Original Article

Combination effects of baicalin with levofloxacin against biofilm-related infections

Zhongye Du1*, Yingying Huang1*, Yan Chen2, Yiqiang Chen1

1Department of Pulmonary and Critical Care Medicine, First Affiliated Hospital of Guangxi Medical University, Guangxi Medical University, Nanning, China; 2Department of Respiratory Disease, Second Affiliated Hospital of Guangxi Medical University, Guangxi Medical University, Nanning, China. *Equal contributors.

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Abstract: It is important to improve the existing techniques and develop new strategies to prevent bacterial biofilm formation. In this in vitro study, biofilms were established by a clinically isolated strain of Staphylococcus aureus 17546 (t037). Different concentrations of baicalin were added to 3- and 7-day biofilms. Based on colony counts and quantitative analysis of the biomass, sub-minimum inhibitory concentrations (sub-MICs) (1024, 512 or 256 μg/mL) of baicalin clearly decreased the number of bacterial colonies and biomass in vitro. Fluorescence microscopy revealed that sub-MICs (1024, 512, or 256 μg/mL) of baicalin inhibited bacterial adherence to the carrier surface and decreased polysaccharide production. Moreover, baicalin disrupted biofilms and exhibited synergistic effects with levofloxacin. Virulence factors were assessed by western blotting and real-time quantitative polymerase chain reaction, confirming that staphylococcal enterotoxin A, α-haemolysin and coagulase production decreased after baicalin treatment. Additionally, baicalin increased production of thermonuclease in S. aureus, and baicalin at 1024 and 512 μg/mL downregulated agrA expression. Based on these findings, the combination of baicalin with levofloxacin might be a new, feasible strategy for treating S. aureus biofilm-related infections. Baicalin may serve as a new inhibitor that modulates S. aureus virulence factors.

Keywords: Biofilm, staphylococcus aureus, baicalin, virulence factors

Introduction

Antimicrobial efficacy in Staphylococcus aureus biofilm-related infections is decreasing because due to the high tolerance of biofilms to antibiotics. Biofilm formation is a protective mechanism that prevents bacteria from eradication. Indwelling medical devices [1-3] and infected lung, trachea, and urinary tract tissues serve as suitable sites for biofilm formation, and once established, the dose of antibiotic required increases multifold [4]. Novel strategies are therefore required to address such biofilm-related infections.

The formation of biofilm by S. aureus is regulated by the quorum sensing (QS) system [5], a universal mechanism for the transmission of information between bacterial cells [6-8]. Bacteria can regulate the density and behaviours of the entire colony by synthesizing and secreting signalling molecules (also called self-inducers) [9]. When these signalling molecules accumulate to a certain threshold, expression of certain specific genes is activated, and some regulatory proteins are secreted [10]. The Agr system is a major density signal induction system in S. aureus [11] that regulates not only the growth of the entire biofilm but also the production and release of virulence factors, such as polysaccharide intercellular adhesin (PIA), phenol-soluble modulin peptide, and enterotoxin. Therefore, the status of the regulatory QS system in S. aureus is indirectly reflected by the levels of virulence factors produced.

Baicalin (a major constituent of the roots of Scutellaria baicalensis) is widely used clinically for treating fever, bronchitis and upper respiratory tract infections [12, 13]. Baicalin also exerts antifungal activity against Candida albicans, antiviral activity against enteroviruses and antibacterial activity against methicillin-resistant S. aureus. In addition, baicalin was reportedly inhibits acyl homoserine lactone (AHL)-based QS-regulated gene expression in
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Burkholderia cenocepacia [14]. However, there is no specific evidence to date demonstrating the effects of baicalin on S. aureus biofilm morphology or combinatorial effects with other classes of antibiotics, such as fluoroquinolones. Furthermore, data regarding the effects of baicalin on QS-controlled virulence and gene expression in S. aureus are still lacking. Thus, the aim of our research was to establish the antibiofilm effect of baicalin on S. aureus and determine its effects on virulence factor and agr gene expression.

Materials and methods

Bacterial strains and reagents

S. aureus 17546 (t037) and standard S. aureus ATCC 29213 (supplied by The First Affiliated Hospital of GuangXi Medical University) strains were selected for this in vitro study. Baicalin, clarithromycin (CLR) and levofloxacin (LEV) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and were dissolved in dimethyl sulfoxide (DMSO, Amresco, Solon, OH, USA).

Detection of MICs and growth curve assay

The minimum inhibitory concentrations (MICs) of baicalin, levofloxacin and clarithromycin were determined using the microtitre broth dilution method according to the Clinical and Laboratory Standards Institute (CLSI, 2012), and growth curves were generated based on spectrophotometry. Briefly, S. aureus was cultured overnight in tryptic soy broth (TSB) supplemented with 0.5% glucose (TSB-G, glucose is a known additive that positively impacts S. aureus biofilm formation [15, 16]) and diluted to an absorbance of OD600 = 0.05. Baicalin at sub-MICs (1024, 512, 256 or 128 μg/mL) were added to the cultures, followed by incubation at 37°C (200 rpm), absorbance at OD600 was measured every 2 hours.

Antibiofilm effect assay in vitro

Colony counts assay: The inhibitory and synergistic effects of the combination of baicalin with levofloxacin were evaluated using the plate counting method. A bacterial culture at an absorbance of OD600 = 0.1 was prepared, and 2 mL of the bacterial suspension with baicalin (final concentrations of 1024, 512, 256 or 128 μg/mL) were added to the cultures, followed by incubation at 37°C (200 rpm), absorbance at OD600 was measured every 2 hours.

Fluorescence microscopy assay: Biofilms were constructed as inhibitory effects assay, and planktonic bacteria were removed by washing three times with PBS. The carriers were stained with 2 mL SYTO-9 (Sigma, USA) in the dark for 15 min, and unbound dye was removed with PBS. Fluorescent images were acquired under a fluorescence microscope (Olympus, Japan).

Scanning electron microscopy (SEM)

S. aureus biofilms were grown as synergistic effects. After treatment with baicalin and antibiotics, carriers were washed with sterile saline and with 2.5% glutaraldehyde and then dehydrated in increasing concentrations of ethanol (70%, 80%, 90% and 100%). The carriers were dried at room temperature, coated with gold, and subsequently analysed by SEM (TM-1000, Hitachi, Tokyo, Japan) at 30 kV.
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Confocal laser scanning microscopy (CLSM)

Synergistic bactericidal effects were also observed by CLSM. Biofilms were established as above, and after incubation, each biofilm carrier was washed with sterile saline to remove planktonic bacteria. The carriers were stained with 2 mL of LIVE/DEAD Viability kit reagent (Invitrogen, MA, USA) in saline (0.9% NaCl) containing a mixture of SYTO-9 and propidium iodide (Sigma, USA, 1.5 μL/mL of each) and incubated for 15 min in the dark. A confocal laser scanning microscope (Nikon A1, Japan) was used to observe the biofilm, with green representing a live biomass and red a dead biomass.

Detection of virulence factors

Suspension separation: S. aureus 17546 was cultivated overnight, and the bacterial suspension was diluted to an absorbance of OD600 = 0.1, after which baicalin was added to final concentrations of 0, 1024, 512, 256 or 128 μg/mL. The suspensions were continuously cultured for 15 min in the dark. A confocal laser scanning microscope (Nikon A1, Japan) was used to observe the biofilm, with green representing a live biomass and red a dead biomass.

Detection of staphylococcal enterotoxin A (SEA)

The supernatant obtained above containing 30 mg protein was mixed 3:1 with sulfone polycrylamide gel electrophoresis (SDS-PAGE) loading buffer and denatured by boiling for 5 min. Then the samples were electrophoresed (constant voltage of 110 V for 2 hours) in a Mini-Protean II vertical dual-cell apparatus (Bio-Rad). The protein bands were stained with Coomassie brilliant blue R (Sigma, USA) and imaged. The gel was incubated with Towbin transfer buffer, and the proteins were transferred to a membrane (Pall Corporation USA), followed by blocking non-specific proteins. The membrane was probed with rabbit polyclonal antibodies and secondary antibodies (Sigma, USA) and rinsed three times with TBST for 10 min each. The immunoreactive bands were exposed to X-ray film, and densitometric analysis of the bands was performed using Quantity One (Bio-Rad, USA). The experiments were repeated three times.

Detection of α-haemolysin (hla) and agrA genes

Real-time quantitative polymerase chain reaction (RTQ-PCR) was used to determine the expression levels of the genes hla and agrA. The primers used are shown in Table 1.

RNA extraction and reverse transcription reaction

The bacterial pellet was washed with diethylpyrocarbonate (DEPC)-treated water and centrifuged at 10,000 rpm for 1 min (twice) at 4°C. The supernatant was discarded, and the bacterial pellet was suspended in 60 μL of lysozyme (10 mg/L, Sigma, USA) containing 5 μL of lyso-staphin (Sigma, USA) + TE buffer (pH 8.0) and incubated for 40 min at 37°C. The suspension was centrifuged at 12,000 rpm for 1 min at 4°C, and the supernatant was discarded. One millilitre of Trizol was added, and the pelleted was homogenized (5 min) with vigorous shaking for 15 seconds after the addition of 200 μL of chloroform. Next, the mixture was centrifuged at 12,000 rpm for 15 min at 4°C. The supernatant was transferred to a new tube, thoroughly mixed with 480 μL of isopropanol and shaken vigorously. The mixture was incubated at 4°C for 20 min and centrifuged at 12,000 rpm for 15 min. The supernatant was discarded, and the white pellet at the bottom of the tube was considered RNA. One millilitre of 75% ethanol was added to resuspend the RNA, followed by centrifugation at 12,000 rpm for 4 min and drying at room temperature. The concentration and purity of the total RNA sample were measured spectrophotometrically by calculating the 260/280 nm ratio (1.9-2.1 target), and the RNA was reverse transcribed into cDNA. A mixture of 2 μL of RNA treated with RNase-free DNase (Roche), 1 μL of oligo (dT)18 primers and DEPC water in a final volume of 12 μL was incubated at 65°C for 5 min and at 4°C for 5 min. Next, 4

Table 1. The primers for Real-time quantitative PCR

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<th>Primers</th>
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<th>Length</th>
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<td>83 bp</td>
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<tr>
<td>16sRNA reverse</td>
<td>CATGCTGATCACAGAAGCT</td>
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<td>Hla forward</td>
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<td>agrA forward</td>
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</tr>
<tr>
<td>agrA reverse</td>
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</table>
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μL of 5 × reaction buffer (40 mg/L), 1 μL of RNase inhibitor (20 U/μL), 2 μL of 10 mM dNTP mix, 1 μL of reverse transcriptase (200 U/μL) and DEPC water to a final volume of 20 μL were added, and the mixture was incubated at 42°C for 60 min and 70°C for 5 min and then cooled at 4°C. The RevertAid First Strand cDNA synthesis kit was used (Fermentas, Thermo Fisher Scientific, Waltham, MA, USA).

RTQ-PCR

The total reaction mixture of 25 μL contained 12.5 μL of SYBR® Premix Ex Taq™ (2 ×) (Sigma, USA), 0.5 μL of the forward primer, 0.5 μL of the reverse primer, 2 μL of cDNA templates and 9.5 μL of sterile DEPC water. The PCR programme involved pre-denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 94°C for 15 seconds, annealing at 58°C for 1 min and extension at 60°C for 1 min. FastStart Universal SYBR Green Master (Roche) and StepOne Plus Real-Time PCR System (Applied Biosystems, USA) were employed for amplification and detection. The results were analysed using the relative quantitative (2^ΔΔCt) method. The experiments were repeated three times.

Detection of S. aureus thermonuclease

Toluidine blue nucleic acid agar was prepared, and a puncher was used to generate a 3-mm hole. The supernatant was boiled for 15 min and incubated in the hole at 37°C for 16 hours. The diameter was measured for comparison (a diameter >1 mm indicates a positive result).

Detection of S. aureus coagulase

Four millilitres of fresh rabbit blood [17] was centrifuged at 3,000 rpm for 10 min; the cells pelleted were discarded, and the plasma was diluted with PBS (1:4). A bacterial culture was diluted to different concentrations with TBS-G, and 100 μL of medium and 0.5 mL of rabbit blood plasma were mixed and incubated at 37°C until coagulation was observed. The largest fold dilution of the bacterial culture that resulted in coagulation was determined as the coagulase valency.

Statistical analysis

All experiments were repeated at least three times in duplicate to validate reproducibility. All values are presented as the mean ± standard error. Graphs were constructed using GraphPad Prism software (version 5.0; GraphPad Software, Inc., La Jolla, CA, USA). A one-way ANOVA with Games-Howell post-hoc test was employed to examine differences between groups for statistical significance. P-values of less than 0.05 were considered statistically significant.

Results

Determination of MICs and the effects of baicalin on S. aureus growth in vitro

The MICs of baicalin, clarithromycin and levofloxacin against S. aureus 17546 are shown in Table 2. S. aureus was grown in the presence of baicalin (1024, 512, 256 or 128 μg/mL) for 24 hours to examine the antibacterial activity of sub-inhibitory concentrations of baicalin. As indicated by the growth curves (Figure 1), sub-inhibitory concentrations of baicalin had no inhibitory effect on the growth of S. aureus.

<table>
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<th>Antimicrobial</th>
<th>MICs (µg/ml)</th>
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<td></td>
<td>S. aureus 17546</td>
</tr>
<tr>
<td>Baicalin</td>
<td>2048</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>32</td>
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<td>Clarithromycin</td>
<td>128</td>
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Figure 1. Growth curves of S. aureus at sub-MIC concentrations of baicalin. S. aureus with different concentrations of baicalin (1024-128 µg/ml) or without baicalin (control) was incubated at 37°C for 24 hours, and OD600 was measured every 2 hours. Experiments were performed in triplicate. Mean values are shown. Baicalin concentrations < 1024 µg/mL did not inhibit S. aureus growth.
Antibiofilm effect by baicalin

Baicalin inhibits S. aureus biofilm formation

The antibiofilm activity of baicalin was examined using the plate counting method and crystal violet staining. S. aureus 17546 was grown in the presence of baicalin (1024 μg/mL, 512 μg/mL, 256 μg/mL and 128 μg/mL) on polystyrene carriers for 3 and 7 days, and the biofilm mass was stained with crystal violet and measured spectrophotometrically (at OD595); the number of bacteria in biofilms was calculated by the colony count assay. Sub-MICs (1024, 512, or 256 μg/mL) of baicalin significantly reduced cell attachment and biofilm biomass development, but concentrations less than 128 μg/mL did not exert an effect (Figure 2A and 2B). Fluorescence microscopy revealed a similar finding: sub-MICs (1024, 512, or 256 μg/mL) of baicalin inhibited adhesion of the bacteria to the carrier surface and decreased polysaccharide production compared to the control treatment, whereas 128 μg/mL baicalin did not have an effect (Figure 2C).

Synergistic effects of baicalin and levofloxacin on S. aureus biofilm formation

Biofilms were established as described above, and baicalin alone or in combination with levofloxacin was applied on the 3rd and 7th days.
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followed by incubation for 12 hours; clarithromycin was used as a positive control. Treatment with baicalin, clarithromycin and levofloxacin alone did not reduce the number of bacteria within the biofilm regardless of whether the compounds were added on the 3rd or 7th day of biofilm formation. However, treatment with baicalin + levofloxacin or clarithromycin + levofloxacin reduced the number of bacteria, with fewer viable counts in these groups than in the control group and the levofloxacin group. Furthermore, bacterial counts for biofilms treated with baicalin + levofloxacin were lower than for those treated with clarithromycin + levofloxacin (Figure 3).

SEM

Biofilms were treated with baicalin, clarithromycin, levofloxacin, baicalin + levofloxacin, or clarithromycin + levofloxacin on days 3 and 7, and SEM (Figure 4) revealed bacterial adhesion to the carriers as well as secretion of an extracellular matrix to form a biofilm. The structure of the 7-day biofilm was denser than that of the 3-day biofilm, and more extracellular matrix was observed. Regardless of whether a 3-day or 7-day biofilm was examined, baicalin or antibiotics alone did not eradicate S. aureus from pre-existing biofilms. However, combined treatment with baicalin and antibiotics was effective at disrupting biofilms: very few S. aureus cells and extracellular matrix remained, and those that were present exhibited a scattered distribution.

CLSM

Biofilm was established as prepared for SEM and visualized by CLSM (Figure 5; green fluorescence represents a live biomass). Similar to the SEM results, S. aureus formed biofilms on the surface of the carrier after 3 and 7 days. The 7-day biofilm was denser than was the 3-day biofilm. Regardless of whether a 3-day or 7-day biofilm was examined, baicalin, clarithromycin and levofloxacin alone did not eradicate the bacteria within the biofilm, and little red fluorescence (representing dead cells) was observed. However, red fluorescence was primarily observed in the baicalin + levofloxacin and clarithromycin + levofloxacin groups, indicating that baicalin disrupted the biofilm and promoted penetration of levofloxacin to kill the bacteria.

Western blotting

Western blot analyses were performed to examine S. aureus exotoxin A (SEA) expression. The SEA band for cells treated with baicalin (1024, 512 or 256 μg/mL, respectively) was less intense than that in the control group (Figure 6A). Moreover, SEA was expressed at lower levels in the treated group than in the control group (Figure 6B).

Relative expression of the Hla and agrA genes

Expression of hla and agrA was quantified by RTQ-PCR. After treatment with baicalin (concentrations ranging from 1024 to 128 μg/mL), lower relative expression of the hla gene was observed in the baicalin group than in the control group, and the 512 μg/mL baicalin group exhibited the lowest expression (Figure 7). Levels of the agrA transcript were also significantly decreased (P < 0.05) (Figure 8).

S. aureus thermonuclease

The diameters of the different baicalin groups were larger than those in the control group after 16 hours of incubation with toluidine blue nucleic acid agar (Figure 9).
In contrast to the results from the experiments described above, treatments with higher concentrations of baicalin (1024 and 512 μg/mL) inhibited the activity of *S. aureus* coagulase (Figure 10).

**Discussion**

The difficulty in controlling *S. aureus* infections is not only related to the improper use of antibiotics but is also closely related to the production of bacterial biofilm (BF) [18, 19]. Biofilms comprise a very dynamic system, and their formation is a layer delivery process [20, 21]. First, the bacteria must be close to a solid surface to form a mature biofilm, accordingly, attachment to the surface is the key step in the process by which a biofilm forms from single cells in suspension [22]. Zeng *et al.* and Luo *et al.* reported the ability of baicalin to inhibit *Pseudomonas aeruginosa* attachment to a...
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glass or polystyrene microtiter plate [23, 24]. In our study, baicalin effectively prevented S. aureus biofilm formation at sub-inhibitory concentrations. Fluorescence microscopy revealed that fewer bacteria had attached to the carrier surface after baicalin treatment, an observation that may be considered an extension of the research of Zeng et al. and Luo et al. We speculate that baicalin inhibits S. aureus adhesion, disrupting the biofilm formation. Therefore, baicalin shows promise for prophylactic treatment of early-stage S. aureus biofilm-related infections.

The treatment of mature bacterial biofilm infections in the clinical environment is mainly dependent on a large number of combined antibiotics. According to Jorge Parra-Ruiz et al., a combination of linezolid plus daptomycin is a more effective treatment for S. aureus biofilms than is either agent alone [25]. In addition, Dong Chai et al. reported that the combination of linezolid and fosfomycin has good therapeutic effects on biofilm-embedded MRSA infections both in vitro and in vivo [26]. Nonetheless, the combined use of antibiotics is easily circumvented by antibiotic resistance. Baicalin is a major component of Scutellaria extracts [27]; it has the advantage of low toxicity and is not associated with drug resistance. Based on our current SEM and CLSM results, baicalin destroyed mature biofilms, as the biofilm structure was looser and the amount of extracellular matrix was significantly reduced. Moreover, according to colony counts, the combination of baicalin and levofloxacin effectively killed bacteria within biofilms, and this combination resulted in a greater reduction in viable count than did levofloxacin alone. Our data indicate a potential application of baicalin as an adjuvant therapeutic agent for preventing S. aureus mature biofilm-related infections.

The pathogenicity of S. aureus mainly depends on its virulence factors [28]. Therefore, treatments that target bacterial virulence factors are being increasingly studied. SEA causes food poisoning, shock, and allergy [29], and Shimamura Y et al. demonstrated that plant-
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Figure 8. Effect of sub-MIC concentration of baicalin on agrA gene expression. After S. aureus 17546 was cultured with different sub-MIC concentrations of baicalin, the levels of specific RNA transcripts in different samples were measured. Total RNA extracts were used as a template for cDNA synthesis, and cDNA was assessed by RTQ-PCR. Data shown are the average of 3 experiments, and relative quantitative values were calculated using the $2^{-\Delta\Delta C_{t}}$. *P < 0.05, compared to the control.

sub-MICs of baicalin increased thermonuclease production in a dose-dependent manner, indicating that baicalin may be considered a secretagogue that enhances thermonuclease production.

When S. aureus invades the human body, coagulase causes fibrin in the blood or plasma to deposit or coagulate on the surface of the bacteria, hindering phagocytosis and causing abscess formation and adhesion of S. aureus to catheters during biofilm-associated infections [37-39]. S. aureus also produces toxic shock syndrome toxin-1, gelatinase, protease, lipase, peptidase and other proteins. Based on the results of our assays, coagulase was inhibited by baicalin at concentrations of 1024 and 512 μg/mL.

The QS system not only plays a regulatory role in the formation of S. aureus biofilms at various stages but is also important for coordinating the production of virulence factors [5]. Such regulatory networks are composed of various systems and pathways that are both independent of and interact with each other. The accessory gene regulator (agr) system is considered a QS system that plays a key role in S. aureus [40], and Qiu et al. confirmed that thymol significantly inhibited agrA transcription [41]. The agr system, which is activated by self-encoded polypeptides, has an important role in regulating many extracellular proteins and cytoplasmic protein genes. The agr locus consists of two divergent transcriptional loci, RNAII and RNAIII, driven by promoters P2 and P3, respectively. RNA II contains four open reading frames, agrA, agrB, agrC and agrD, encoding four proteins, AgrA, AgrB, AgrC and AgrD, respectively. AgrD is the precursor of autoinducing polypeptide (AIP), which is processed by AgrB to generate mature AIP and then transported to the extracellular space. When AIP accumulation reaches a certain threshold, AgrA is activated by agrC-dependent phosphorylation, significantly accelerating the transcriptional efficiency of P2 and P3. P3 initiates the transcription of RNA III, a regulatory RNA molecule that controls

derived polyphenols interact with SEA and inhibit toxin activity [30]. In the present study, sub-MICs of baicalin decreased SEA production in a dose-dependent manner.

Hla forms a micro-channel in the hydrophobic region of the cell membrane, resulting in an imbalance in intracellular and extracellular ions and subsequent cell necrosis [31-33]. In a study involving in vitro biofilm formation on polystyrene, an S. aureus hla mutant exhibited dramatically reduced biofilm formation based on a standard microtiter plate assay and under flow conditions. Initial attachment to the surface was also decreased, indicating that an inability to bind to the surface contributes to decreased formation of a mature biofilm. However, the exact mechanism remains unclear. Based on our RTQ-PCR results, baicalin (1024, 512 and 256 μg/mL) inhibited expression of hla compared with the control treatment.

Nucleic acids degrades by S. aureus thermonuclease when a tissue or the cell infected by S. aureus undergoes apoptosis, promoting a diffuse infection [34], and activity of this enzyme has been shown to correlate with biofilm formation. Extracellular DNA (eDNA) [35] is one of most important components of the biofilm matrix: it promotes the connection between cells and helps to stabilize the mature biofilm. S. aureus with a Sig B mutation rarely forms biofilms because it secretes thermonuclease to degrade eDNA [36]. Therefore, we postulate that S. aureus thermonuclease exerts negative effects on biofilms. Our research revealed that...
expression of many virulent factors. Although the agr system is considered to play a leading role, many genes interact with it to form a complex regulatory network that coordinates the regulation of virulence factor expression. For example, the dual regulation system of ArlS/ArlR regulates expression of beta-hemolysin, lipase, coagulase and other virulence factors, and The SAR system is activated by AgrA, up-regulating expression of various toxins. Jia, P et al. confirmed that clindamycin differentially inhibits the transcription of exoprotein genes in S. aureus, partly through sar [42]. In the present study, 1024 and 512 μg/mL baicalin down-regulated agrA expression. However, the mechanisms by which S. aureus controls virulence gene expression are fairly intricate. We hypothesize that baicalin regulates expression of virulence factors by inhibiting the agr system, though the specific mechanism needs further in-depth research.

In conclusion, baicalin inhibits S. aureus biofilm formation and exerts a synergistic effect with levofloxacin. Baicalin might be considered to act as a QS inhibitor that in vitro regulates S. aureus virulence factors by inhibiting the agr system.

Acknowledgements

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Disclosure of conflict of interest

None.

Address correspondence to: Yiqiang Chen, Department of Pulmonary and Critical Care Medicine, First Affiliated Hospital of Guangxi Medical University, Guangxi Medical University, Shuangyang Road No. 6, Nanning, China. E-mail: chenylq0708@163.com
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References


[27] Zhao Q, Chen XY and Martin C. Scutellaria baicalensis, the golden herb from the garden of Chinese medicinal plants. Sci Bull (Beijing) 2016; 61: 1391-1398.


