before.

the 2 groups after adjusting for the baseline scores in a regression model. For measurements with a high correlation ($r > 0.8$) between the baseline and 6-month measurements, similar results were obtained for the 2 methods of analysis. However, for measurements in which the treatment effect was not in the same direction for both groups (eg, possible baseline by treatment interaction), the *a priori* statistical method is preferred. Therefore, the reported tests of significance were based on the 2-sample *t* test or Wilcoxon rank-sum test. All calculated *P* values were 2-sided, and *P* values $< .05$ were considered statistically significant. Statistical analyses were performed by using SAS software (SAS Institute, Cary, NC).

RESULTS

Patient information

Table II summarizes the patient demographics at enrollment. The study did not reach the desired enrollment for 2 reasons: slower than expected enrollment and a national shortage of amphotericin B.^{23,24} The slower enrollment was a result of an Institutional Review Board requirement to include intranasal antifungal treatment as an alternative to participation in the study; thus, numerous patients elected to be treated directly outside of this study and not to take the chance of being treated with placebo. The decision to stop enrollment in the study was made before data analyses. Thirty patients with CRS were recruited; although 24 patients completed the trial, 5 patients on amphotericin B and 1 patient on placebo did not. In the amphotericin B group, 2 patients had a strong burning sensation on intranasal application and stopped

after 5 weeks, 2 patients were from other areas of the country and were unwilling to return at the 3-month follow-up, and 1 patient disliked the intranasal administration and stopped after 2 weeks. One placebo group patient stopped because of increased nasal congestion after 9 weeks. On the basis of our previous report,¹⁵ a treatment response is not expected until approximately 10 to 12 weeks. Because all 6 patients discontinued their participation before any expected improvement, an intent-to-treat analysis was not performed. In addition, given the small sample size, it was not feasible to attempt imputation techniques or to summarize the outcome as success or failure. Physicians not involved in the study prescribed the following interventions. Two patients in the placebo group each reported 1 acute exacerbation during the trial and were treated with a course of antibiotics. Two patients, both in the amphotericin B group, required a short course of systemic steroids because of a flare of their asthma.

Inflammatory mucosal thickening by CT and endoscopy

Computed tomography scans were performed at baseline and 6 months. Table III summarizes the percent of inflammatory mucosal thickening for the 24 patients, and Fig 2 shows their relative changes. The amphotericin B group showed a mean decrease of 8.8%, and the placebo group showed a mean increase of 2.5% ($P = .030$; 11.3% between-group comparison; 95% CI, 1.2-21.4).

Table III shows that after 3 and 6 months, 70% of the patients receiving amphotericin B had improved endoscopy scores (change < 0). Overall, the amphotericin B group showed significantly improved median endoscopy scores versus no change in the placebo group (3 months, $P = .047$; 6 months, $P = .038$).

Alternaria proteins, eosinophil-associated mediators, and eosinophilia

In addition, Table III shows the results from the mucus and blood specimens; the analyses were performed on the basis of the availability of paired samples. All nasal mucus supernatants contained *Alternaria* proteins, indicating that fungi were present. However, the changes in *Alternaria* concentrations at 6 months were not statistically different between groups. EDN concentrations in mucus, as a marker of eosinophilic inflammation, decreased significantly (median change, $-15.7 \mu\text{g/mL}$) in the amphotericin B group compared with the placebo group ($+6.3 \mu\text{g/mL}$; $P = .046$). Likewise, IL-5 in mucus tended to decrease in the amphotericin B group (median change, -47 pg/mL) compared with the placebo group ($+4.8 \text{ pg/mL}$; $P = .082$). The peripheral blood eosinophil counts showed a tendency to be lower in the amphotericin B group compared with placebo, but did not show significance ($P = .16$).

Subjective symptom scores

After 6 months, 90% (9/10) of the amphotericin B group improved their SNOT-20 scores (change < 0)

TABLE III. Effect of amphotericin B treatment on inflammatory mucosal thickening, *Alternaria* protein, inflammatory mediators, eosinophilia, and SNOT-20 scores

	Placebo (N = 14)	Amphotericin B (N = 10)	P value
Percent of airspace occluded by inflammatory mucosal thickening on CT scan*			
Baseline			.49†
Mean (SD)	65.3 (24.0)	57.8 (28.3)	
Range	10.1, 91.8	9.9, 87.3	
6 mo			.11†
Mean (SD)	67.8 (23.4)	49.0 (31.5)	
Range	0.8, 97.4	1.5, 91.0	
Change (6 mo-baseline)‡			.030†
Mean (SD)	2.5 (10.3)	−8.8 (13.6)	
Range	−13.5, 21.8	−31.7, 5.5	
Endoscopy score§			.33 ¶
Baseline			
Median	4.0	2.8	
Range	2, 6	1, 8	
Score ≤4, n (%)	9 (64)	8 (80)	
Change (3 mo-baseline)‡			.047 ¶
Median	0	−1.0	
Range	−2, 0	−2, 0	
Patients improved (change <0), n (%)	4 (29)	7 (70)	
Change (6 mo-baseline)‡			.038 ¶
Median	0	−1.3	
Range	−2, 2	−2, 0	
Patients improved (change <0), n (%)	5 (36)	7 (70)	
<i>Alternaria</i> , µg/mL			
Baseline			1.00 ¶
N¶	7	9	
Median	6.77	6.56	
Range	3.91, 10.06	1.33, 25.81	
Change (6 mo-baseline)			.73 ¶
N¶	7	6	
Median	−0.30	−0.30	
Range	−1.91, 4.81	−4.64, 3.21	
EDN, µg/mL			
Baseline			.24 ¶
N¶	9	8	
Median	14.45	28.29	
Range	1.82, 49.94	3.13, 121.76	
Change (6 mo-baseline)			.046 ¶
N¶	8	5	
Median	6.35	−15.72	
Range	−13.94, 29.38	−26.38, −2.08	
IL-5, pg/mL			
Baseline			.60 ¶
N¶	9	9	
Median	28.5	54.0	
Range	0.0, 168.5	0.0, 301.0	
Change (6 mo-baseline)			.082 ¶
N¶	8	6	
Median	4.8	−47.0	
Range	−24.0, 226.5	−161.5, 5.5	

TABLE III. Continued

	Placebo (N = 14)	Amphotericin B (N = 10)	P value
Eosinophil count (×10 ⁹ /L)			
Baseline			
N¶	14	8	.29 ¶
Median	0.3	0.5	
Range	0.1, 0.7	0.1, 1.0	
Change (6 mo-baseline)			.16 ¶
N¶	13	7	
Median	0.0	−0.2	
Range	−0.2, 0.4	−1.0, 0.3	
SNOT-20 score#			.50 ¶
Baseline			
Median	1.8	2.3	
Range	0.3, 4.5	0.7, 2.8	
Change (3 mo-baseline)‡**			.65 ¶
Median	−0.08	−0.4	
Range	−1.5, 0.7	−1.4, 0.5	
Patients improved (change <0), n (%)	8 (57%)	5 (56%)	
Change (6 mo-baseline)‡			.72 ¶
Median	−0.3	−0.3	
Range	−1.8, 0.8	−1.3, 0.3	
Patients improved (change <0), n (%)	9 (64%)	9 (90%)	

*Percentage of mucosal thickening was determined from CT scans that were digitized and objectively measured.

†Analysis on the basis of a 2-sample *t* test assuming equal group variances.

‡A negative change indicates improvement.

§The endoscopy score is the sum of the stages (0-4) of each side made by 1 observer (D.A.S.).

¶Analysis on the basis of an exact Wilcoxon rank-sum test.

¶Because of limited quantities of mucus, paired specimens were not available from all patients.

#Each patient subjectively evaluates 20 symptoms; each is rated from 0 = “no problem” to 5 = “problem as bad as it can be” at beginning, 3 months, and 6 months.

**One patient receiving amphotericin B did not complete the SNOT-20 scores at 3 months.

compared with 64% (9/14) of the placebo group, but overall there were no significant differences (Table III).

Adverse events

Headache, trouble sleeping, nasal congestion, fatigue, postnasal drip, cough, and nasal inflammation are CRS symptoms and were not judged as adverse events. Two patients in the amphotericin B group had nasal burning on application and discontinued. None of the remaining 28 patients with CRS reported any major or minor adverse events or side effects.

DISCUSSION

This randomized, placebo-controlled, double-blind trial in patients with CRS found that direct mucoadministration of intranasal amphotericin B reduced both the inflammatory mucosal thickening by CT scan and the disease stage

CT Scan Analysis

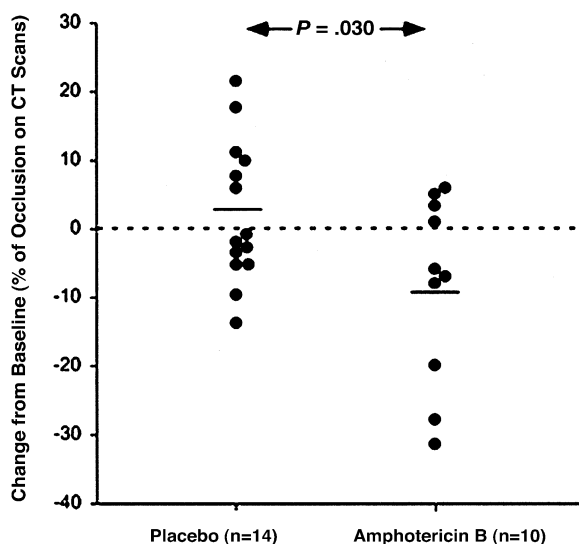


FIG 2. Changes from baseline in the percentage of inflammatory mucosal thickening. As measured by CT scan, the relative changes in thickening that occlude the nasal and paranasal cavities are shown after 6 months of treatment (primary endpoint). Each dot represents an individual patient. A negative change from baseline indicates a decrease in mucosal thickening. Horizontal lines represent the means for each group.

by endoscopy. In addition, amphotericin B reduced an intranasal marker of eosinophilic inflammation, EDN, compared with placebo. Interestingly, daily intranasal instillation with the placebo solution (colored sterile water, 20 mL each nostril, twice daily) tended to worsen the CT scans, did not improve the endoscopic staging, and increased the intranasal inflammation (ie, increased levels of EDN). Thus, we did not observe a therapeutic benefit in the mechanical cleansing effect from instillation.

The SNOT-20 did not detect any significant overall difference in symptoms with treatment. Because the SNOT-20 was developed in patients with a variety of sinonasal symptoms, including those with acute rhinosinusitis,¹⁸ its validity and specificity for CRS are questionable. Indeed, it did not correlate with objective findings such as CT scans or nasal endoscopy in this and other studies.²⁵ This information was unavailable when our trial began.

Exposure to light and room temperature reduces the antifungal potency in reconstituted amphotericin B in a time-dependent manner.^{26,27} Therefore, we anticipated that efficacy might be reduced in this trial, in which patients received a 3-month supply at enrollment and at their 3-month follow-up visit, compared with our original open-label trial, in which patients' amphotericin B solutions were fresh every 2 weeks.¹⁵ Unfortunately, it would have been prohibitively expensive to provide new solutions every 2 weeks (overnight shipping on dry ice). To compensate for the anticipated loss in potency, we started with a higher concentration of amphotericin B (250 µg/mL). Overall, we still prefer the 100 µg/mL ampho-

tericin B, which is kept refrigerated and is freshly made every 2 to 4 weeks.¹⁵

In an earlier report, *Alternaria* (more than other fungal species) stimulated PBMCs from patients with CRS to produce high levels of IL-13 and IL-5; this immune response was strikingly absent in healthy controls.²⁸ As judged by their physiologic activities²⁹ and their presence in tissue from patients with CRS,^{11,30} these cytokines likely promote the eosinophilic inflammation in CRS. Thus, an empiric treatment with antifungal drugs, which are directly administered to the sinonasal mucus, could reduce the antigenic stimulus arising from these non-invasive fungal organisms and indirectly reduce the eosinophilic reaction directed towards the fungi.

Detectable *Alternaria* protein levels in all mucus specimens from patients with CRS confirmed the presence of fungi; however, the intranasal fungal burden (estimated only by total *Alternaria* protein) was not reduced after treatment. The levels of *Alternaria* protein at 6 months could be artificially high for 2 reasons: patients without mucus at the end of the study could not be analyzed, and the mucus collection was not timed relative to the nasal instillation. In addition, uncontrolled environmental exposure to *Alternaria* may affect the results. Because ubiquitous colonizing fungi do not cause inflammation in healthy individuals, patients with CRS may have a distinct immune response to these organisms that differs from an IgE-mediated allergy. Moreover, although these organisms are likely to be present continuously, the amphotericin B may attenuate the antigen production needed to instigate inflammation.

Indeed, intranasal amphotericin B significantly reduced a marker of eosinophilic inflammation (EDN) in the treatment group compared with placebo. Interestingly, the peripheral blood eosinophil count and the intranasal IL-5 levels tended to be reduced in the amphotericin B group compared with the placebo group, but in this small trial, these trends did not show significance. Certainly, when used as an infusion, amphotericin B can have an immunomodulatory effect on lymphocytes and macrophages.³¹ Because intranasal amphotericin B is only minimally or not absorbed through mucus membranes,^{32,33} the decreased inflammation is probably not a direct anti-inflammatory effect of amphotericin B on IL-5 producing lymphocytes in the mucosa.

This pilot trial tested the hypothesis that objective signs of CRS could be positively altered after 6 months of treatment with an intranasal antifungal. However, the clinical relevance of such objective outcome measures needs to be further validated. In obstructive diseases, such as CRS, topical delivery of any drug has limitations in the sinonasal anatomy; future studies need to address mucosal disease in frontal, posterior ethmoid, and sphenoid sinuses. Our results suggest that extramucosal fungi, which do not invade the tissue and therefore are not infectious, might play an important role in the pathophysiology of CRS by triggering the eosinophilic inflammation in susceptible individuals. Larger, multicenter trials with amphotericin B and other antifungal drugs with different

mechanisms of action are now warranted to examine further the potential of intranasal antifungal therapy in patients with CRS.

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REFERENCES

- Kaliner MA, Osguthorpe JD, Fireman P, Anon J, Georgitis J, Davis ML, et al. Sinusitis: bench to bedside: current findings, future directions. *Otolaryngol Head Neck Surg* 1997;116:S1-20.
- Benninger MS, Ferguson BJ, Hadley JA, Hamilos DL, Jacobs M, Kennedy DW, et al. Adult chronic rhinosinusitis: definitions, diagnosis, epidemiology, and pathophysiology. *Otolaryngol Head Neck Surg* 2003;129:S1-32.
- Lethbridge-Cejku M, Schiller JS, Bernadel L. Summary health statistics for U.S. adults: National Health Interview Survey, 2002. National Center for Health Statistics. *Vital Health Stat* 2004;10:23.
- Gliklich RE, Metson R. The health impact of chronic sinusitis in patients seeking otolaryngologic care. *Otolaryngol Head Neck Surg* 1995;113:104-9.
- Hamilos DL, Leung DY, Wood R, Meyers A, Stephens JK, Barkans J, et al. Chronic hyperplastic sinusitis: association of tissue eosinophilia with mRNA expression of granulocyte-macrophage colony-stimulating factor and interleukin-3. *J Allergy Clin Immunol* 1993;92:39-48.
- Hamilos DL. Chronic sinusitis. *J Allergy Clin Immunol* 2000;106:213-27.
- Ponikau JU, Sherris DA, Kephart GM, Kern EB, Gaffey TA, Tarara JE, et al. Features of airway remodeling and eosinophilic inflammation in chronic rhinosinusitis: is the histopathology similar to asthma? *J Allergy Clin Immunol* 2003;112:877-82.
- Harlin SL, Ansel DG, Lane J, Myers J, Kephart TM, Gleich GJ. A clinical and pathologic study of chronic sinusitis: the role of eosinophils. *J Allergy Clin Immunol* 1988;81:867-75.
- Ponikau JU, Sherris DA, Kern EB, Homburger HA, Frigas E, Gaffey TA, et al. The diagnosis and incidence of allergic fungal sinusitis. *Mayo Clin Proc* 1999;74:877-84.
- Lopez AF, Sanderson CJ, Gamble JR, Campbell HD, Young IG, Vadas MA. Recombinant human interleukin 5 is a selective activator of human eosinophil function. *J Exp Med* 1988;167:219-24.
- Hamilos DL, Leung DY, Huston DP, Kamil A, Wood R, Hamid Q. GM-CSF, IL-5 and RANTES immunoreactivity and mRNA expression in chronic hyperplastic sinusitis with nasal polyposis (NP). *Clin Exp Allergy* 1998;28:1145-52.
- Hisamatsu K, Ganbo T, Nakazawa T, Murakami Y, Gleich GJ, Makiyama K, et al. Cytotoxicity of human eosinophil granule major basic protein to human nasal sinus mucosa in vitro. *J Allergy Clin Immunol* 1990;86:52-63.
- Braun H, Buzina W, Freudenschuss K, Beham A, Stammberger H. "Eosinophilic fungal rhinosinusitis": a common disorder in Europe? *Laryngoscope* 2003;113:264-9.
- Taylor MJ, Ponikau JU, Sherris DA, Kern EB, Gaffey TA, Kephart G, et al. Detection of fungal organisms in eosinophilic mucin using a fluorescein-labeled chitin-specific binding protein. *Otolaryngol Head Neck Surg* 2002;127:377-83.
- Ponikau JU, Sherris DA, Kita H, Kern EB. Intranasal antifungal treatment in 51 patients with chronic rhinosinusitis. *J Allergy Clin Immunol* 2002;110:862-6.
- Ricchetti A, Landis BN, Maffioli A, Giger R, Zeng C, Lacroix JS. Effect of anti-fungal nasal lavage with amphotericin B on nasal polyposis. *J Laryngol Otol* 2002;116:261-3.
- Weschta M, Rimek D, Formanek M, Polzehl D, Podbielski A, Riechelmann H. Topical antifungal treatment of chronic rhinosinusitis with nasal polyps: a randomized, double-blind clinical trial. *J Allergy Clin Immunol* 2004;113:1122-8.
- Piccirillo JF, Merritt MG Jr, Richards ML. Psychometric and clinimetric validity of the 20-item Sino-Nasal Outcome Test (SNOT-20). *Otolaryngol Head Neck Surg* 2002;126:41-7.
- Abu-Ghazaleh RI, Dunnette SL, Loegering DA, Checkel JL, Kita H, Thomas LL, et al. Eosinophil granule proteins in peripheral blood granulocytes. *J Leukocyte Biol* 1992;52:611-8.
- Kita H, Jorgensen RK, Reed CE, Dunnette SL, Swanson MC, Bartemes KR, et al. Mechanism of topical glucocorticoid treatment of hay fever: IL-5 and eosinophil activation during natural allergen exposure are suppressed, but IL-4, IL-6, and IgE antibody production are unaffected. *J Allergy Clin Immunol* 2000;106:521-9.
- Swanson MC, Bubak ME, Hunt LW, Yunginger JW, Warner MA, Reed CE. Quantification of occupational latex aeroallergens in a medical center. *J Allergy Clin Immunol* 1994;94:445-51.
- Vickers AJ, Altman DG. Statistic notes: analysing controlled trials with baseline and follow up measurements. *BMJ* 2001;323:1123-4.
- Drug shortages. U.S. Food and Drug Administration, Center for Drug Evaluation and Research; 2003. Available at: <http://www.fda.gov/cder/drug/shortages/default.htm>. Accessed June 12, 2003.
- Companies announce shortage of anti-fungal drug. *Drug Industry Daily* 2003;2(115):5.
- Kenny TJ, Duncavage J, Bracikowski J, Yildirim A, Murray JJ, Tanner SB. Prospective analysis of sinus symptoms and correlation with paranasal computed tomography scan. *Otolaryngol Head Neck Surg* 2001;125:40-3.
- Gallelli JF. Assay and stability of amphotericin B in aqueous solutions. *Drug Intell Clin Pharm* 1967;1:102.
- Cheung SC, Medoff G, Schlessinger D, Kobayashi GS. Stability of amphotericin B in fungal culture media. *Antimicrob Agents Chemother* 1975;8:426-8.
- Shin S-H, Ponikau JU, Sherris DA, Congdon D, Frigas E, Homburger HA, et al. Chronic rhinosinusitis: an enhanced immune response to ubiquitous airborne fungi. *J Allergy Clin Immunol* 2004;114:1369-75.
- Kita H, Adolphson CR, Gleich GJ. Biology of eosinophils. In: Adkinson NF Jr, Bochner BS, Yunginger JW, Holgate ST, Busse WW, Simons FE, editors. *Middleton's allergy principles & practice*. 6th ed. Philadelphia: Mosby; 2003. p. 305-32.
- Simon HU, Yousefi S, Schranz C, Schapowal A, Bachert C, Blaser K. Direct demonstration of delayed eosinophil apoptosis as a mechanism causing tissue eosinophilia. *J Immunol* 1997;158:3902-8.
- Lowery MM, Greenberger PA. Amphotericin-induced stridor: a review of stridor, amphotericin preparations, and their immunoregulatory effects. *Ann Allergy Asthma Immunol* 2003;91:460-6.
- Bennett JE. Antimicrobial agents: antifungal agents. In: Hardman JG, Limbird LE, editors. *Goodman & Gilman's the pharmacological basis of therapeutics*. 9th ed. New York: McGraw-Hill; 1996. p. 1175-90.
- Product information: Fungizone(R). Princeton, NJ: ER Squibb & Sons Inc; 1994. p. 1.