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Influence of Amphotericin B on the Ciliary Beat Frequency of Nasal Mucosa

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Objective: **A new therapeutic approach in eosinophilic fungal rhinosinusitis is topical application of antifungals such as amphotericin B. The tolerability of a recently applied concentration of this drug by human nasal mucosa was tested in vitro.** ***Methods:*** **Ciliary beat frequency (CBF) was measured by a photometric technique, combining a light microscope, a photometer, a photograph-multiplier, and a computed analyzing unit.** ***Results:*** **Treatment for 20 minutes with amphotericin B finally diluted in saline to 0.1 mg/mL caused no decrease in CBF. By contrast, both amphotericin B diluted in distilled water and distilled water without additives lowered CBF about 50%. This decrease was not reversible after reincubation for 20 minute in saline.** ***Conclusions:*** **The results confirm the good tolerability of topical amphotericin B in the nasal and paranasal cavity. Because of the negative effect of distilled water, final dilution in physiologic solvents should be considered.** ***Key Words:*** **Amphotericin B, ciliary beat frequency, rhinosinusitis.**

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INTRODUCTION

Allergic fungal rhinosinusitis has recently been shown to be associated not with a type I hypersensitivity but with eosinophilic attack of fungal antigens in nasal mucus in most cases.[1,2] Therefore, it has been proposed to change the terminology to *eosinophilic fungal rhinosinusitis*.[2] Based on the hypothesis that fungal antigens within the mucus might induce the production of cytokines and activate eosinophilic granulocytes to migrate, antifungal treatment has been taken into consideration to reduce the antigenic load and therefore the inflammatory reaction.

In two open studies, intranasal irrigation with amphotericin B at a concentration of 0.1 mg/mL appeared to reduce symptoms.[2,3] 20 mL of the aqueous solution were applied in each nostril twice daily. In the first study, treatment for 1 month in 74 subjects in addition to saline nasal lavage and corticosteroid spray cured 62% of the patients with stage I of nasal polyposis, 44% with stage II, and 0% with stage III.[3] Tolerability was excellent without side effects. Nasal polyposis disappeared in 39% of all patients. The best results were gained in subjects who had previously undergone endoscopic polypectomy and endoscopic sinus surgery, probably due to better penetration of the drug and improved application.[3]

In the second study, 51 patients were treated in the same way for 3 to 17 months (average, 11.3 mo).[2] On application of the solution, 20% noted a burning sensation, which was the only side effect reported. Nasal obstruction and discharge were improved in 75% of subjects (stages I-III), and in 49%, the symptoms were completely resolved at the end of the therapy.[4]

Two-thirds of the study population were using systemic or intranasal glucocorticoids, and 13 of 16 patients discontinued their systemic corticoid use or reduced the dosage.[4] Endoscopy demonstrated improvement by at least one stage in 75%, and computed tomography scans available from some patients disclosed reduction of occlusion of the paranasal sinuses.[4] Although corticoids were used in parallel in the majority of the subjects and definite conclusions cannot be drawn to date, these results are encouraging for the concept of antifungal therapy.

Possible toxicity of topically applied drugs to the epithelium has to be considered, particularly during long-term treatment. In a previous study, nasal respiratory cells harvested from the inferior turbinate were perfused for hours with cell culture medium containing the investigated drugs.[5] The applied concentrations of amphotericin B caused little reduction in the ciliary beat frequency of the cells after a few hours (0.125 and 0.25 mg/mL) and significant reduction after 1 hour (0.5 mg/mL) of perfusion. As a conclusion, careful selection of the concentration was recommended for clinical use.

The aim of this study was to compare the tolerability of 0.1 mg/mL amphotericin B in distilled water and in 0.9% saline by human nasal mucosa samples.

MATERIALS AND METHODS

Chemicals

Amphotericin B (50 mg amphotericin B dry substance plus 41 mg sodium desoxy cholate, Bristol-Myers Squibb, Vienna,

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Austria, charge A090/2003) was dissolved with 10 mL distilled water to 5 mg/mL. Aliquots of this stock solution were 50-fold diluted in 0.9% sodium chloride or in distilled water to a final concentration of 0.1 mg/mL containing 0.108 mmol/L amphotericin B (MW 924.11) and 0.198 mmol/L sodium desoxycholate (MW 414.57).

Ciliated Epithelium Samples and Course of Toxicity Testing

Epithelium mucosa samples of approximately 10 mm^2 were excised after conchotomy from removed tissue and put into 0.9% saline. Each sample was from a different patient. All tests were done at a constant temperature of 22°C. After 20 minutes in saline, CBF was determined. Subsequently, saline was replaced by 0.1 mg/mL amphotericin B in saline or distilled water for 20 minutes. CBF was measured again, and the samples were rinsed with saline. After further 20 minutes in saline, CBF was measured for the last time. In control experiments, the samples were exposed to distilled water.

Similar to a previous report,[6] the biopsy samples taken for measurement of CBF were used for evaluation within 3 hours to exclude any damage of the cells by storage. According to our experiences, storage of the bioptic material for days would damage the cells, so that it was not possible to perform repeated exposure of amphotericin B over time in vitro.

Determination of the Ciliary Beat Frequency

We used the same detection method as in a recent study,[7] which was in accordance with a previous description.[6] Briefly, the deflection of light caused by the ciliary beats was measured photometrically with a Laborvert FS light microscope and a MPV combi-photometer (Leitz, Jena, Germany). The magnification was 500, and the diameter of the photosensitive field on the sample was 5 μm. The signal was photograph-multiplied, digitalized at a sample frequency of 400 Hz, and transformed into a time-amplitude signal. A Fast Fourier transformation analysis was performed every 1.6 seconds. For each sample, CBF values were detected at five different sites. Results are presented as the mean values of these single measurements. Student paired or unpaired *t* test was used to test for statistical significance, and *P* values of less than .05 were considered significant.

RESULTS

Solutions of amphotericin B in physiologic saline did not induce a reduction in CBF after 20 minutes on average ($P = .13$, Table I). The minimal decrease in some samples did not exceed that of pure saline (mean reduction of 2.4%), presented in our recent publication.[7]

By contrast, solutions of amphotericin B (0.1 mg/mL) in distilled water caused a marked, statistically highly significant reduction in the CBF after 20 minutes (Table I). This effect occurred in each sample and was reversible only to a small extent after 20 minutes re-exposure to saline ($P = .006$ for reversibility). A similar, even more pronounced, irreversible effect could be observed using distilled water without additives (Table I).

DISCUSSION

Amphotericin B diluted in saline did not cause any effect on the ciliary function in the present study. This finding is in accordance with the results of a previous investigation using a perfusion model, in which 2.5 times higher concentrations diluted in cell medium had only little influence after several hours, and only the fivefold concentration decreased CBF rapidly.[5] In both clinical trials on topical application in cases of rhinosinusitis, 0.1 mg/mL amphotericin B was nontoxic,[3,4] which is also supported by our results. Since repeated dosing over time in vitro reveals unreliable results, at least in our system, further investigation should be considered for the CBF of samples removed from patients who have been treated for months with amphotericin B to definitely exclude long-term toxicity on the epithelium.

When aqueous solution was instilled, 20% of the patients noted burning sensation.[4] This was related to the low osmolarity, an explanation confirmed by our observation that the CBF was significantly impaired by aqueous amphotericin B. Since water without additives had the same effect in vitro, no toxicity can be connected with the antifungal agent.

TABLE I.
Ciliary Beat Frequency Response to Amphotericin B in Saline and Distilled Water.

	Single values												% Reduction from Start Value*		
Sample no	1	2	3	4	5	6	7	8	9	10	Mean	SD	Mean %	Min %	Max %
NaCl	5.7	6.2	6.3	5.7	6.2	6.0	6.4	6.1	6.4	5.7	6.07	0.28			
A-saline†	5.8	6.1	6.3	5.6	6.3	5.9	6.3	6.2	6.3	5.5	6.03	0.31	0.7	0.0	3.5
NaCl	5.7	6.0	6.2	5.6	6.2	5.9	6.4	6.2	6.4	5.5	6.01	0.32	1.0	0.0	3.5
NaCl	6.2	5.9	5.5	6.0	6.0	5.9	6.2	6.7	5.9	6.7	6.1	0.37			
A-water†	3.0	2.8	3.5	4.1	4.9	3.6	4.1	3.9	3.1	2.9	3.59	0.67	41.1‡	18.3	56.7
NaCl	3.5	2.8	4.4	4.8	5.2	3.9	4.0	4.2	3.4	3.1	3.93	0.75	35.6†	13.3	53.7
NaCl	6.7	6.0	6.2	5.9	6.4	5.5	6.4	6.3	6.4	6.3	6.21	0.33			
Water†	3.1	3.3	3.8	3.1	3.8	2.8	3.1	4.1	3.5	4.1	3.47	0.46	44.1‡	34.9	53.7
NaCl	3.2	3.4	4.1	3.3	3.7	3.3	3.2	4.0	3.7	3.9	3.58	0.34	42.4‡	33.9	52.2

*All mucous membrane samples were treated for 20 min with saline (start value), followed by 20 min test solution (italic), and by 20 min saline. Values are CBFs (Hz) at the end of each of these periods.

†*A-water* = amphotericin B in distilled water. *A-saline* = amphotericin B in saline. *Water* = distilled water without additives.

‡$P < .01$ versus start value.

CONCLUSION

Concentrations of amphotericin B of about 0.1 mg/mL can be considered safe, as confirmed in vitro and in vivo. Solvents with approximately physiologic osmolarity should be preferred to distilled water.

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