Efficacy of NVC-422 against *Staphylococcus aureus* biofilms in a sheep biofilm model of sinusitis

Deepti Singhal, PhD[1], Andreas Jekle, PhD[2], Dmitri Debabov, PhD[2], Lu Wang, PhD[2], Bez Khosrovi, PhD[2], Mark Anderson, PhD[2], Andrew Foreman, PhD[1] and Peter-John Wormald, MD[1]

Background: **Bacterial biofilms are a major obstacle in management of recalcitrant chronic rhinosinusitis. NVC-422 is a potent, fast-acting, broad-spectrum, nonantibiotic, antimicrobial with a new mechanism of action effective against biofilm bacteria in in vitro conditions. The aim of this study was to investigate the safety and efficacy of NVC-422 as local antibiofilm treatment in a sheep model of rhinosinusitis.**

Methods: **After accessing and occluding frontal sinus ostia in 24 merino sheep via staged endoscopic procedures, *S. aureus* clinical isolate was instilled in frontal sinuses. Following biofilm formation, ostial obstruction was removed and sinuses irrigated with 0.1% and 0.5% NVC-422 in 5 mM acetate isotonic saline at pH 4.0. Sheep were monitored for adverse effects and euthanized 24 hours after treatment. Frontal sinuses were assessed for infection and changes in mucosa after the treatment. *S. aureus* biofilms were identified with Baclight-confocal scanning microscopy protocol and the biofilm biomass assayed by applying the COMSTAT2 program to recorded image stacks.**

Results: **After 2 irrigations with 0.1% NVC-422, *S. aureus* biofilm biomass was reduced when compared to control sinuses ($p = 0.0001$), though this effect was variable in samples. NVC-422 0.5% solution irrigations reduced biofilm even more significantly and consistently over all samples ($p < 0.0001$). NVC-422 0.5% was also more effective than 0.1% NVC-422, vehicle control, and normal saline sinus irrigations in reducing biofilm biomass ($p < 0.05$ for all subgroups). No adverse events were observed in sheep after sinus irrigations with 0.1% and 0.5% NVC-422 solutions.**

Conclusion: **NVC-422 is an effective topical agent against *S. aureus* biofilms, with dose-dependent efficacy in this animal model of biofilm-associated sinusitis.** © 2012 ARS-AAOA, LLC.

Key Words:
chronic rhinosinusitis; *S. aureus*; biofilm; confocal scanning laser microscopy; NVC-422; sheep model; COMSTAT

How to Cite this Article:
Singhal D, Jekle A, Debabov D, et al. Efficacy of NVC-422 against *Staphylococcus aureus* biofilms in a sheep biofilm model of sinusitis. *Int Forum Allergy Rhinol*, 2012; 2:309–315.

Chronic rhinosinusitis (CRS) is a recurring, persistent inflammation of the sinonasal tissues, and is known to cause significant physical symptoms, negatively affect quality of life, and substantially impair daily functioning. Whereas most patients do well after endoscopic sinus surgery (ESS), there is a recalcitrant subgroup of CRS patients who continue to have persistent sinonasal inflammation and recurrent acute exacerbations despite long-term, culture-directed antibiotic therapy and well-performed sinus surgery. With the detection of biofilms on the sinonasal mucosa, it has been extensively speculated that these structures may be responsible for propagating recalcitrant and chronic pathophysiological processes seen in CRS patients.[1–7] Biofilms are organized communities of bacteria, attached to a biotic or abiotic surface embedded and protected in a mosaic of self-produced extracellular polymeric substance.[8] These biofilms are a highly self-sufficient fortress, and can persist within tissues, leading to chronic inflammation in the surrounding tissues with intermittent acute exacerbations, which are hallmarks of biofilm-mediated diseases.

[1]*Department of Surgery–Otolaryngology Head and Neck Surgery Adelaide University, South Australia;* [2]*NovaBay Pharmaceuticals, Inc., Emeryville, CA*

Correspondence to: Peter-John Wormald, MD, Department of ENT-Surgery, The Queen Elizabeth Hospital, 28 Woodville Rd, Woodville South, South Australia 5011, Australia; e-mail: peterj.wormald@adelaide.edu.au

Funding sources for the study: This study was supported by a grant from NovaBay Pharmaceuticals, Inc.
Potential conflict of interest: P.J.W. is a consultant for Neilmed and receives royalties for design of instruments from Medtronic ENT. A.J., D.D., L.W., B.K., and M.A. are employees of NovaBay Pharmaceuticals, Inc.

Received: 15 August 2011; Revised: 21 November 2011; Accepted: 19 January 2012
DOI: 10.1002/alr.21038
View this article online at wileyonlinelibrary.com.

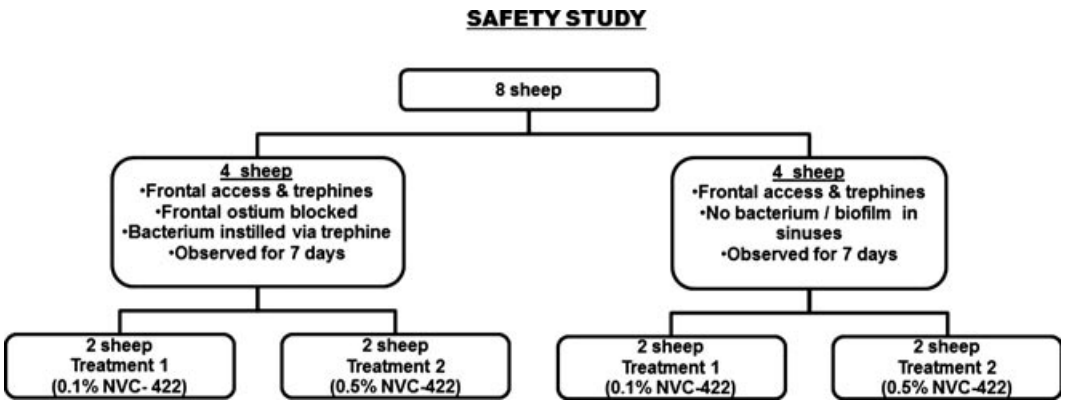

FIGURE 1. Treatment 1: 2 doses (4 hours apart) of 100 mL of 0.1% NVC-422 flushed slowly through the trephine into 1 of their frontal sinuses. Treatment 2: 2 doses (4 hours apart) of 100 mL of 0.5% NVC-422 flushed slowly through the trephine into 1 of their frontal sinuses.

N,N-dichloro-2,2-dimethyltaurine (NVC-422) is a novel, nonantibiotic antimicrobial agent that was developed by NovaBay Pharmaceuticals, Inc. (Emeryville, CA). NVC-422 is a stable analog of the *N*-chlorotaurines (NCT) and *N,N*-dichlorotaurine (NNDCT) formed within phagocytic leucocytes during oxidative burst.[9] It has been described as a fast-acting, potent, and broad-spectrum agent that effectively kills pathogens such as *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), *Streptococcus spp*., *Escherichia coli*, *Candida albicans*, adenovirus, herpes simplex virus, and many other microorganisms.[9] In vitro experiments have also shown that it can effectively kill microbes within a biofilm.[10] Furthermore, no drug resistance is observed with NVC-422 in multiple passage studies against multiple pathogens (unpublished results). NVC-422 gel has been used for the treatment of impetigo.[11]

The sheep model for CRS associated with biofilms[12] has been previously standardized by our department and it provides the opportunity to test novel biofilm treatments under in vivo conditions in which the dynamic interaction between the host and biofilm exist. To further the use of this model, this pilot study was planned to establish the safety and efficacy of NVC-422 in the sheep model of biofilm-associated sinusitis.

Materials and methods

Animals used in the study

The study was approved by the Animal Ethics Committees of the University of Adelaide and the Institute for Medical and Veterinary Science and conducted at The Queen Elizabeth Hospital surgical workshop facility in Adelaide, South Australia. As part of the standardized biofilm treatment model developed in our department, frontal sinuses of 24 adult merino sheep were used for *S. aureus* biofilm formation in vivo.[12]

Bacterial inoculum

A pure strain of *S. aureus* clinical isolate with described in vitro biofilm-forming capacity was procured from the Department of Microbiology at The Queen Elizabeth Hospital in Adelaide. The frozen glycerol stocks were first subcultured overnight at 37°C in 3 mL cerebrospinal fluid (CSF) broth (Oxoid Australia, Thebarton, South Australia, Australia) on a shaker and again on Columbia Horse Blood Agar (Oxoid Australia, Thebarton, South Australia, Australia) for 12 to 18 hours at 37°C. To prepare the inoculum, single colony units from the plate cultures were added to 0.45% sterile saline and adjusted to a 0.5 McFarland units (MFU), and transported on ice for instillation into sheep sinuses.

Surgery study protocols

As per protocol the middle turbinates of the sheep were removed and the sheep were allowed to recover for 3 to 4 weeks of convalescence after this procedure. Under general anesthetic the frontal sinuses were trephined and the biofilm-forming bacteria (1 mL of 0.5 MFU *Staphylococcus aureus*, Clinical isolate #1) was inoculated into both of their frontal sinuses. The frontal ostium was occluded with petrolatum gauze (Vaseline, Kendall, tyco healthcare, Mansfield, MA, USA) and the bacteria was allowed to grow into the biofilm form over the next 7 days. Thereafter, the petrolatum gauze was removed and 1 sinus was treated as per the protocol in Figures 1 and 2. The other sinus served as a control. Sheep were monitored following the procedure for the next 24 hours and on day 8 the sheep were euthanized. In this study, 8 of the 24 sheep were used for the safety analysis and the remaining 16 of the 24 were used for the efficacy evaluation of the study.

Tissue collection and sample preparation

The sheep were euthanized 24 hours after receiving the sinus treatment and the mucosa lining the inoculated frontal sinus was exposed by removing the forehead skin and anterior bony table of the sinus. The sinus mucosa was carefully dissected off the bony walls, taking care to maintain its structural integrity. It was transported in Dulbecco's Modified Eagle's Medium (Gibco/Invitrogen, Grand Island, NY) to the laboratory for further analysis. In the laboratory, under sterile conditions, the sinus mucosa

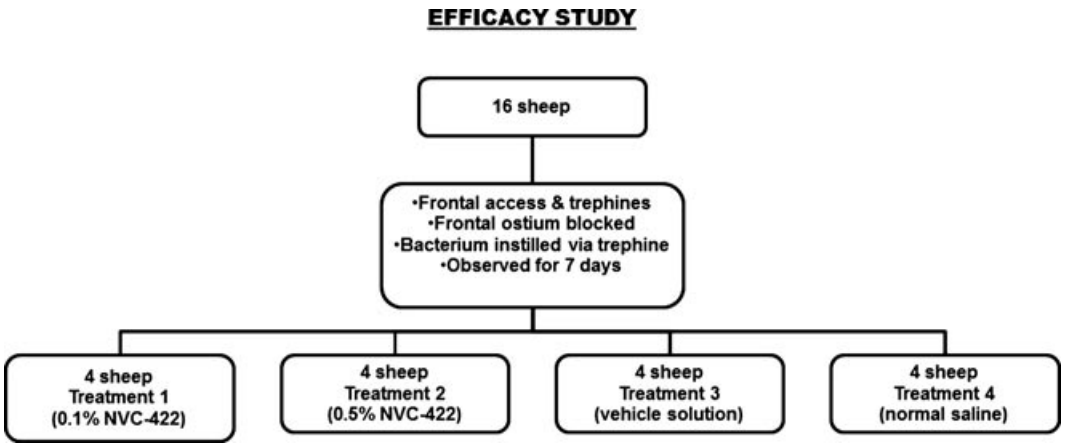

FIGURE 2. Treatment 1: 2 doses (4 hours apart) of 100 mL of 0.1% NVC-422 flushed slowly through the trephine into 1 of the frontal sinuses. Treatment 2: 2 doses (4 hours apart) of 100 mL of 0.5% NVC-422 flushed slowly through the trephine into 1 of the frontal sinuses. Treatment 3: 2 doses (4 hours apart) of 100 mL of vehicle solution (0.9% NaCl, 5 mM acetate) flushed slowly through the trephine into 1 of the frontal sinuses. Treatment 4: 2 doses (4 hours apart) of 100 mL of normal saline flushed slowly through the trephine into 1 of the frontal sinuses.

was carefully dissected into small pieces of approximately 10 mm $\times$ 10 mm, and further processed for microbiology stains and cultures, histopathology examinations, and biofilm analysis.

Outcome measures

The primary outcome measures for the safety study were the overall condition of the sheep (change in appetite/thirst, respiration, behavior, posturing, elevated temperature) and evaluation of sinus mucosa (gross and histopathological changes seen in mucosa). The primary outcome measure for the efficacy study was change in biomass of the biofilm after treatment.

Biofilm imaging and analysis

The specimens were stained for biofilms using the LiveDead Baclight stain-confocal scanning laser microscopy (CSLM) protocol.[13] Briefly, each tissue sample was washed thoroughly in 3 separate beakers of sterile water to remove any planktonic bacteria. The sample was then immersed in 1 mL of sterile MQ water, to which 1.5 μL aliquots of component A (Syto 9) and component B (propidium iodide) of the BacLight LIVE/DEAD kit (Invitrogen, Molecular Probes) are added. After incubation in darkness at room temperature for 15 minutes, each sample was rinsed in sterile MQ water to remove excess stain. The specimens were mounted on coverslips for analysis with a Leica TCS SP5 confocal scanning laser microscope (Leica Microsystems, Wetzlar, Germany). The entire area of each specimen was scanned for evidence of biofilm structures using a 488-nm argon laser and water immersion lens at $\times$20 magnification. Biofilms were identified as clusters and towers of immobile, irreversibly attached, intensely fluorescing, live green cocci-shaped bacteria, approximately 0.5 to 2 μm in diameter.

Two random Z-stacks, with a total thickness of each Z-stack at 85 μm, at a distance of 0.5 μm per slice, were recorded from each of the 2 samples from each sinus; thus making a total of 4 image-stacks imaged for biofilm evidence from each sinus. These image stacks were taken randomly from different positions on the specimen where maximal biofilm was seen. The physical dimensions of each stack as recorded by the Leica Confocal microscope at $\times$20 magnification were 775.0 μm $\times$ 775.0 μm $\times$ 85 μm, with a volume of 5.1 $\times$ 10^7 μm^3 of tissue being imaged per image stack. Using the COMSTAT2 software (Lyngby, Denmark; http://www.COMSTAT2.dk),[14,15] a threshold was set for each Z-stack by which the fluorescence emitted by epithelial debris in the image background was minimized, while maintaining the biofilm fluorescence as much as possible. After applying the individually set thresholds to each stack, biofilm biomass (volume/area or $\mu m^3/\mu m^2$) was then calculated using the same software. Biovolume or biomass as calculated by COMSTAT2 is defined as the number of biomass pixels in all images of a stack multiplied by the voxel size [$(\text{pixel size})_x \times (\text{pixel size})_y \times (\text{pixel size})_z$] divided by the substratum area of the image stack.

Statistical analysis

Statistical analysis was performed using Graph Pad Prism 5.0 software (San Diego, CA). All data was considered nonparametric, and hence differences were analyzed using the Mann-Whitney U test and analysis of variance (ANOVA) model tests. Mean values and standard deviations are reported in the results, and all statistical tests were considered to be significant at $p = 0.05$.

Results

Safety analysis

Condition of the sheep

The 4 sheep with no biofilm infection did not show any change in thirst/appetite, behavior, respiration, or

posturing after instilling the treatment. The 4 bacterium-inoculated sheep showed some decline in food and water intake, which has been an expected response to the bacterial infection in the designed sheep model. However, the appetite and thirst did not improve or worsen after the treatment was given to the sinuses. Other parameters (respiratory movements, general behavior, posturing, and temperature) were all maintained during the biofilm formation stage and after the treatment were given.

Evaluation of sinus tissue and contents

Gross examination. All the 4 non–biofilm-inoculated sheep had pale-white mucosa lining both the treated and untreated sinuses, with no mucopurulent collection in either of the sinuses. The 4 biofilm-inoculated sheep had friable and necrotic mucosa lining the untreated sinuses, with mucopurulent secretions filling them. The biofilm-inoculated sinuses that were treated with 0.1% and 0.5% NVC-422 showed lesser congestion and edematous mucosal linings with mild to moderate thick mucoid secretions in the sinuses.

Histopathology

Among the 4 non-biofilm-inoculated sheep, 2 types of samples for analysis were taken. First, there were the 4 "untreated uninfected sinuses": 1 sinus in each of these 4 sheep was not treated to serve as the control for the other side. The epithelium lining of these untreated uninfected sinuses was the expected pseudostratified ciliated epithelium seen structurally on normal epithelium lining the sinuses. The second type of sample was the "treated uninfected sinuses": the 4 uninfected sheep had treatment instilled in 1 of the sinuses. These 4 uninfected treated sinuses had extensive segments of a different type of epithelium, which can be described as "multilayered" or "stratified," with patchy loss of the ciliary layer. In the sheep inoculated with biofilm-forming bacterium, there was necrosis of the mucosal layer with sections of ulcerated-denuded epithelium, with a change from normal pseudostratified epithelium to a stratified epithelium and an increase in the goblet cells. There was also extensive acute inflammatory reaction in the submucosa. In the biofilm-infected sheep who were not treated with the study agent, or "infected-untreated sheep," the epithelial changes were very extensive, with an intense neutrophilic reaction seen in the submucosa. In the biofilm-infected sheep that were treated with the study agents, or "infected-treated sheep," the epithelial changes were present but the neutrophilic infiltrate was less in the submucosa as compared to the infected-untreated sheep.

Efficacy analysis

S. aureus biofilms were identified using the Baclight-CSLM protocol on the sinomucosal samples as clusters and towers of immobile, irreversibly attached, intensely fluorescing, live green cocci-shaped bacteria, approximately 0.5 to 2 μm in diameter. They were seen interspersed between the viable (green fluorescence) epithelium and dead necrotic (red fluorescence) tissue (Fig 1). The main outcome measure for the efficacy study was the effect of the treatments on the biofilm present on the sinonasal mucosa sampled from the frontal sinuses of the sheep 24 hours after the treatment was given. The 4 different treatments applied to the sinuses were 0.1% NVC-422, 0.5% NVC-422, NVC-422 vehicle, and normal saline. The number of sheep from which data is available for analysis for the NVC placebo and normal saline treatments is 4 in each subgroup. The number of sheep from which data was analyzed for 0.1% NVC-422 and 0.5% NVC-422 subgroups was 6 (4 sheep from the efficacy arm of the study and 2 sheep from the safety arm of the study in each subgroup).

NVC-422 (0.1%)

The mean biomass of biofilms in the sinuses treated with 0.1% NVC-422 was 0.71 $\pm$ 0.8 $\mu m^3/\mu m^2$, compared to the mean biomass of biofilms on the untreated (control) sides, which was 1.94 $\pm$ 1.1 $\mu m^3/\mu m^2$ ($p = 0.0001$, Mann-Whitney *t* test). As shown in Figure 3, the 2-way ANOVA analysis of the mean biomass of the treated and untreated sinus of each sheep showed that though 0.1% NVC-422 does have a significant effect in reducing biofilm biomass when compared to untreated sides ($p < 0.0001$, ANOVA); the treatment had significantly variable effects in the individual sheep.

NVC-422 (0.5%)

The mean biomass of biofilms in the sinuses treated with 0.5% NVC-422 was 0.11 $\pm$ 0.11 $\mu m^3/\mu m^2$, and this was very significantly lower than the biofilm biomass of 2.01 $\pm$ 2.7 $\mu m^3/\mu m^2$ on the untreated (control) side ($p < 0.0001$, Mann-Whitney *t* test). By the 2-way ANOVA analysis of the mean biomass of the treated and untreated sinus of each sheep, it was seen that 0.5% NVC-422 reduces the biofilm biomass very significantly when compared to untreated sinuses. Variability within this treatment group was minimal ($p = 0.0013$, ANOVA; Fig. 4).

NVC placebo

The mean biomass of biofilms in the untreated (control) sinuses was 1.14 $\pm$ 0.9 $\mu m^3/\mu m^2$, which was not significantly different from the mean biofilm biomass of 1.1 $\pm$ 0.8 $\mu m^3/\mu m^2$ in the sinuses treated with vehicle ($p = 0.86$, Mann-Whitney *t* test), as shown in Figure 5A.

Normal saline irrigation

The mean biomass of biofilms in the sinuses irrigated with normal saline was 1.16 $\pm$ 0.5 $\mu m^3/\mu m^2$, which was similar to the biofilm biomass of 1.15 $\pm$ 0.6 $\mu m^3/\mu m^2$ on the untreated (control) side ($p = 0.6625$, Mann-Whitney

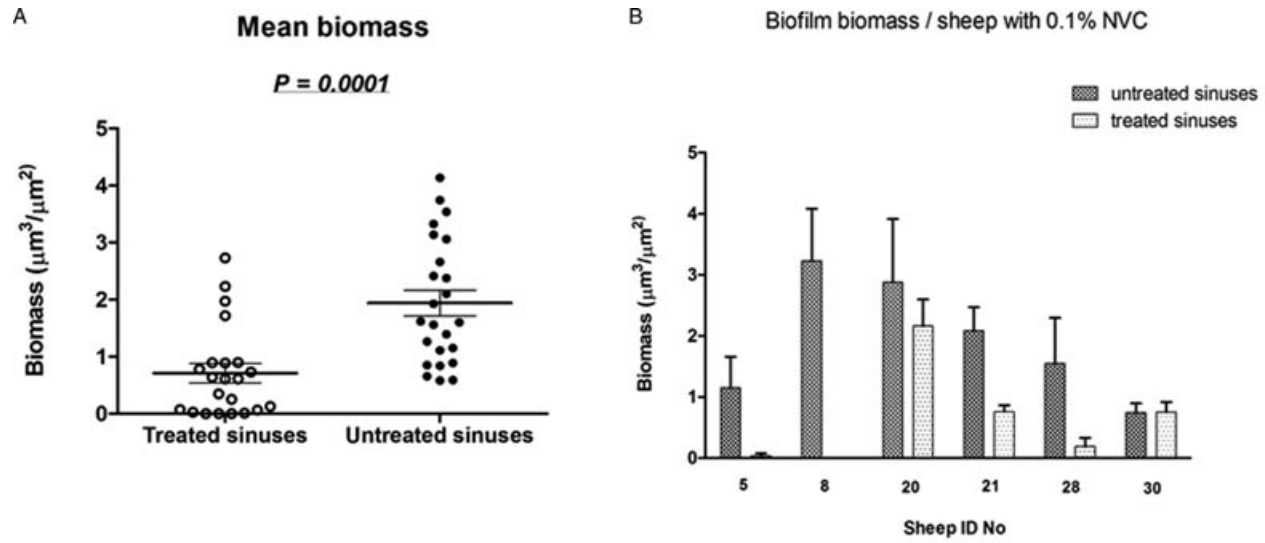

FIGURE 3. Efficacy of 0.1% NVC 422 on *S. aureus* biofilms. (A) Each point represents the biomass assessed from each Z-stack image recorded from the 6 sheep in the 0.1% NVC 422 subgroup. (B) Mean biofilm biomass in the untreated and treated sinuses in each sheep in the 0.1% NVC-422 treatment subgroup.

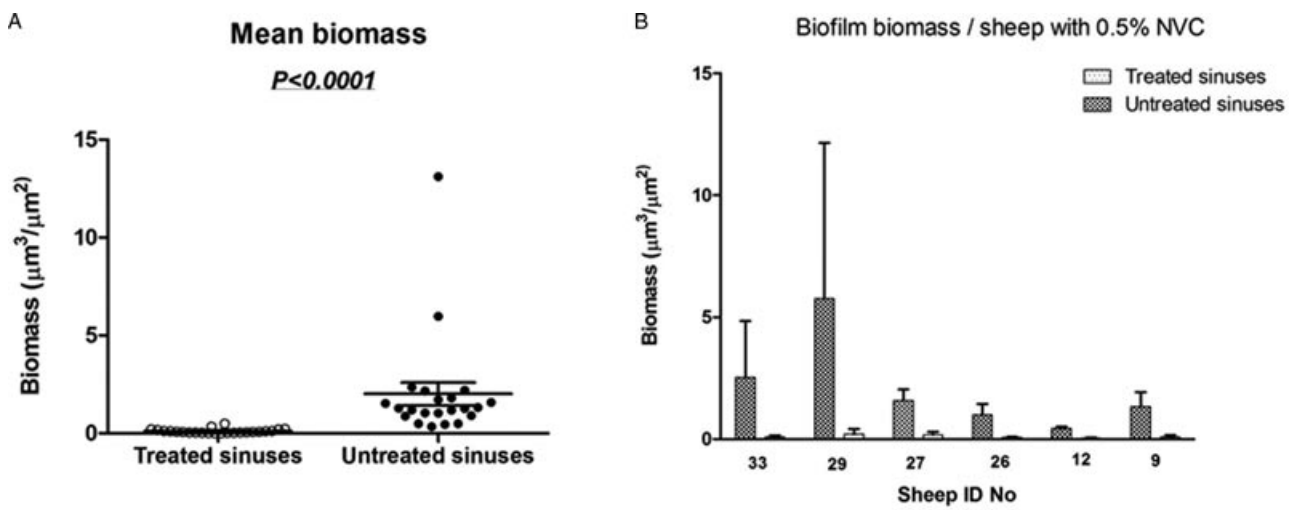

FIGURE 4. Efficacy of 0.5% NVC-422 on *S. aureus* biofilms. (A) Each point represents the biomass assessed from each Z-stack image recorded from the 6 sheep in the 0.5% NVC-422 subgroup. (B) Mean biofilm biomass in the untreated and treated sinuses in each sheep in the 0.5% NVC-422 treatment subgroup.

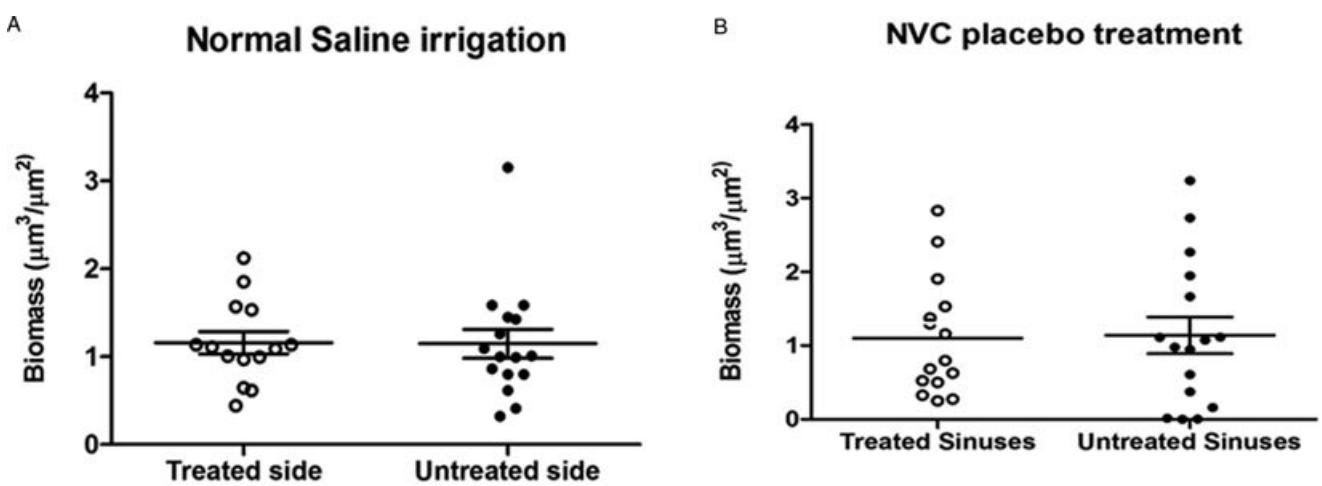

FIGURE 5. (A) Efficacy of vehicle treatment on *S. aureus* biofilms. (B) Efficacy of normal saline irrigation on *S. aureus* biofilms.

TABLE 1. Results of posttest Dunn's multiple comparison test

Dunn's multiple comparison test[a]	Difference in rank sum	Significant at $p < 0.05$
0.1% NVC-422 vs 0.5% NVC-422	17.95	Yes
0.1% NVC-422 vs vehicle	−15.51	No
0.1% NVC-422 vs NS washes	−19.76	Yes
0.5% NVC-422 vs vehicle	−33.47	Yes
0.5% NVC-422 vs NS washes	−37.71	Yes
Vehicle vs NS washes	−4.248	No

[a]NVC-422 = antibiofilm agent under study.
NS = normal saline.

t test), as shown in Figure 5B. The 2-way ANOVA analysis shows that there is no statistically significant difference in the mean biomass of the untreated and normal saline–treated sinus of each.

Comparison of all treatment subgroups

There was a significant difference in the mean biomass of the biofilms from all the treated sinuses in the 4 subgroups ($p < 0.0001$, Kruskal-Wallis test). Table 1 shows the further posttest (Dunn's multiple comparison test) to compare each pair of columns. The subgroup analysis clearly indicates that although both 0.1% and 0.5% concentrations of NVC-422 significantly decreased the biofilm biomass when compared to control saline washes, the decrease in biomass brought about by 0.1% NVC-422 did not significantly differ from the decrease in biomass brought about by the vehicle, as seen in Figure 6. However, 0.5% NVC-422 was found to have reduced the biofilm biomass more significantly when compared to control normal saline irrigation, vehicle, and 0.1% NVC-422.

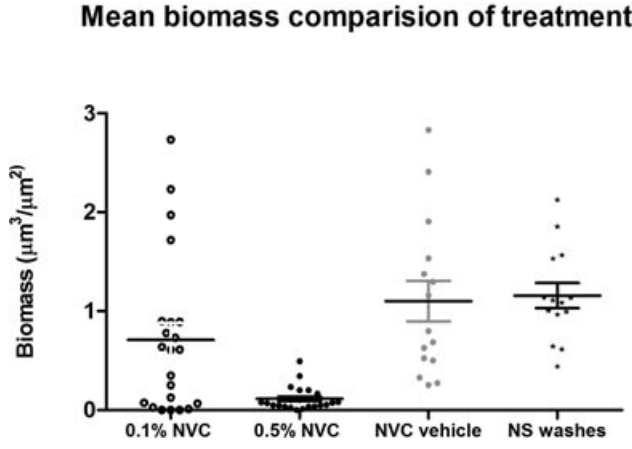

FIGURE 6. Mean biomass of *S. aureus* biofilms after the 4 different treatments.

Discussion

This pilot study to investigate the safety and efficacy of NVC-422 as a local antibiofilm treatment in the sheep model of rhinosinusitis showed that NVC-422 was a very effective topical agent against *S. aureus* biofilms, with a 0.5% concentration of the solution being more efficacious in this small study sample.

The approach to management of biofilm-associated CRS has mostly focused on topical administration of antimicrobials, which can possibly be administered in larger doses to achieve higher concentrations in the sinonasal tissues, while minimizing systemic absorption and avoiding ensuing adverse effects. Mupirocin is 1 such agent that has shown 90% reduction in *S. aureus* biofilms in vitro and in animal models,[16,17] with significant improvement in signs and symptoms in CRS patients[18] when used as an adjunct to nasal lavages. Lavages with chemical surfactants such as baby shampoo have also shown to be of benefit in postsurgical CRS patients,[19] whereas a combination of soap-like surfactant and a calcium ion sequestrating agent, aiming to break the bonds in the exopolysaccharide matrix (EPS) have also been reported to effectively eradicate *S. aureus* and *P. aeruginosa* biofilms under controlled in vitro settings.[20] Concerns about development of resistance to classical antibiotics such as mupirocin have been well-documented.[21]

NovaBay Pharmaceuticals, Inc., has completed preliminary nasal clinical evaluations of NVC-422 as a fast-acting antimicrobial for the rapid decolonization of *S. aureus*, including MRSA, in 78 subjects for which treatment was well-tolerated. NVC-422 is a stable derivative of the naturally occurring chlorotaurines formed within white blood cells during phagocytosis. In vitro studies with NVC-422 have also shown that it is capable of penetrating a biofilm and effectively killing the contained microbes.[10] In this pilot study in an animal model of biofilm-associated sinusitis, NVC-422 was found to be effective in reducing the biomass of the *S. aureus* biofilms at both the 0.1% and 0.5% concentrations used for local sinus irrigations.

Topical application of 0.1% NVC-422 was seen to have a variable effect in decreasing the biomass of the *S. aureus* biofilm in the sinuses of the sheep. The mean biomass values in individual animals indicate that in some the treatment very effectively eradicated the biofilm to show completely healthy epithelium, whereas in some it seemed to have had no affect at all (Fig. 3). Hence, the effect that 0.1% NVC-422 has on biofilm biomass is difficult to interpret with such low sample numbers. But overall treatment with 0.1% NVC-422 does have a significant effect in reducing biofilm biomass when compared to untreated sinuses and sinuses treated with nasal saline irrigations. At the same time there was no significant difference between biofilm biomass reduced by 0.1% NVC-422 and NVC-422 vehicle, which essentially is its diluting solution. There is a possibility that the effect of biomass reduction with 0.1% concentration may be contributed to by the diluting solution.

NVC-422 at a concentration of 0.5% is very effective in decreasing the biomass of *S. aureus* biofilms. The effect of NVC-422 at a concentration of 0.5% is fairly constant over the treatment subgroup, with significant decline in biofilm biomass seen in all samples as compared to the control untreated sides. The concentration of 0.5% is more effective than the concentration of 0.1% NVC-422 in reducing the biomass of *S. aureus* biofilms, as well as being more effective in reducing the biofilm biomass when compared with vehicle. NVC-422 at 0.5% concentration is also significantly more effective in reducing the biofilm biomass when compared with normal saline sinus irrigations, indicating that it is the NVC-422 component of the solution acting against the biofilm and the biofilm is not being removed solely by the flushing action.

No immediate adverse events were observed in the sheep after the instillation of 2 doses (4 hours apart) of 100 mL of either of the 2 solutions. Whether the presence of a stratified epithelium with increased goblet cells and neutrophils in submucosa was a transient reaction or long-lasting change could not be assessed in this study because the animals were euthanized within 24 hours of instilling the study agent. At the structural level there appears to be no difference in the type of reaction seen in the mucosa of the sinuses treated with 0.1% and 0.5% NVC-422. The changes described above are, however, only structural, and based only on histology it is difficult to comment on the viability or functional aspects of the cilia.

Conclusion

NVC-422 as a nasal irrigation solution at the study concentrations of 0.1% and 0.5% was found to be an effective in reducing the biomass of the *S. aureus* biofilms in the sheep model of sinusitis. The safety of the NVC-422 solutions requires further evaluation, including assessment of ciliary function as well as mucosal changes after more doses and prolonged periods of administration of the agent. NVC-422 at 0.1% concentration was seen to have a variable effect in decreasing the biomass, whereas the 0.5% concentration was more consistent in reducing the biomass over all the samples. The NVC-422 at 0.5% concentration was more efficacious than the NVC-422 at 0.1% concentration, vehicle control, or normal saline sinus irrigations in reducing the biomass of *S. aureus* biofilms.

References

1. Cohen M, Kofonow J, Nayak JV,et al. Biofilms in chronic rhinosinusitis: a review. *Am J Rhinol Allergy*. 2009;23:255–260.
2. Harvey RJ, Lund VJ. Biofilms and chronic rhinosinusitis: systematic review of evidence, current concepts and directions for research. *Rhinology*. 2007;45: 3–13.
3. Hunsaker DH, Leid JG. The relationship of biofilms to chronic rhinosinusitis. *Curr Opin Otolaryngol Head Neck Surg*. 2008;16:237–241.
4. Palmer JN. Bacterial biofilms: do they play a role in chronic sinusitis? *Otolaryngol Clin North Am*. 2005;38:1193–1201.
5. Suh JD, Ramakrishnan V, Palmer JN. *Biofilms. Otolaryngol Clin North Am*. 2010;43:521–530.
6. Kilty SJ, Desrosiers MY. The role of bacterial biofilms and the pathophysiology of chronic rhinosinusitis. *Curr Allergy Asthma Rep*. 2008;8:227–233.
7. Kilty SJ, Desrosiers MY. Are biofilms the answer in the pathophysiology and treatment of chronic rhinosinusitis? *Immunol Allergy Clin North Am*. 2009;29:645–656.
8. Hall-Stoodley L, Stoodley P. Evolving concepts in biofilm infections. *Cell Microbiol*. 2009;11:1034–1043.
9. Wang L, Belisle B, Bassiri M,et al. Chemical characterization and biological properties of NVC-422, a novel, stable N-chlorotaurine analog. *Antimicrob Agents Chemother*. 2011;55:2688–2692.
10. Celeri C, Debabov D, Flagella M,et al. *Biological characterization of N,N-dichloro-2,2-dimethyltaurine (NVC-422)*. Poster presented at the American Society for Microbiology and the Infectious Diseases Society of America, 48th Annual ICAAC/IDSA 46th Annual Meeting, Washington, DC, October 25-28, 2008. Emeryville, CA: NovaBay Pharmaceuticals, Inc.; 2008.
11. 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;126:41–47.
12. Ha KR, Psaltis AJ, Tan L, Wormald PJ. A sheep model for the study of biofilms in rhinosinusitis. *Am J Rhinol*. 2007;21:339–345.
13. Psaltis AJ, Ha KR, Beule AG, Tan LW, Wormald PJ. Confocal scanning laser microscopy evidence of biofilms in patients with chronic rhinosinusitis. *Laryngoscope*. 2007;117:1302–1306.
14. Heydorn A, Ersbøll BK, Hentzer M, Parsek MR, Givskov M, Molin S. Experimental reproducibility in flow-chamber biofilms. *Microbiology*. 2000;146(Pt 10):2409–2415.
15. Heydorn A, Nielsen AT, Hentzer M,et al. Quantification of biofilm structures by the novel computer program COMSTAT. *Microbiology*. 2000;146:2395–2407.
16. Ha KR, Psaltis AJ, Butcher AR, Wormald PJ, Tan LW. In vitro activity of mupirocin on clinical isolates of *Staphylococcus aureus* and its potential implications in chronic rhinosinusitis. *Laryngoscope*. 2008;118:535–540.
17. Le T, Psaltis A, Tan LW, Wormald PJ. The efficacy of topical antibiofilm agents in a sheep model of rhinosinusitis. *Am J Rhinol*. 2008;22:560–567.
18. Uren B, Psaltis A, Wormald PJ. Nasal lavage with mupirocin for the treatment of surgically recalcitrant chronic rhinosinusitis. *Laryngoscope*. 2008;118:1677–1680.
19. Chiu AG, Palmer JN, Woodworth BA,et al. Baby shampoo nasal irrigations for the symptomatic postfunctional endoscopic sinus surgery patient. *Am J Rhinol*. 2008;22:34–37.
20. Desrosiers M, Myntti M, James G. Methods for removing bacterial biofilms: in vitro study using clinical chronic rhinosinusitis specimens. *Am J Rhinol*. 2007;21:527–532.
21. Patel JB, Gorwitz RJ, Jernigan JA. Mupirocin resistance. *Clin Infect Dis*. 2009;49:935–941.