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CONFLICT OF INTEREST

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REFERENCES

1. Eckert L, Gupta S, Gadkari A, et al. Burden of illness in adults with atopic dermatitis: analysis of national health and wellness survey data from France, Germany, Italy, Spain, and the United Kingdom. *J Am Acad Dermatol*. 2019;81:187-195.
2. Silverberg JI. Comorbidities and the impact of atopic dermatitis. *Ann Allergy Asthma Immunol*. 2019;123:144-151.
3. Patel KR, Immaneni S, Singam V, et al. Association between atopic dermatitis, depression, and suicidal ideation: a systematic review and meta-analysis. *J Am Acad Dermatol*. 2019;80:402-410.
4. Gupta MA. Somatization disorders in dermatology. *Int Rev Psychiatry*. 2006;18:41-47.
5. Prasad KM, Desai G, Chaturvedi SK. Somatization in the dermatology patient: some sociocultural perspectives. *Clin Dermatol*. 2017;35:252-259.
6. Lee S-C. Various diagnostic criteria for atopic dermatitis (AD): a proposal of reliable estimation of atopic dermatitis in childhood (REACH) criteria, a novel questionnaire-based diagnostic tool for AD. *J Dermatol*. 2016;43:376-384.

SUPPORTING INFORMATION

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In vitro safety and anti-bacterial efficacy assessment of acriflavine

To the Editor,

Chronic rhinosinusitis (CRS) is a complex sinus disease defined as inflammation of the nasal mucosa and paranasal sinuses.[1] It has been shown that the bacterial biofilm formation is one of the major factors involved in recalcitrant CRS.[2] The most frequently isolated biofilm-forming species in patients with CRS are *Staphylococcus*

Please contact Shari Javadiyan if further documents/editing is required.

Shari Javadiyan and Kitty C. Germein Equal contributions.

aureus and *Pseudomonas Aeruginosa*, and both appear to be associated with more severe and surgically recalcitrant disease.[3] The biofilm extracellular polymeric substances (EPS) and the slow growth rate of biofilm bacteria both contribute to a biofilm's inherent antibiotic resistance, thought to be 10- 1000x that of their planktonic counterparts.[4,5] The utilization of oral antibiotics against this condition is limited by low-quality supporting evidence when weighed against the risk of adverse effects and antibiotic resistance. Novel anti-biofilm drugs that could be topically applied to the nasal mucosa safely and without promoting antibiotic resistance could address multiple current unmet needs.

Acriflavine is a topical antiseptic that was first reported in the literature as a medicine in the early 20th century and used in the treatment of wounds.[6] However, due to the antibiotic discovery revolution, the use of acriflavine has been very limited for the past 50 years. Acriflavine interacts with DNA through its insertion

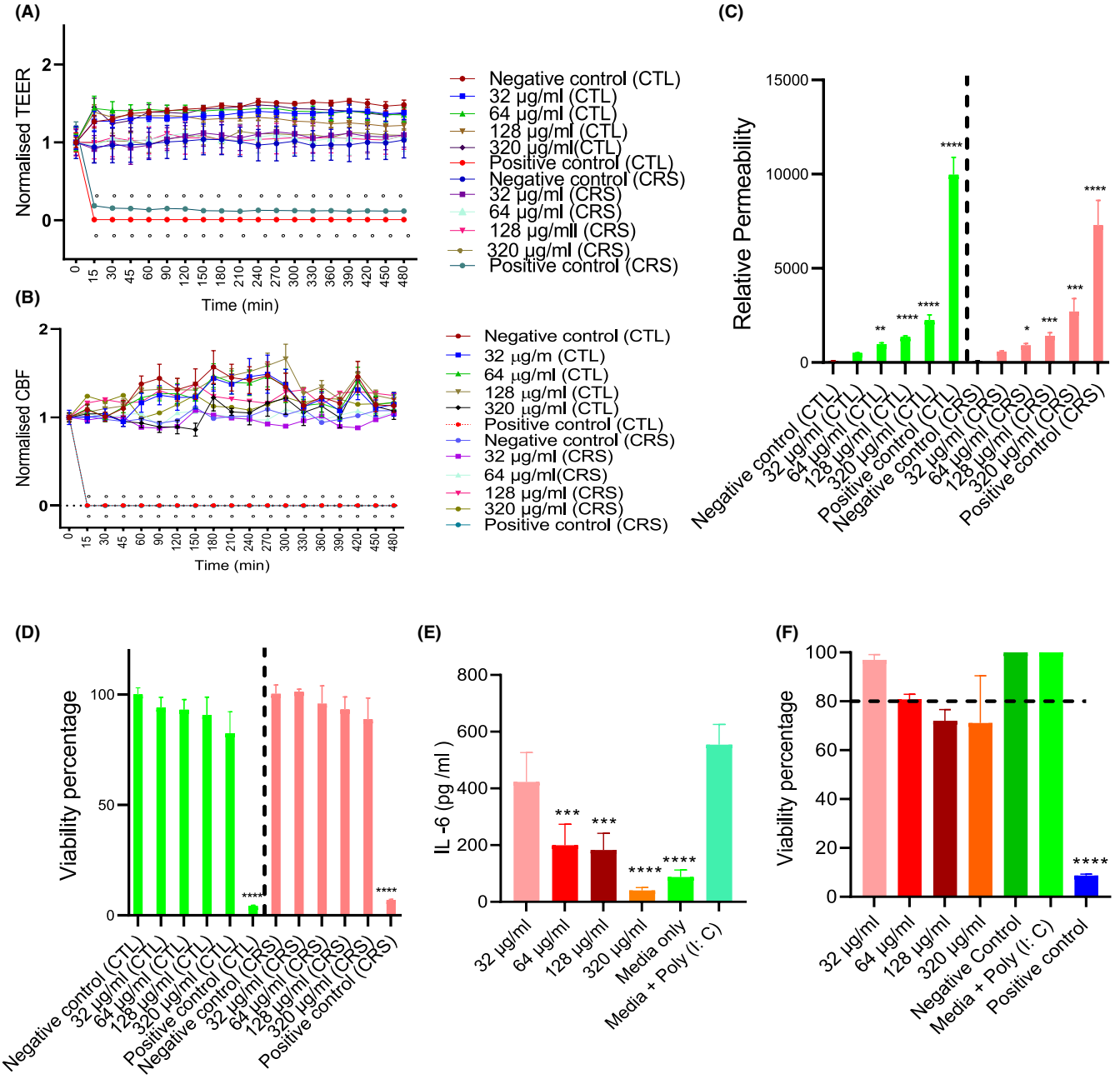

FIGURE 1 Impact of varying concentrations of acriflavine on ALI-HNEC. Negative control, acriflavine and positive control were applied to the control and CRS cells for 8 hours followed by measurement of TEER (A) or CBF (B) values or the passage of FITC-dextran (C). The viability was determined by the LDH assay (D). The secretion of IL-6 and cytotoxicity were measured on samples from the basal chamber of the treated CRS cells (E and F). The values are shown as means ±SD ($n = 6$). TEER and CBF values were normalized against time 0. * and ˚ indicate statistical significance. HNEC, human nasal epithelial cells; LDH, lactate dehydrogenase; ALI, air–liquid interface; CTL, control; CRS, chronic rhinosinusitis; SD, standard deviation. Positive control =10% triton X-100 in ALI medium; negative control =ALI medium

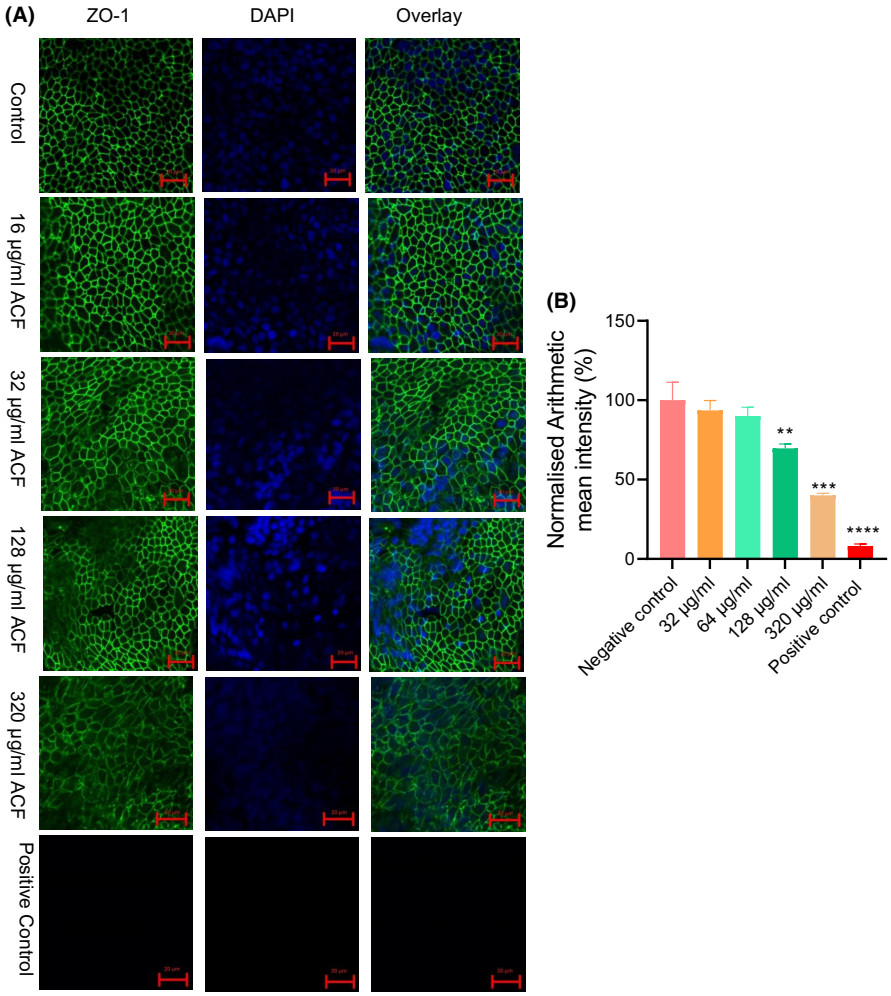

FIGURE 2 Effect of acriflavine treatment on the localization of ZO-1 in ALI HNECs. ZO-1 and DAPI stained green and blue, respectively (A). Imaging was performed using 20x objective power and immunofluorescence confocal laser scanning microscopy (LMS700) 8 hours after application of acriflavine (red bar =20 μm). Arithmetic mean intensity of ZO-1 was normalized against DAPI and reported as a percentage in comparison with untreated controls (B). DAPI =4′,6-diamidino-2-phenylindole; HNEC =human nasal epithelial cell; ZO-1 = zona occludens-1; ALI: air–liquid interface). Positive control =10% triton X-100 in ALI medium; negative control =ALI medium. * indicates statistical significance

between base pairs. This non-covalent fashion of DNA intercalating is mutagenic in bacteria through inhibition of DNA replication and transcription, thus interfering with cell division and growth.[7] Acriflavine's theoretical promise in CRS stems from two factors. The first is its connection to one of the disease's pathophysiological components, bacterial biofilm; the second is the potential to avoid exacerbating antibiotic resistance. The current study aimed to assess acriflavine's anti-bacterial/biofilm properties when used against Methicillin-resistant *Staphylococcus aureus* (MRSA) and *P. aeruginosa* clinical isolates from recalcitrant CRS patients in their planktonic and biofilm stages and to investigate *in vitro* safety application of acriflavine on human nasal epithelial cells (HNECs).

Minimum inhibitory concentration (MIC) of acriflavine against clinical isolates of MRSA and *P. aeruginosa was* determined to be 32 μg/ml (Figure S1A,B). The minimum biofilm eradication concentration (MBEC) was determined (320 μg/ml) using AlamarBlue (Figure S1C,D) and crystal violet assays (Figure S1E,F), which resulted in the significant killing of biofilms and reduction in biofilm biomass of matured biofilm ($p < .05$, Figure S1). Detailed methodology is included in Appendix S1.

Trans-epithelial electrical resistance (TEER, Figure 1A) and the fluorescein-isothiocyanate labelled dextran assay (FITC, Figure 1C) were utilized to assess the effect of acriflavine on integrity of tight junctions of human nasal epithelial cells using air–liquid interphase (ALI) culture. The application of acriflavine to HNECs at concentrations up to 320 μg/ml did not cause a significant difference in the TEER (Figure 1A), cilia beat frequency (Figure 1B) or caused any cytotoxicity as measured by lactate dehydrogenase assay (Figure 1D). However, at concentrations ≥64 μg/ml, an increase in paracellular permeability detected via FITC assay (Figure 1C) and an altered ZO- pattern of expression (Figure 2) was observed. Acriflavine demonstrated an anti-inflammatory effect at concentrations ≥64 μg/ml and caused a decrease in Interleukin 6 (IL-6) secretion in inflammation induced cells (Figure 1E,F). Detailed methodology is included in Appendix S1.

We have shown anti-bacterial and anti-biofilm properties of acriflavine when applied against MRSA and *P. aeruginosa* clinical isolates from recalcitrant CRS patients. For the first time, we have demonstrated the non-cytotoxic effect of acriflavine on HNECs in an *in vitro* setting, with preservation of CBF and epithelial integrity at concentrations ≤32 μg/ml. Whilst we also observed additional anti-inflammatory at concentrations ≥64ug/ml, there did appear to be disruption of tight junction proteins and increased paracellular permeability at these higher concentrations. The findings of this study support the possible utility of topical acriflavine for the treatment of recalcitrant CRS. The topical delivery of this agent

may circumvent the systemic side effects commonly seen with oral antibiotic therapy and address the significant issue to worldwide antibiotic resistance.

Limitations of this study include its sample size, in vitro nature and the use of poly-IC rather than the bacteria themselves as pro-inflammatory stimulants for nasal epithelium. This may not be completely reflective of what occurs in the in vivo setting, but we hope that it provides useful preliminary information for future in vivo animal and clinical trials to establish safe and effective dosing for topical acriflavine use in human CRS patients.

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REFERENCES

1. Orlandi RR, Kingdom TT, Hwang PH, et al. International consensus statement on allergy and rhinology: rhinosinusitis. *Int Forum Allergy Rhinol*. 2016;6(Suppl 1):S22-S209.
2. 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(7):1302-1306.
3. Cleland EJ, Bassiouni A, Wormald PJ. The bacteriology of chronic rhinosinusitis and the pre-eminence of *Staphylococcus aureus* in revision patients. Paper presented at: International forum of allergy & rhinology2013.
4. Römling U, Balsalobre C. Biofilm infections, their resilience to therapy and innovative treatment strategies. *J Intern Med*. 2012;272(6):541-561.
5. De la Fuente-Núñez C, Reffuveille F, Fernández L, Hancock RE. Bacterial biofilm development as a multicellular adaptation: antibiotic resistance and new therapeutic strategies. *Curr Opin Microbiol*. 2013;16(5):580-589.
6. Bond C. Acriflavine paste as a dressing for infected wounds. *BMJ*. 1917;2(2949):6.
7. Doudney C, White BF, Bruce BJ. Acriflavine modification of nucleic acid formation, mutation induction and survival in ultraviolet light exposed bacteria. *Biochem Biophys Res Comm*. 1964;15(1):70-75.

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