

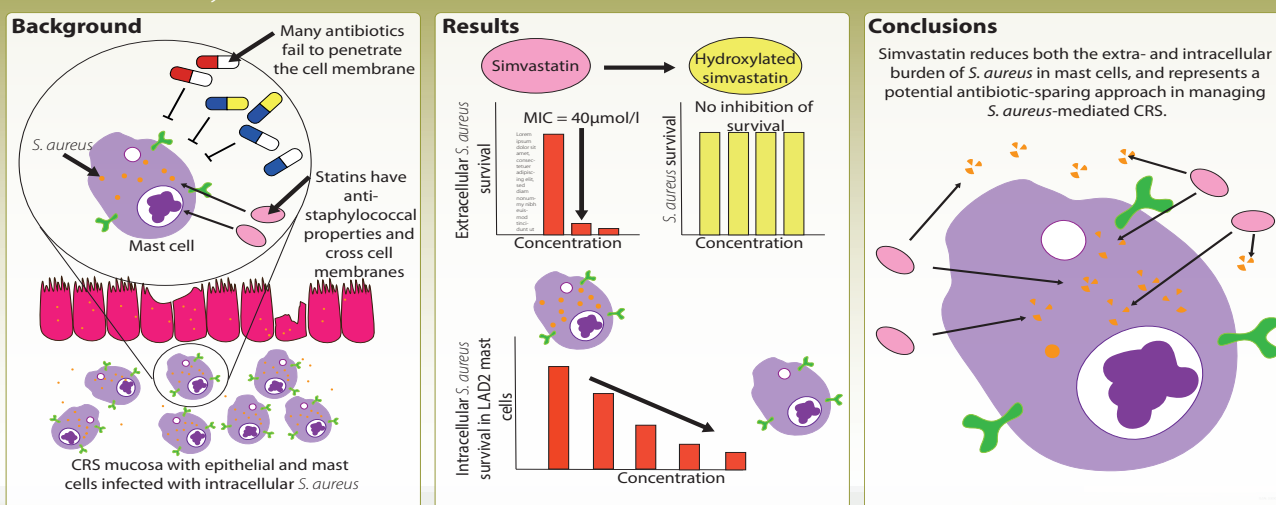
Antimicrobial activity of simvastatin against chronic rhinosinusitis-related *Staphylococcus aureus*: an in vitro study

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Antimicrobial activity of simvastatin against chronic rhinosinusitis-related *Staphylococcus aureus*: an in vitro study



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Abstract

Introduction: *Staphylococcus aureus* (*S. aureus*) in chronic rhinosinusitis (CRS), particularly when localised intracellularly, is linked to disease recalcitrance and poor post-surgical outcomes. Antibiotics frequently fail to penetrate the mammalian cell membrane, resulting in an inability to address the intracellular component of *S. aureus*. This contributes to treatment failure and development of antimicrobial resistance. We investigated the antimicrobial effects of simvastatin, a widely used, inexpensive medication with extracellular and intracellular antimicrobial properties, against CRS-related *S. aureus*.

Methods: Simvastatin's antimicrobial activity, in prodrug and hydroxy acid forms, was assessed against *S. aureus* using the broth dilution method to determine the minimal inhibitory concentration (MIC). Intracellular activity of simvastatin was evaluated by pre-treating *S. aureus*-infected LAD2 mast cells with simvastatin and performing colony forming unit (CFU) enumeration and confocal microscopy. Cell viability was assessed using lactate dehydrogenase (LDH) assays.

Results: Simvastatin exhibited an extracellular MIC of 40 µmol/l against *S. aureus*. Intracellularly, it significantly reduced the bacterial burden by 46-fold in a dose-dependent manner between concentrations of 0.1–100 µmol/l. Toxicity to LAD2 cells was observed at 100 µmol/l. Confocal microscopy revealed a lower percentage of infected cells in the group pretreated with 30 µmol/l simvastatin (15.3%) compared to untreated cells (32.8%). Simvastatin hydroxy acid demonstrated no antimicrobial activity against *S. aureus*.

Conclusions: Simvastatin demonstrates in vitro antimicrobial activity against CRS-related *S. aureus* with the potential for repurposing as a novel antibiotic-sparing topical agent for the treatment of refractory CRS. This could improve surgical outcomes and reduce the risk of antimicrobial resistance.

Key words: drug resistance, bacterial, drug repositioning, rhinosinusitis, simvastatin, *Staphylococcus aureus*

Introduction

Staphylococcus aureus (*S. aureus*) colonises the nasal cavity in 64% of patients with chronic rhinosinusitis and nasal polyps (CRSwNP) compared with 33% of those without polyps (CRS-sNP) and 20% in those without disease^(1,2). Culture of *S. aureus* pre- and post-operatively in patients with chronic rhinosinusitis (CRS) is a poor prognostic indicator for disease recurrence and recalcitrance⁽³⁾. *S. aureus* can persist in the nasal cavity of CRS patients, evading the immune system and the effects of antimicrobials⁽⁴⁾. This is achieved through internalisation within host cells, sequestering it within the intracellular space or by creating extracellular biofilms^(5,6). In 2015, our group made the novel observation that *S. aureus* internalises within mast cells in nasal polyps, serving as a reservoir of bacteria that seeds the extracellular space and perpetuates chronic inflammation in CRSwNP patients⁽⁷⁾. Intracellular *S. aureus* is challenging to treat, as the mammalian cell wall prevents diffusion of many commonly used antibiotics⁽⁸⁾. Furthermore, intracellular *S. aureus* often forms small colony variants (SCVs), which exhibit reduced metabolism and increased cell wall thickness⁽⁹⁾. Consequently, anti-metabolic antibiotics have limited efficacy on these resistant variants. In CRS, *S. aureus* appears to exist extracellularly and can transition phenotype into an intracellular SCV within epithelial and mast cells in the nasal mucosa^(10,11). Interestingly, *S. aureus* cultured from antibiotic-treated tissue and nasal swabs of the middle meatus demonstrate identical genotypes, suggesting the extracellular bacteria can switch phenotype to thrive within cells⁽¹⁰⁾. Furthermore, a significant association has been observed between the presence of intracellular *S. aureus* in the nasal mucosa and the need for revision endoscopic sinus surgery, with patients harbouring intracellular *S. aureus* at a higher risk of requiring additional surgery compared to those without (85% vs 33%, $P=0.0083$)⁽⁵⁾. Consequently, intracellular *S. aureus* in CRS is commonly associated with refractory disease and antibiotic resistance often resulting in the need for multiple surgical procedures.

Given the challenges associated with intracellular *S. aureus* persistence and antibiotic resistance, alternative therapeutic strategies are being explored. Statins, widely used for their lipid-lowering effects, are now being investigated for diverse therapeutic applications, including cancer prevention⁽¹²⁾, neuroprotection in Parkinson's disease⁽¹³⁾, treatment of chronic obstructive pulmonary disease⁽¹⁴⁾, and as an antimicrobial-sparing therapy for tuberculosis⁽¹⁵⁾. Notably, their potential role in *S. aureus*-related conditions is gaining interest with studies showing efficacy in treatment of *S. aureus* pneumonia, skin wound infections and biofilm formation on simulated joint implants in rats⁽¹⁶⁻¹⁹⁾. In relation to CRS, regular statin use has been associated with a reduced incidence in two large-scale studies. Gilani et al. retrospectively analysed 10,965 patients and demonstrated a reduced odds ratio (OR) of being diagnosed with CRS (0.716; 95%

CI, 0.612–0.838) among those taking statin medications⁽¹²⁾. Similarly, Wilson et al. demonstrated a reduced OR of CRS for patients taking statins on univariate (0.53; $P<0.001$) and multivariate (0.79; $P=0.03$) regression analyses using over 10 million records from the National Ambulatory Medical Survey of North America^(20,21). Lipophilic statins including simvastatin, atorvastatin, lovastatin and fluvastatin have the capacity to cross cell membranes and have exhibited anti-bacterial properties both extra- and intracellularly⁽²²⁾. In vitro studies have shown these statins to be active against *S. aureus* at various concentrations, however simvastatin demonstrates particularly potent activity characterised by the lowest observed minimal inhibitory concentration (MIC) ranging between 16 to 63 mg/L⁽²²⁾. In vivo studies have demonstrated that topical treatment of MRSA-infected mice wounds with simvastatin reduces the bacterial load and significantly improves wound healing with reductions in pro-inflammatory cytokines IL-6, TNF- α and IL-1 β ^(23,24).

Lipophilic statins such as simvastatin are administered orally as an inactive prodrug which is metabolised in the liver into its active β -hydroxy acid form. Simvastatin is 95% protein bound and 5% is free in the serum and eliminated by hepatic metabolism^(22,25). They mediate their effects through inhibition of the mevalonate pathway, which is essential for isoprenoid synthesis in humans and bacterial species, including *S. aureus*. By inhibition of 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMG-CoA reductase), statins reduce cholesterol and isoprenoid synthesis required for protein prenylation⁽²⁶⁾. In bacterial cells, lipophilic statins decrease cholesterol, directly affecting bacterial growth and protein production via a reduction in signalling protein prenylation^(23,27). They also show broad antimicrobial effects when directly applied to bacteria including virulent strains of *S. aureus*, as well as methicillin and vancomycin resistant strains^(23,26,28).

In mammalian cells, statins reduce cholesterol in lipid rafts, diminishing areas involved in bacterial translocation and intracellularisation, as well as the pro-inflammatory response associated with it. They also appear to modulate mast cell signalling, reducing degranulation in response to IgE-dependent stimulation and protecting cells from the effects of bacterial toxins⁽²⁹⁻³²⁾. Simvastatin is one of the most commonly used statins, well known to reduce the risk of coronary deaths, myocardial infarctions, ischemic strokes, and coronary revascularisation procedures, in patients with elevated LDL cholesterol with infrequent adverse effects reported, including myalgia, new-onset type 2 diabetes, and haemorrhagic stroke⁽³³⁾.

Given these findings, we hypothesised that simvastatin, with its low MIC, well-characterised pharmacokinetics and low-cost, may reduce the burden of intracellular *S. aureus* in CRS. This represents an exciting opportunity to develop a novel targeted topical therapy for intracellular *S. aureus* in patients with refractory CRS, which could also reduce our dependence on antibiotics and

the risk of antimicrobial resistance which has reached epidemic proportions worldwide.

Materials and methods

S. aureus receipt and culture

A well characterised strain of *S. aureus* ^(11,34-36), cultured from the intracellular space of polyp tissue from a patient with CRSwNP was used for further study. Ethical approval for the receipt of patient isolated strains of *S. aureus* was granted by Southampton and South-West Hampshire Research and Ethics committee (reference code: REC 09/HO501/74).

Minimal inhibitory concentration of prodrug and activated simvastatin

Prodrug simvastatin (Sigma-Aldrich) was dissolved in dimethylsulphoxide (DMSO) to create a 10mmol/l stock solution in 41.8% v/v DMSO. Activated simvastatin was prepared by dissolving 4mg of simvastatin in 100µL of ethanol and 150µl of 0.1M NaOH, followed by incubation at 50°C for 2 hours. The pH was adjusted to 7 and the total volume was made up to a 10mmol/l solution as described by McKay et al. ⁽³⁷⁾.

The MIC of prodrug and activated simvastatin against the CRSwNP strain of *S. aureus* was calculated using the international standard broth microdilution method ⁽³⁸⁾. *S. aureus* was grown to the exponential growth phase, with absorbance at 600nm extrapolated using absorbance vs colony forming unit (CFU) enumeration graphs and diluted to create a stock concentration of 10⁷ CFU/ml in Mueller Hinton broth, pH 7.0 (Sigma-Aldrich). Wells contained 90µl of Mueller Hinton broth (Sigma Aldrich) with serially decreasing concentrations of simvastatin. Each well was inoculated with 10⁵ CFUs of CRSwNP *S. aureus* and incubated at 37°C in the presence of 5% CO₂ for 16 hours. Absorbance was measured at 600nm using a microplate reader (Molecular Devices).

Intracellular survival of *S. aureus* in LAD 2 cells with simvastatin treatment

CRSwNP *S. aureus* was grown in RPMI 1640 (Fisher Scientific) at 37°C in the presence of 5% CO₂ to the exponential growth phase. Absorbance at 600nm was calculated and extrapolated using absorbance vs CFU enumeration graphs.

Laboratory of Allergic Diseases 2 (LAD2) human mast cells were grown in antibiotic-free conditions in StemPro-34 media (Life Technologies) containing 0.1mM Stem Cell Factor (SCF; Pepro-Tech). LAD2 cells (5x10⁵ cells in 1 ml) were pre-incubated with simvastatin at concentrations ranging from 0 - 100µmol/l for 16 hours. Each condition was co-cultured with RPMI 1640 (control) or CRSwNP *S. aureus* (5x10⁵ CFUs) and incubated for 6 hours. Cultures were centrifuged at 250g for 10 minutes and supernatants were collected for lactate dehydrogenase (LDH) assay.

Cell pellets were resuspended with 1ml 20µg/ml lysostaphin (Sigma-Aldrich) containing StemPro-34 media with 0.1mM SCF for 60 minutes. LAD2 cells were then centrifuged at 250g for 10 minutes and washed three times in antibiotic free media. Supernatants were streaked on Columbia blood agar (CBA) plates to ensure no growth. Pellets were resuspended in STEMPro-34 media with SCF and 0.5% Triton-X100, vortexed for 10 minutes and used for serial CFU assessments using CBA plates.

Lactate dehydrogenase assay

A colorimetric LDH cytotoxicity assay (Sciencell Research Laboratories, USA) was performed per the manufacturer's instructions. Control LAD2 cells (5x10⁵ cells) were incubated in media alone for 6 hours and centrifuged at 250g for 10 minutes, removing the supernatant to calculate the spontaneous release. Maximal release was calculated by lysing cells after centrifugation in the presence of 0.5% Triton X-100 containing media with vortexing and rolling for 30 minutes. The subsequent lysate was centrifuged for 10 minutes at 250g and the supernatant was extracted to determine maximal release.

For the assay, 150µl of controls and culture supernatants were plated in flat-bottom 96-well plates (Greiner Bio-One, Austria) and 60µL of reaction mixture was added. The reaction was incubated at room temperature in the dark for 20 minutes and the reaction was stopped using 20µl of sodium oxamate per well. Absorbance was measured at 490nm using a spectrophotometer (Molecular Devices). Net release was calculated by subtracting the spontaneous release from each value and dividing by maximal release to determine percentage LDH release.

Confocal microscopy

LAD2 cells were preincubated with simvastatin at concentrations of 0, 1, 30 and 50µmol/l for 16 hours, then co-cultured with CRSwNP *S. aureus* for 6 hours. Cells were resuspended in 0.5ml 20 µg/ml lysostaphin for 60 minutes and washed three times in calcium and magnesium free phosphate-buffered saline (PBS). Cells were resuspended in 15 µM Syto9 and 40µM propidium iodide in 1 ml PBS (Thermo-Fisher, UK). A 50µl aliquot of each suspension was placed on an Ibidi 8-well glass-bottom slide (Thistle Scientific, UK) and imaged using a Leica TCS SP5/8 inverted confocal microscope (Leica Microsystems, UK) with a 63x glycerol immersion lens. Images were collected with Leica LAS-AF software and analysed using Fiji 2 ⁽³⁹⁾.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 9 software (GraphPad Software Inc, USA). Data was assessed for normality using histogram plots and normality tests. One-way ANOVA tests with Tukey's multiple comparisons was used to compare data between experiments.

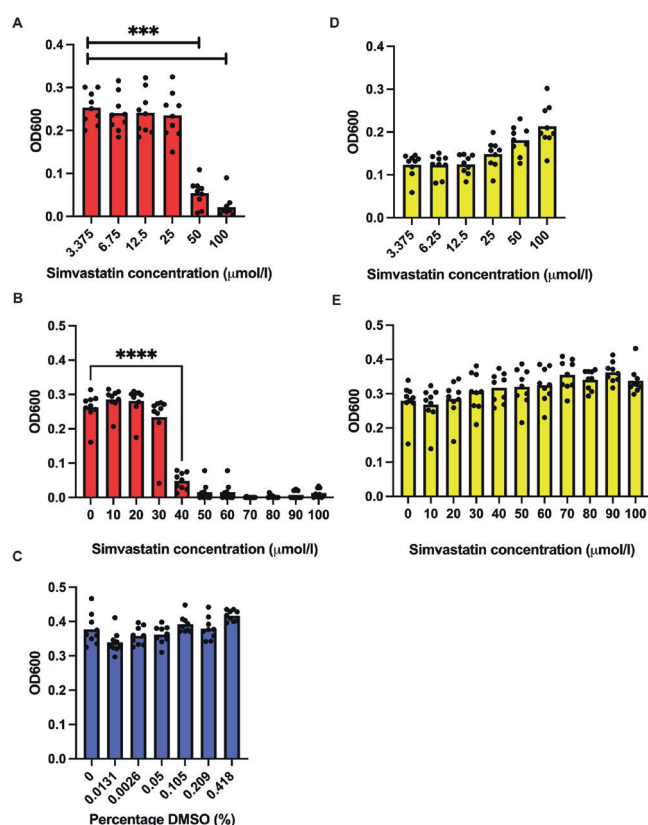


Figure 1. Minimal inhibitory concentration of prodrug and simvastatin hydroxy acid. Minimal inhibitory concentrations of simvastatin (A+B), DMSO (C) and simvastatin hydroxy acid (D+E) was calculated against CRS *S. aureus*. Optical density at 600nm was used as a measure of bacterial density. DMSO concentrations of 0.013, 0.026, 0.052, 0.105, 0.209, 0.418 v/v correspond to that used to dissolve 3.375, 6.75, 12.5, 25, 50, 100 μmol/L simvastatin, respectively. Nine experimental repeats were completed for each variable with statistical analysis using one-way ANOVA and Tukey's multiple comparisons test. Bars represent the mean of each experiment with dots showing the result of each experimental repeat (***) $P \leq 0.001$ **** $P \leq 0.0001$.

Results

Minimal inhibitory concentration of activated and prodrug simvastatin against *S. aureus*

To evaluate the concentration of simvastatin needed to inhibit the growth of CRS-related *S. aureus*, we performed MIC calculations using the internationally standardised broth dilution method (ISO 20776-1:2019) with a well-studied CRS strain of *S. aureus* (11, 38). Our results demonstrated a MIC of between 25-50 μmol (Figure 1A), further refined to 40 μmol (Figure 1B). As simvastatin was dissolved in DMSO, which has been shown to affect biofilm formation and bacterial growth, we performed a control experiment to rule out DMSO-related effects. The results indicated no significant bactericidal activity from DMSO alone at concentrations up to 0.418% v/v, corresponding to the 100

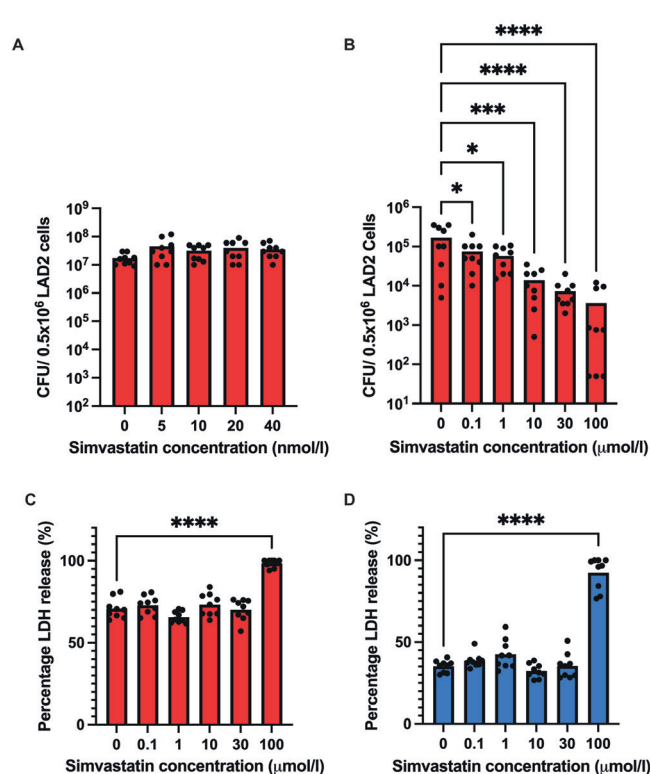


Figure 2. Intracellular infection of simvastatin pretreated LAD2 cells with *S. aureus*. Mean intracellular survival of CRSwNP *S. aureus* in co-culture with LAD2 cells pretreated with simvastatin for 16 hrs at typical serum (A) and topical application (B) concentrations. Mean of nine experimental repeats are displayed showing *S. aureus* CFU/ 5x10⁵ LAD2 cells represented by bars with each dot demonstrating the result of each experimental repeat. One-way ANOVA used with Tukey's multiple comparisons test used to determine statistical significance (* $P \leq 0.05$, ** $P \leq 0.01$, **** $P \leq 0.0001$). Net LDH release of *S. aureus* infected cells was measured for topical application concentrations (C) and net LDH release for uninfected cells exposed to identical topical application concentrations of simvastatin are displayed (D). Mean of nine experimental repeats are displayed showing percentage net LDH release represented by bars with the result of each experimental repeat demonstrated by dots. One-way ANOVA used with Tukey's multiple comparisons test used to determine statistical significance (**** $P \leq 0.0001$).

μmol/l simvastatin solution (Figure 1C).

Most simvastatin exists in its prodrug form (68-77%), with 95% bound to serum proteins (25). However, a small proportion is present in its hydroxy acid active form. As few studies have examined the antimicrobial activity of activated simvastatin, we converted simvastatin to its hydroxy acid form and repeated the MIC calculation. Activated simvastatin demonstrated no antimicrobial activity against the CRSwNP strain of *S. aureus* (Figure 1D,E).

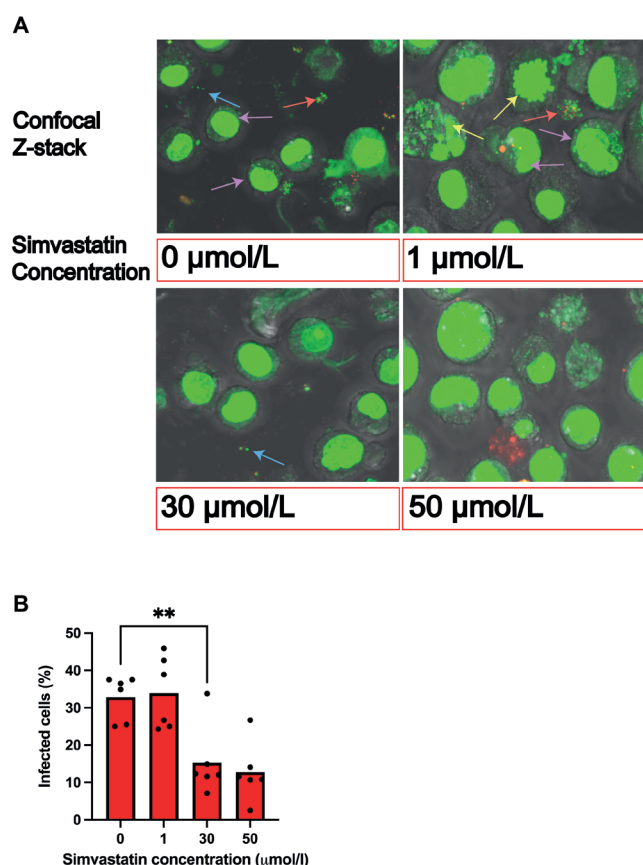


Figure 3. LAD2 mast cell infection with *S. aureus* after pre-treatment with simvastatin. LAD2 cells were pre-treated with simvastatin and subsequently cultured with CRS *S. aureus* for 6 hrs, followed by staining with BacLight™ LIVE/DEAD™ staining. A) Representative confocal z-stacks of each experimental condition are displayed. The percentage of infected cells was calculated from six separate z-stack images, each containing between 74–152 cells. Purple arrows indicate infected cells, blue arrows highlight extracellular bacteria, yellow arrows denote apoptotic cells, and red arrows show infected apoptotic cell bodies. B) A graphical representation of the percentage of infected cells. Bars represent the mean number of cells infected, with each dot demonstrating the percentage of infected cells in each experimental repeat. Statistical analyses were performed using one-way ANOVA and Tukey's multiple comparisons test (** $P \leq 0.01$).

Simvastatin at oral administration serum concentrations does not affect intracellular *S. aureus* survival

The concentrations of simvastatin used for MIC determination were beyond those typically observed in human serum. Nevertheless, given the hydrophobic and lipophilic properties of simvastatin which may cause it to localise to the cell membrane, we hypothesised that it might still exert activity at these concentrations in co-culture.

To test this, LAD2 mast cells were pre-treated for 16 hours with simvastatin at serum concentrations typically observed in patients taking the drug orally (19–31 nmol) ⁽²⁵⁾. The mast cells were

then inoculated with *S. aureus*. Our results showed no significant effect on intracellular *S. aureus* survival at simvastatin concentrations of 0–40 nmol/l (Figure 2A).

Simvastatin at topical administration concentrations reduces intracellular *S. aureus* survival

As simvastatin showed no effect at oral administration serum concentrations, we tested higher concentrations that could be achieved topically, as described by Horn et al. and Thangamani et al. to evaluate intracellular antimicrobial activity and cellular toxicity ^(23, 40). A sequential reduction in intracellular survival was observed, ranging from 1.7×10^5 to 3.6×10^3 CFUs, between simvastatin concentrations of 0 to 100 µmol/l (Figure 2B).

Cytotoxicity assays

To assess cytotoxicity, LDH assays were performed on supernatants. LAD2 cells treated with CRS *S. aureus* showed similar, non-significant changes in LDH release between 0–30 µmol/l concentrations of simvastatin (65.5–73.1%). However, at concentrations of 100 µmol/l, LDH release increased (98.4%; $P \leq 0.0001$) (Figure 2C). A parallel experiment using uninfected LAD2 cells treated with simvastatin for 6 hours confirmed no significant toxicity below 30 µmol/l with LDH release around 32.3–42.4% which rose to 92.3% at 100 µmol/l concentrations ($P \leq 0.001$). As the LDH levels were stable below 30 µmol/l, these findings suggest that the reduction in intracellular *S. aureus* survival was due to simvastatin reducing bacterial internalisation and intracellular survival rather than the number of viable host cells (Figure 2D). Furthermore, simvastatin appeared toxic to LAD2 cells at concentrations of 100 µmol/l.

Confocal microscopy demonstrates reduced LAD2 cell infection in simvastatin-treated LAD2 cells

To validate these findings and determine whether the reduction in intracellular CFUs was due to there being fewer infected cells, we used confocal microscopy and BacLight™ LIVE/DEAD™ imaging. LAD2 cells were pretreated with 0, 1, 30 and 50 µmol/l simvastatin and co-cultured with CRS-related *S. aureus*. At simvastatin concentrations of 0 and 1 µmol/l, 32.8–33.9% of LAD2 cells were infected, compared to 15.3–17.1% at 30, and 50 µmol/l ($P \leq 0.01$; Figure 3). These results confirmed a significant reduction in infection rate with increasing simvastatin concentrations.

Discussion

Statins have been shown to possess significant anti-staphylococcal properties, with patients taking oral statins demonstrating a significantly reduced odds ratio of being diagnosed with CRS ^(20, 21). Based on this, we hypothesised that statins could potentially be repurposed as a novel anti-staphylococcal treatment to reduce dependence on antibiotics in recalcitrant *S. aureus*-related CRS. To explore this possibility, we focused on

simvastatin, a widely prescribed statin with a lower MIC against *S. aureus* compared to other statins^(22,41).

In our study, we determined the MIC of prodrug simvastatin against a well-characterised, virulent CRSwNP isolate of *S. aureus* to be below 40 µmol/l, consistent with previously reported MIC values ranging from 38.1–398 µmol/l (16–167 mg/l)^(22,23,26,28,42,43). However, this concentration far exceeds the levels typically observed in the serum of patients taking oral simvastatin (19–31 nmol/l)⁽²⁵⁾. We also found that the hydroxy acid form of simvastatin exhibited no direct antimicrobial activity. Previous studies have shown that simvastatin reduces intracellular translocation and survival of *S. aureus* in HEK293A epithelial cells at concentrations of 0.1–1 µmol/l, in a process that could be reversed by addition of HMG-CoA reductase products⁽⁴⁰⁾. Similarly, we found that simvastatin significantly reduced intracellular *S. aureus* in LAD2 mast cells at concentrations of 1–100 µmol/l. At 30 µmol/l, simvastatin reduced the percentage of infected cells from 32.8% vs 17.1%, highlighting its potential to inhibit bacterial internalisation and survival.

The mechanisms underlying this effect likely involve the inhibition of HMG-CoA reductase, which produces cholesterol and isoprenoids in mammalian and bacterial cells^(22,40). Cholesterol is a major component of lipid raft domains, which act as docking sites for bacteria, and facilitate energy-efficient endocytosis^(44,45). By reducing cholesterol, simvastatin may disrupt lipid raft domains, potentially impairing *S. aureus* internalisation. Furthermore, simvastatin inhibits the prenylation of small GTPases including CDC42 and Rac preventing their localisation to the cell membrane and p85 and PI3K activation of actin-mediated caveolation and endocytosis^(40,46,47).

S. aureus has an HMG-CoA reductase enzyme (*mvaA*) which is essential for its survival and is inhibited by statins, such as fluvastatin⁽²²⁾. Statins reduce the production of isoprenoid intermediates involved in prenylation, a critical post-translational protein modification of bacterial toxins, antibiotic efflux pumps and cell wall components which are essential for bacterial survival, growth and antimicrobial resistance⁽¹²⁾. Simvastatin has been shown to reduce *S. aureus* toxin production, including panton-valentine leukocidin and α -haemolysin at concentrations similar to those tested in our study⁽⁴³⁾. Alpha-haemolysin plays a critical role in intracellular translocation by assisting escape from phagosomes^(48,49). Recent reports have shown that statins induce disassembly of functional membrane microdomains in MRSA which stabilise proteins during infection via recruitment of flotilin. This leads to denatured antimicrobial resistance proteins such as penicillin binding protein 2a and accumulation of unfolded proteins, which affect bacterial cell viability and induce penicillin susceptibility⁽⁵⁰⁾.

Given its well-established safety profile, affordability and ease of manufacture, these preliminary findings support the potential for simvastatin to be repurposed as a novel topical

anti-staphylococcal agent for use in refractory *S. aureus*-related CRS. The accessibility of the nasal cavity to topical treatments such as creams, ointments, sprays and drops, further supports the feasibility of achieving the required concentrations for anti-staphylococcal effects.

This study has some limitations. Statins have been reported to reduce IgE-mediated signalling in RBL-2H3 cell lines, leading to reduced degranulation and potentially reduced bacterial entry via membrane recycling^(30,51). We were unable to evaluate this mechanism due to a common loss-of-function mutation in the high affinity receptor for IgE in LAD2 cells. Nevertheless, we tested the MRGPRX2 receptor which uses similar signalling pathways and found no effect of simvastatin on degranulation (data not included). Whilst tissue-derived nasal polyp mast cells could have been used, these are notoriously difficult to isolate with a typically low yield and inter-patient heterogeneity. This would have rendered this approach both costly and impractical. Delivering lipophilic simvastatin to the sinuses at therapeutically relevant concentrations is likely to be problematic. While simvastatin ointments at 1% and 3% have been formulated and tested on human skin in previous trials, delivering this at optimal antimicrobial concentrations in a high-volume nasal saline rinse may be more challenging⁽⁵²⁾.

Evidently clinical validation of these in vitro findings, including safety and tolerability profiles, will be required. Future in vivo antimicrobial efficacy clinical studies in patients with *S. aureus*-related CRS will be needed to prove therapeutic efficacy. Investigation of the absorption distribution and retention of topically applied simvastatin to the nasal mucosa will be also required to establish optimal dosing strategies. These studies are currently underway, and the results will be reported in due course.

Conclusion

At typical serum concentrations observed in patients taking oral simvastatin, neither the prodrug nor hydroxy acid forms of simvastatin exhibited significant anti-staphylococcal effects. However, at concentrations achievable through the topical application route, simvastatin demonstrated a direct anti-staphylococcal effect. Treatment of mast cells with simvastatin significantly reduced both the *S. aureus* intracellular burden and the proportion of infected cells. Given the accessibility of the nasal cavity to topical treatments, topical simvastatin offers a promising approach for treating refractory *S. aureus*-related CRS, and could help reduce the need for revision sinus surgery and the risk of antimicrobial resistance which has reached epidemic proportions globally.

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Authorship contribution

SPG, AFW, RJS contributed to study conception and design. RJS, PGH, HASJ were responsible for retrieving bacterial samples. Sample preparation, data collection and analysis were performed by SPG, LCL. The first and final draft of the manuscript was written by SPG and all authors advised on previous drafts. All authors approved the final manuscript.

Conflict of interest

The authors have no conflicts of interest to disclose.

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Availability of data and materials

The datasets generated and analysed during the current study are available in the University of Southampton Institutional Repository, <https://eprints.soton.ac.uk/490561/>.

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