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Nasal lavage with sodium hypochlorite solution in *Staphylococcus aureus* persistent rhinosinusitis*

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SUMMARY

Objective: *To determine a selected concentration of sodium hypochlorite (NaOCl) in saline solution for nasal lavage and evaluate its clinical efficiency in the treatment of symptomatic patients with persistent, Staphylococcus aureus (SA) associated rhinosinusitis (RS).*

Material and Methods: *In vitro tests for cilia and epithelial cell viability were done on reconstituted primary epithelial cells in vitro. Cells were exposed for 5 and 15 minutes twice daily for 5 consecutive days to one of the following conditions, 1) saline, 2) 0.5% NaOCl in saline, and 3) 0.05% NaOCl in saline. In order to evaluate tolerance, immunostaining was done for ezrin and F-actin network and observed with confocal microscopy. The patients (n = 20) were all persistent SA symptomatic carriers, with unique patient-specific SA clonotypes, and multiple infection recurrence despite effective systemic antibiotic therapy. Each patient applied first saline alone for 3 months followed by saline + 0.05 % NaOCl solution, as nasal lavage twice daily on both nostrils for 3 months. Symptom intensity and endoscopic findings were recorded with visual analogue scale (VAS). Nasal airway resistance (NAR) and nasal Nitric Oxide (NO) levels were measured before and after the saline lavage regimen, and after the saline + NaOCl treatment.*

Results: *F-actin network loss and decreased expression of ezrin were significant in cells exposed to 0.5%, but not in those exposed to 0.05% NaOCl. These changes were more obvious when exposed for 15 min. than 5 min. daily. The nasal lavage with 0.05% NaOCl in saline was well tolerated and a significant improvement in nasal obstruction (p = 0.001), posterior nasal discharge (p = 0.018), olfaction (p = 0.007) and headache (p = 0.009) was demonstrated. Significant improvement was also recorded in nasal endoscopic grading of oedema (p = 0.001), erythema (p = 0.001), purulent discharge (p = 0.002), nasal crusts (p = 0.001), and NAR (p = 0.05) as measured by rhinomanometry. There was no significant improvement in nasal NO production or subjective anterior nasal discharge. Bacteriological cultures of middle meatus secretions collected one month after the end of the treatment revealed the persistence of SA.*

Conclusion: *Nasal lavage with 0.05% NaOCl solution in saline is suitable for long-term use and seems to be a good alternative to lavage with saline alone in the management of symptomatic RS associated with recurrent SA infections due to patient-specific SA clonotypes.*

Key words: sodium hypochlorite, nasal lavage, Staphylococcus aureus, chronic rhinosinusitis

INTRODUCTION

Chronic rhinosinusitis (CRS) is one of the most common health care problems with major economic implications. The etiology of this disease is most likely multifactorial. The role of bacteria in the pathogenesis of CRS is still a matter of debate. Recently, it was suggested that the inflammation of the nasal and paranasal sinus mucosa could be related to an immunological response toward enterotoxins released by *Staphylococcus aureus* (SA) colonizing the nose [(1)]. *SA* is one of the most common pathogenic aerobic bacteria cultured from patients with CRS [(2)]. This bacterium was shown to colonize the nasal vestibule in up to 40% of the population [(3)]. The binding properties of *SA* to mucosal epithelial cells or mucosal nasal secretions may explain the chronic carriage. Recently, the long-term persistence of *SA* was shown to be associated with its intracellular persistence. Intracellular *SA* reservoir was found in the

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nasal middle meatus mucosa biopsies of patients with CRS [4]. The presence of *SA* in the nasal mucosa epithelial cells was proposed to be a risk factor for recurrent episodes of *SA*. Persistent *SA* symptomatic carriers with unique patient-specific *SA* colonotype appear to be refractory to antimicrobial and surgical therapy [5]. Thus, symptoms of recurrent RS episodes in patients yielding intracellular *SA* reservoirs are difficult and problematic. The intracellular location combined with the lack of efficient bactericidal mechanisms in non-professional phagocytes are assumed to protect intracellular bacteria from professional phagocytes and from antimicrobial agents whose action is mainly extracellular [6]. This represents a future challenge for rhinologists, pathologists and infectious disease specialists because a novel and alternative therapy for *SA* associated CRS is clearly becoming more necessary.

The conventional medical therapy of CRS includes antibacterial treatment. However, no placebo-controlled studies on the effect of antibiotic treatment are available. On the other hand, topical corticosteroids and saline nasal lavage have gained good beneficial evidence in the treatment of CRS [7]. When CRS is resistant to traditional medical therapy, endoscopic sinus surgery (ESS) can be proposed. This is a reasonably successful treatment [7]. However, a certain percentage of patients (5% to 20%) do not improve or have recurrences of severe symptoms, even in the hands of very experienced surgeons. Many studies attribute this persistence of symptoms to multiple factors including local and/or systemic immunological factors as well as environmental circumstances such as pollution, dust, pollens and cigarette smoke [8]. No data are yet available regarding the treatment of *SA* persistent symptomatic carriers.

Sodium hypochlorite (NaOCl) is a well-known bleaching and disinfecting agent that has been found to be effective against several organisms including *S. aureu*s and *P. aeruginosa* [9-17]. Its antiseptic property has been observed by Koch as early as the 1880s and Dakin had used various NaOCl solutions for treating wounds during World War I [12,16,18]. The exact mechanism of microbial killing of NaOCl has never been determined. We know that NaOCl dissociates in water to Na^+ and OCl-/HOCl (hypochloric acid). The active chlorine is a strong oxidizing agent. Substantial evidence suggests that chlorine exerts its antibacterial effect by the irreversible oxidation of -SH groups of essential enzymes, disrupting the metabolic functions of the bacterial cell. Chlorine may also combine with cytoplasmic components to form N-chloro compounds that are toxic complexes for the micro-organisms [12,18,19].

Selection of the appropriate concentration of NaOCl is based on a balance between antimicrobial action and tissue toxicity. The choice of NaOCl concentration traditionally ranges from 0.5% to 5.25% and sometimes as high as up to 10% [15]. In addition increasing the time of exposure to NaOCl decreases cell viability. If the irrigation time is increased, 0.5% NaOCl has nearly the same bactericidal effect as 5.25% NaOCl when being used as an endodontic irrigant [20]. It has also been demonstrated that 0.5% NaOCl was inappropriate for long term wound maintenance. After 2 weeks of continuous treatment of burn wound lesions with NaOCl gauze soaks, a marked toxicity was noticed in the epidermal cells of the treated areas. The basal cell isolates from treated areas were inhibited in their growth and tissue culture. Conversely, 0.1% NaOCl was found to be of low toxicity for long-term maintenance [12].

Thus, before applying NaOCl in vivo, we first evaluated its toxicity on reconstituted nasal epithelium, from primary nasal epithelial cells cultured in vitro, to 2 different concentrations and exposure periods to NaOCl. The evaluation of NaOCl potential cytotoxicity was assessed by immunohistochemistry for F-actin and ezrin.

F-actin based cytoskeleton stabilizes the cell shape and plays important roles in the distribution of membrane proteins and in the regulation of transmembrane transport pathways [21,22]. Alteration in the expression of F-actin may have a profound effect on tight junction (TJ) structure and barrier function of epithelial cells [23]. A central role in organizing and regulating specialized apical membrane proteins is played by polarized Ezrin, Radixin and Moesin (ERM) that are derived from a large dormant cytoplasmic pool [24]. Thus, tracking the expression of Ezrin can help to indicate the polarity and differentiation of ciliated nasal epithelial cells. Tight junctions, characteristically located at the apico-lateral borders of epithelial cells, selectively regulate the passage of water, ions, neutral molecules, and inflammatory cells through the paracellular pathway [25]. Since our recent descriptions of the lack of efficient conventional antibiotic treatment for persistent *SA* intracellular symptomatic carriers with unique patient-specific *SA* clonotype [5], we have decided to study, prospectively, the effects of nasal lavage with NaOCl solution.

MATERIAL AND METHODS

In Vitro Study

Biopsies of nasal mucosa were obtained from symptomatic intracellular *SA* carriers undergoing endoscopic sinus surgery according to the guidelines of the Ethical Committee for Clinical Studies of the Geneva University Hospital and informed consent was obtained from all subjects.

Culture of primary epithelial cells from biopsies of the middle turbinate mucosa was obtained according to the protocol described by Karp et al. [26]. In summary, epithelial cells are dispersed from the biopsies and plated at a density of $5x10^5$ cells/cm^2 onto 0.6 cm^2 collagen-coated filters (Millipore, Molsheim, France). Cells are cultured in Dulbecco's modified Eagle's medium (DMEM)-nutrient mixture F-12 (F-12) (Invitrogen, Basel, Switzerland), supplemented with 2% Ultroser G (Biosepra, Ciphergen Biosystems, Cergy-St. Christophe, France), 100 U/ml penicillin and 100 mg/ml streptomycin. After one day, filters are taken at the air-liquid interface for the next 2-3 weeks.

Experimental treatment

The apical surface of reconstituted epithelia, were exposed for 5 or 15 min. to one of the following conditions twice daily for 5 consecutive days: 1) untreated, 2) 0.05% NaOCl in saline 0.9% solution, and 3) 0.5% NaOCl in saline 0.9% solution. The experiments were stopped by washing of the epithelia in DMEM-F12 and metabolically active cells were evaluated using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetra-zolium bromide (MTT) (thiazolyl blue) assay (Sigma).

Immunostaining

The reconstituted epithelia were fixed in 4% paraformaldehyde, permeabilized in 0.1% saponin and incubated for 1 hour to overnight with the rabbit polyclonal ezrin antibodies from Upstake (New York), diluted in (1:800) phosphate-buffer saline-1% BSA. After washing, the tissues were incubated again for 30 min. with an appropriate secondary antibody in presence of Texas-red phalloidin from Molecular Probes (Leiden, The Netherlands), in order to label the F-actin network and delineate the cells. Filters were cut off from the culture inserts, mounted in Vectorshield-DAPI (VECTOR) between glass cover slips, and observed with an LSM 510 confocal microscope (Zeiss).

In Vivo Study

Preparation of NaOCl solution

As a source of NaOCl solution, Amuchina Med® (ACRAF SpA, Genoa, Italy) containing 0.5% NaOCl was used. The 0.5% NaOCl was used pure and diluted with normal saline (0.9% NaCl) to get the desired concentration of 0.05% NaOCl using the following formula: (0.05 x 100) / 0.55 = 9.1ml of 0.5% NaOCl in each 100 ml of solution. The 0.05% NaOCl was put into a Sinus Rinse® bottle (NeilMed Products, Santa Rosa, CA, USA). Each bottle can hold up to 240 ml of the solution. Each patient was taught how to prepare the solution and the method of administration at home. They were given a 20 ml syringe to fill up with Amuchina Med (0.5% NaOCl) and add to the sinus rinse bottle. The bottle was then filled up with normal saline (0.9% NaCl) up to the 240 ml mark. After gently shaking the bottle, they were advised to apply the rinse to each of their nasal cavities two to three times, twice daily for three months. Each patient did first nasal lavage with saline only for 3 months, followed by 0.05% NaOCl in saline nasal lavage twice daily for 3 months. A fresh solution was prepared before each application. Throughout the study, each patient was given, in addition, topically administered nasal corticosteroid (mometasone furoate monohydrate 400 mcg / day) to be applied twice daily 5 min. after the lavage and nasal blowing. None of the patients received systemic antibiotics throughout the period of the study.

Patient selection criteria

Twenty adult patients known as persistent *SA* symptomatic carriers with unique patient-specific *SA* clonotypes [5] with recurrent RS despite classical treatment including saline lavage, topical corticosteroids and several systemic antibiotics were eligible for participating in the study. CRS has been defined according to The European position paper on Rhinosinusitis and Nasal Polyps [7]. They were recruited from the outpatient clinic of the Rhinology-Olfactology Unit of the Service of Otorhinolaryngology of the University Hospital of Geneva, Switzerland. Patients were excluded from the study in case of the presence of any disease or condition associated with a higher risk of developing infection. Follow up visits were scheduled on monthly basis. Bacteriological cultures of middle meatus secretions were undertaken before and after saline lavage and after the end of NaOCl treatment, using the technique described previously by Clement et al. [4].

Symptom evaluation

Patients' nasal symptoms (nasal obstruction, anterior and posterior nasal discharge, olfactory function and headache) were recorded on a visual analog scale (VAS) graded from 0 (= no symptom) to 10 (= maximal intensity of symptom) during the baseline visit and after six months of therapy.

Endoscopic examination

Nasal endoscopy was carried out by the same physician (J.S.L.). Using a VAS from 0 to 10, the nasal mucosal oedema, erythema, purulent discharge, crusting and polyps were evaluated.

Nasal Nitric oxide measurements

The level of nasal NO production was measured by a chemiluminescence's analyzer (Ecomedics, Drunten, Switzerland). The measurement was done according to the recommendations for intranasal measurement of the European Respiratory Society Guidelines [27,28]. NO measurements were taken during baseline visit and after three and six months of treatment.

Rhinomanometry measurements

NAR ($Pa/cm^3/s$) was measured by active anterior Rhinomanometry (Rhinometer 200; ATMOS, Germany), at the baseline and after three and six months, for each nostril after 10 normal breathings. Total NAR (tNAR) was then calculated with the following formula [29]: tNAR= (right NAR x left NAR) / (right NAR + left NAR).

Statistical analysis

All statistical analysis was performed using SPSS (SPSS Inc. Chicago, Illinois, USA) statistical package (version 10.0) for windows. Data are given as mean ± standard error of mean (SEM). A value of $p < 0.05$ was considered to be statistically significant. We used ANOVA test then a Wilcoxon signed rank test of paired differences was performed for all pair-wise combinations of VAS scores for subjective symptom and nasal endoscopic findings. The mean paired difference and p-

value of the Wilcoxon signed rank test were reported. For comparison of pre- and post- treatment NO and NAR measurements, Wilcoxon signed rank test was used.

RESULTS

In vitro study

F-actin network

F-actin network labeling forms a distinctive ring at the periphery of cells. The gradual depletion of this ring in cells is comparatively more obvious when these are treated twice daily for 5 consecutive days for 15 min. (Figure 1) than those for 5 min. (Figure 2). Depletion in cells treated for 5 minutes is less marked but more comparable with the cells at the beginning of treatment. A significant loss of F-actin network and loss of epithelial cells is noticeable in tissues exposed to the more concentrated NaOCl (0.5% NaOCl) after only 5 minutes.

Expression of ezrin in the apical domain of reconstituted epithelia

After exposure to undiluted (0.5% NaOCl) and one-tenth dilution (0.05% NaOCl) for 5 min., the epithelial cells were observed under confocal microscope for ezrin expression. No ezrin was detected in the epithelia exposed to 0.5% NaOCl (Figure 3a) even as early as 5 min. On the other hand expression of ezrin was still observed in the cells exposed to the diluted 0.05% NaOCl for 5 min. (Figure 3b). The Z stack images of confocal microscopy demonstrated polarized apical expression of ezrin in epithelial cells after 5 days of treatment, twice daily for 5 min. with 0.05% NaOCl which is comparable to the control. However, when epithelial cells were treated for 15 min., the thick apical band expression of ezrin was absent. There was redistribution of ezrin indicating loss of polarity of cells. Absence of apical ezrin may be due to loss of ciliated cells.

Figure 1 and Figure 2 compare expression of ezrin in cells treated with 0.05% NaOCl, twice daily for 5 consecutive days, 15 min. and 5 min., respectively. Ezrin expression reduction was relatively more pronounced in the epithelial cells treated for 15 min. It is mentionable that no ezrin was expressed at all in cells exposed to 0.5% NaOCl for 5 min. one time only (Figure 3a).

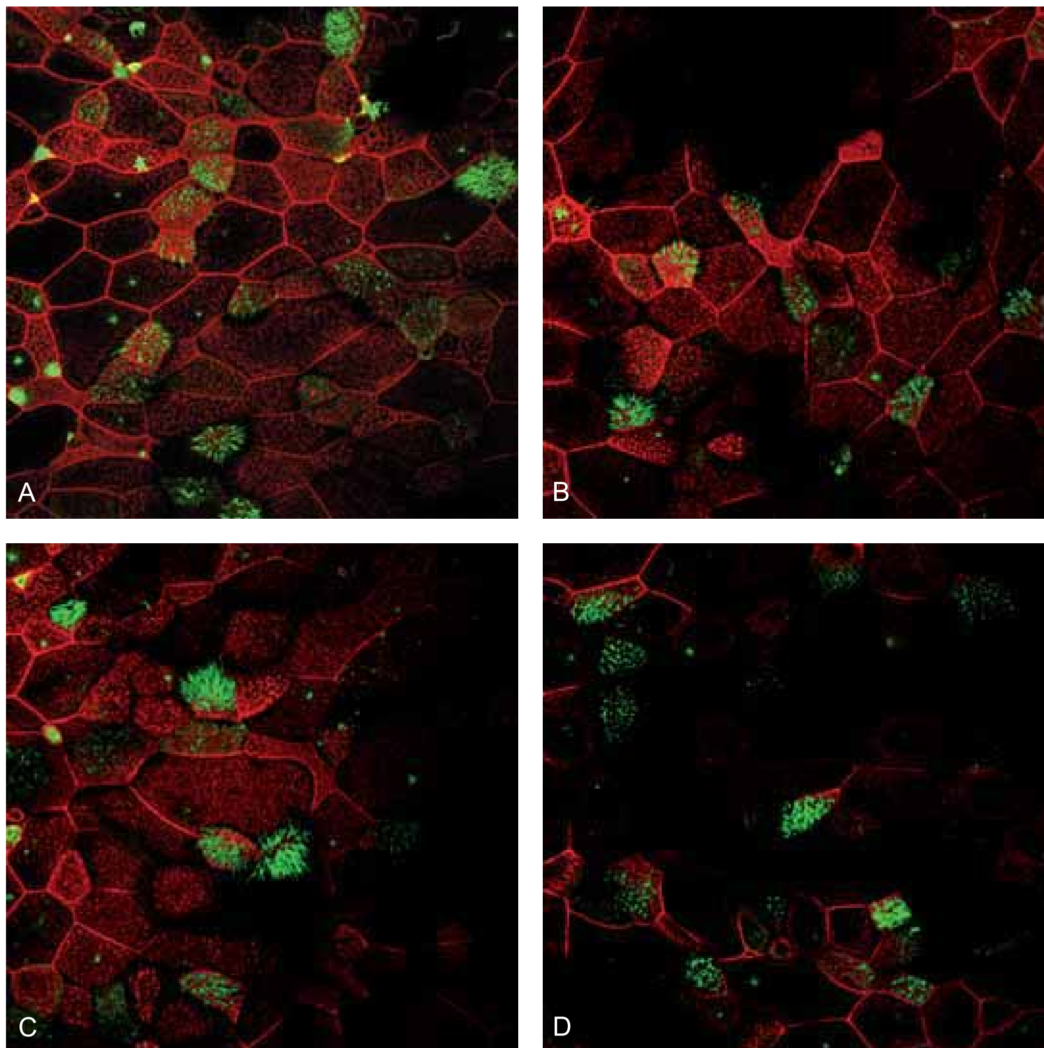

Figure 1. Confocal microscopic images of reconstituted nasal epithelial cells showing expression of ezrin (green) and F-actin network (red) delineating the cell outline after treatment with 0.05% NaOCl (prepared with PBS) exposed for 15 minutes, twice daily for 5 consecutive days. (A) day 1 (B) day 2 (C) day 3 (D) day 4.

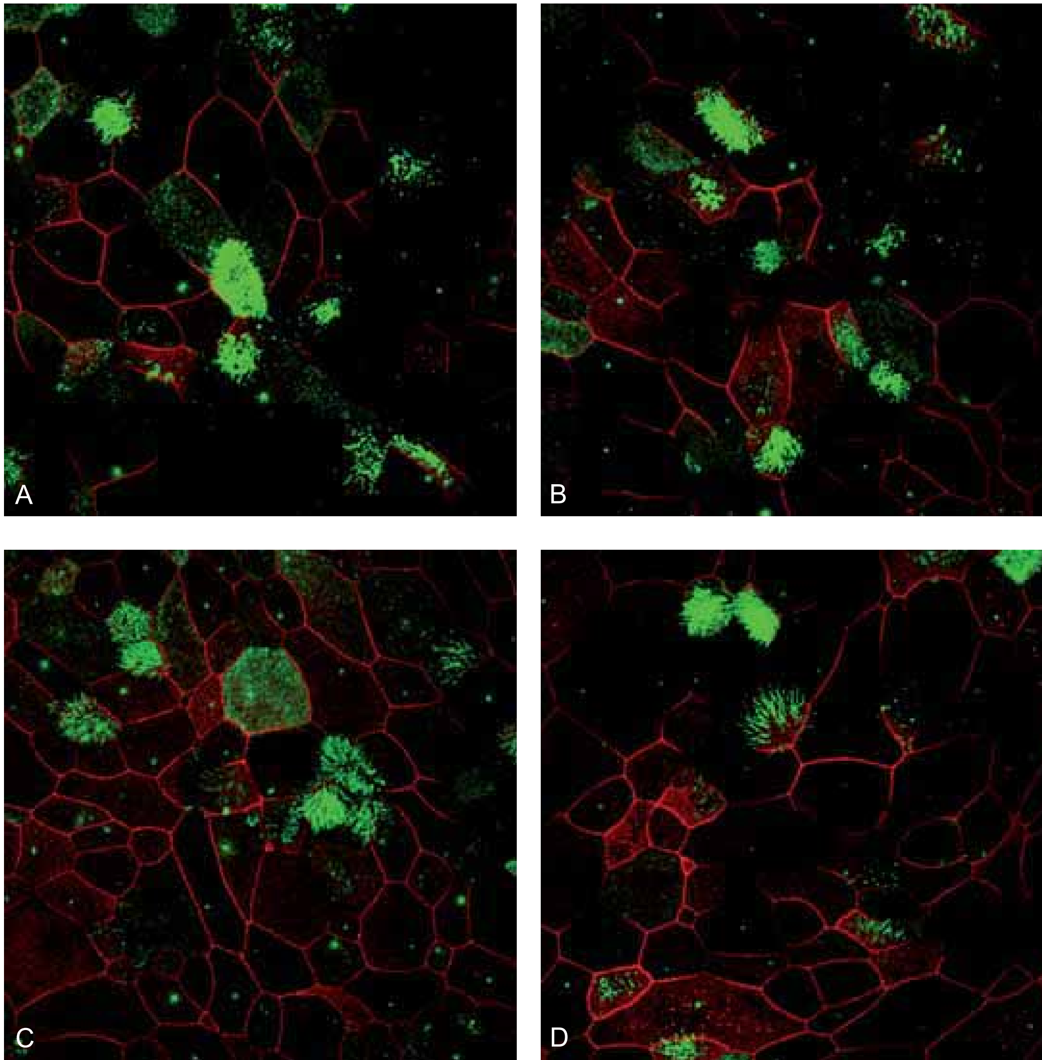

Figure 2. Confocal microscopic images of reconstituted nasal epithelial cells showing expression of ezrin (green) and F-actin network (red) delineating the cell outline after treatment with 0.05% NaOCl solution (prepared with PBS) exposed for 5 minutes, twice daily for 5 consecutive days. (A) day 1 (B) day 2 (C) day 3 (D) day 4.

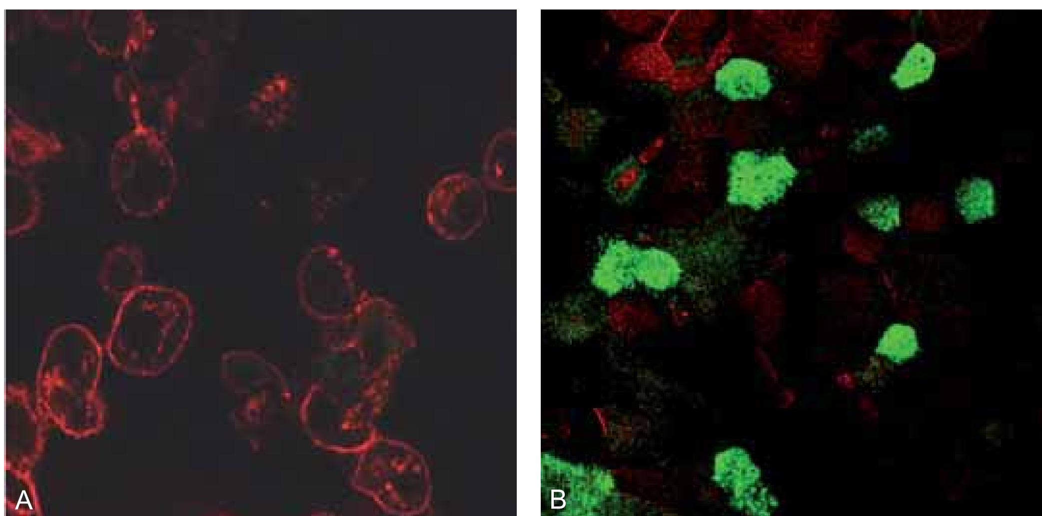

Figure 3. Confocal microscopic images of reconstituted nasal epithelial cells showing expression of ezrin (green) and F-actin network (red) delineating the cell outline after exposure to 0.5% and 0.05% Sodium Hypochlorite solution (prepared with PBS). (A) 0.5% NaOCl, (B) 0.05 NaOCl.

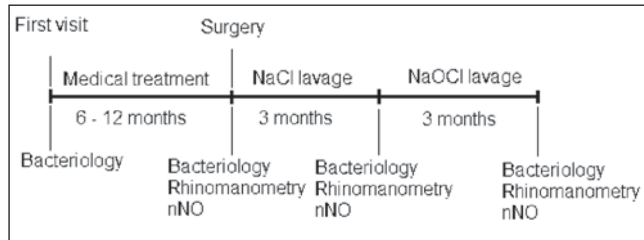

Figure 4. A schematic illustration of the methodology of in vivo study.

Table 1. Mean of the visual analogue scale values of subjective symptoms and nasal endoscopic findings before treatment and after lavage with saline alone or saline + NaOCl.

Subjective Symptom Score		Mean (± SEM)	*p* value
Nasal Obstruction	Pre-treatment	5.8 ± 0.7	} NS
	Post-saline	4.6 ± 0.3	} 0.01
	Post-NaOCl	2.0 ± 0.6	
Anterior Nasal Discharge	Pre-treatment	3.9 ± 0.9	} NS
	Post-saline	3.5 ± 0.2	} NS
	Post-NaOCl	3.5 ± 0.2	
Posterior Nasal Discharge	Pre-treatment	4.8 ± 0.8	} 0.05
	Post-saline	3.8 ± 0.3	} 0.018
	Post-NaOCl	2.0 ± 0.6	
Smell disturbance	Pre-treatment	5.4 ± 1.1	} NS
	Post-saline	5.1 ± 0.5	} 0.007
	Post-NaOCl	1.5 ± 0.9	
Headache	Pre-treatment	4.3 ± 1.1	} NS
	Post-saline	3.5 ± 0.3	} 0.009
	Post-NaOCl	0.9 ± 0.5	

Nasal Endoscopic Findings		Mean (± SEM)	*p* value
Oedema	Pre-treatment	6.0 ± 0.7	} 0.05
	Post-saline	4.8 ± 0.4	} 0.01
	Post-NaOCl	1.3 ± 0.4	
Erythema	Pre-treatment	6.9 ± 0.7	} 0.05
	Post-saline	5.1 ± 0.4	} 0.001
	Post-NaOCl	1.0 ± 0.3	
Purulent discharge	Pre-treatment	7.7 ± 0.9	} NS
	Post-saline	7.1 ± 0.5	} 0.002
	Post-NaOCl	1.0 ± 0.5	
Crusts	Pre-treatment	7.6 ± 0.8	} NS
	Post-saline	5.4 ± 0.4	} 0.001
	Post-NaOCl	0.9 ± 0.4	

In vivo study

Twenty Caucasian patients (8 females and 12 males; mean age 41 years) completed the study.

Nasal subjective and objective evaluation

Significant improvement was found in all nasal endoscopic findings. Moreover, significant improvement was found in all nasal symptoms, except for the anterior nasal discharge (Table 1). Four of our patients had nasal polyps. All of them showed regression of their polyp stage. Two patients had stage 1 nasal polyposis, which disappeared after treatment. The other two had stage 2 and 3, which became stage 1 and 2 respectively after treatment (data not shown).

Nitric Oxide measurement

The pre-treatment NO nasal production was 366.75 ± 97 ppb. This was reduced to 282 ± 73.26 ppb after saline alone and to 271 ± 67 ppb after lavage with saline + 0.05% NaOCl. The difference was not statistically significant.

Nasal Airway Resistance measurement

Mean tNAR decreased from 0.55 Pa/cm^3/s before therapy to 0.45 Pa/cm^3/s ($p < 0.05$) after three months of saline alone lavage. Following lavage with saline and 0.05% of NaOCl, tNAR was further decreased to 0.38 Pa/cm^3/s ($p < 0.05$) (data not shown).

Bacteriological culture

All patients showed persistence of unique patient specific SA clonotype in endoscopically guided cultures of middle meatus secretion done before treatment and at least one month after the end of treatment with 0.05% NaOCl.

DISCUSSION

The main finding of this study is that 0.05% NaOCl solution in saline for nasal lavage improves both symptoms and clinical findings, but not bacterial colonization of the nose in patients with persistent *SA* rhinosinusitis. It seems to be a safe, well tolerated, cheap, and effective alternative to saline nasal lavage alone in the treatment of *SA* rhinosinusitis. Being cheap, it maybe a good option for developing countries, where high budget antibiotics are not suitable. It decreases significantly the intensity of symptoms and clinical status of symptomatic *SA* carriers despite the persistence of the carriage state. Previous studies have demonstrated the efficacy of NaOCl in treating several types of infections. However, no studies were found in the literature concerning the use of NaOCl onto the nasal mucosa of symptomatic patients with infectious CRS. Additionally, the cellular effects of NaOCl may not be comparable in all tissues [30]. Hence, there was a need to perform an in vitro study to find out the appropriate concentration of NaOCl and the duration of exposure, that would not be harmful yet bactericidal, before in vivo application. The 0.05% NaOCl concentration was better tolerated in vitro than the 0.5%. The in vitro study showed that the 5 min. exposure was less cytotoxic than the 15 min exposure. Despite the fact that we do not know exactly how long the lavage solution would stay in contact with the nasal mucosa, we assume that, it would be closer to 5 min. than to 15 min.

The depletion of F-actin network in our study indicated that the cytotoxic effect of NaOCl depends on both its concentration and the duration of exposure. This cytotoxic effect has been suggested to be mediated via DNA or RNA synthesis [30]. Modification of erzin expression corresponded with the alteration in F-actin network. The in vitro response, by F-actin network and ezrin expression of reconstituted nasal epithelium, have demonstrated that 0.05% NaOCl induced less epithelial

cell cytotoxicity than 0.5% NaOCl. We also demonstrated that 5 min. of exposure twice daily to 0.05% NaOCl was less harmful than 15 min. exposure.

The alteration of F-actin network and ezrin expression is frequently associated with changes in the appearance and function of the TJs. The discrepancy between ezrin expression at the cell surface and F-actin network may be due to a change in cells polarity, possibly related to the opening of the TJs of the reconstituted nasal epithelia.

The Sinus Rinse® bottles were used for lavage. These were easy to use and provided an efficient flow of solution in the nasal cavity. A lavage has certain advantages over the administration of topical or systemic antibiotics, as it removes secretions and crusts from the nose [(31)]. It acts also by altering the composition of the mucus and reducing the local concentrations of bacterial or fungal organisms and their toxins or proinflammatory substances released during the inflammatory response [(32)].

Objectively, the tNAR measured by rhinomanometry was significantly reduced at the end of the 3rd month of NaOCl treatment. Active anterior rhinomanometry is a reliable method of assessing the objective functional status of the nasal cavities in different situations [(33)]. It is also well correlated with the subjective evaluation of NAR [(34)].

No significant changes in nasal NO production was observed after treatment. This correlates with a study carried out by Cervin et al. [(35)], who demonstrated no significant improvement in NO level in spite of significant improvement of symptoms in patients who received low dose of the antibiotic erythromycin therapy for CRS during 3 months. However, they mentioned that there was a trend toward an increase in nasal NO after 12 months. This could be explained by the gradual restoration of the normal epithelial functions due to the regrowth of ciliated cells and the reduction of inflammation [(35)].

CONCLUSION

Nasal lavage with 0.05% NaOCl solution in saline is well-tolerated and suitable for use in humans and may be a good alternative to saline lavage alone in the treatment of RS with recurrent *SA* infections due to patient-specific*SA* clonotypes. In the mentioned concentration and method of application it can improve the quality of life of the patients despite the persistence of SA in the post-treatment bacteriological culture.

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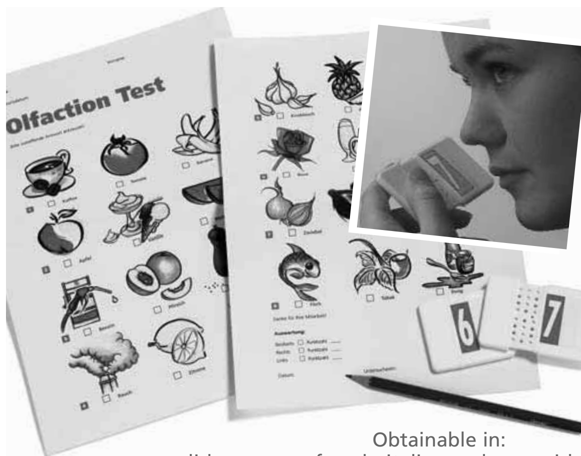

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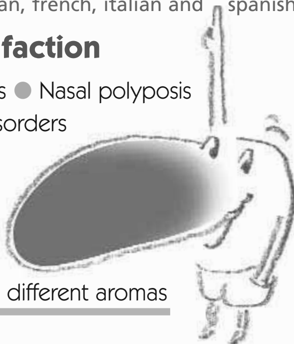

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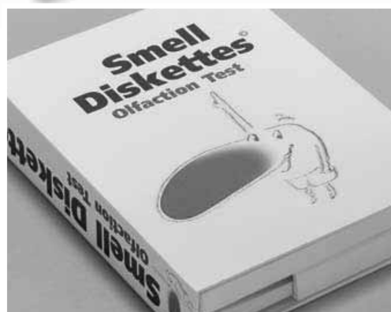

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