International Journal of Nanomedicine

Dovepress
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ORIGINAL RESEARCH

Construction of Baicalein-Loaded Chitosan Nanoparticles for *Staphylococcus aureus*-Induced Lung Infection Remission

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Purpose: In this study, we constructed baicalein (a traditional Chinese medicine)-loaded chitosan nanoparticles (a drug-delivery system) with great biocompatibility for the remission of *Staphylococcus aureus*-induced lung infection.

Methods: The nanoparticles were prepared via a one-step reaction. Baicalein-release rates were studied via ultraviolet absorption assays. Morphology was characterized using AFM and TEM. The antibacterial mechanism of the nanoparticles was studied through ONPG, crystal violet staining, and live/dead bacterial staining assays, anti-inflammatory performance investigated via ELISA and WB assays, and in vivo anti-lung-infection capacity studied via H&E staining and ELISA kits.

Results: The average size of the nanoparticles was uniform (~200 nm), and the zeta potential was about 18.5 ± 0.3 mV. The encapsulation efficiency was about 40%. The release of baicalein was >80% under different temperatures and pH. Dry nanoparticles were also stable. The minimum inhibitory concentration against *S. aureus* was about 15 μg/mL. The maximum tolerable dose in vivo was 300 μg/kg. The nanoparticles exhibited outstanding anti-inflammatory and anti-lung-infection performance.

Conclusion: The in vitro and in vivo results demonstrate that our drug-delivery system could be an efficient platform for the remission of bacterium-induced lung infection.

Keywords: baicalein, *Staphylococcus aureus*, lung infection

Introduction

Bacterial infection has become a serious public health issue worldwide.[1,2] Bacterium-induced lung infection, which can cause further damage to the airway or other organs, is one of the most harmful types, with a 50%–60% mortality rate at the late stage of infection.[3,4] Fortunately, the emergence of antibiotics has greatly alleviated the endangerments caused by lung infections.[5–9] However, it is predicted that by 2030, antibiotic consumption will increase by 52.3% compared to 2023,[10] which brings anther serious threat: the abuse of antibiotics. This would lead to the generation of drug resistance, which is a huge obstacle to the treatment of bacterium-induced lung infection, so developing strategies with low drug resistance for bacterium-induced lung infection is pressing.

The use of antimicrobial peptides (AMPs) brings a new dawn. AMPs have inhibitory effects on a broad range of microorganisms, including bacteria, fungi, parasites, and viruses.[11–13] They usually act on the outer membranes and intracellular targets of pathogenic bacteria, resulting in the death of bacteria. As such, AMPs have positive effects on reducing drug resistance during the treatment period.[14] For lung infections, Sun et al reported that the AMPs LL37 and innate defense regulator 1 can recruit immune cells to infection sites, thereby reducing the expression of the inflammatory factors IL6 and TNFα and alleviating the lung inflammation induced by methicillin-resistant *Staphylococcus*

Received: 14 April 2025
Accepted: 23 September 2025
Published: 1 October 2025

aureus (MRSA).[15] Lau et al developed random peptides that destroy MRSA biofilm, resulting in elimination of the infection.[16] However, unpredictable immunoresponses caused by AMPs still limits their further application.

Recently, in the light of such advantages as perfect biocompatibility, negligible drug resistance, and controllable antimicrobial properties, several nanodelivery systems have been developed for *S. aureus*-induced lung infection. Zhou et al developed the hybrid biomimetic membrane-coated particles which could release cinnamaldehyde and ferrosoferric oxide (Fe_3O_4), resulting in the emergence of MRSA ferroptosis and relieving MRSA-induced lung infection.[17] Wang et al reported on a breathable polymer-based nanodelivery platform loaded with indocyanine green (ICG) and bacteriophages. MRSA was removed by heat produced by the ICG and swallowed by bacteriophages, causing recovery from lung damage.[18] Despite this, some traditional Chinese medicines, such as baicalein, remain attractive due to their huge clinical translation potential.

In this study, baicalein, which has cell-protective and anti-inflammatory effects, was used as cargo in a nanodelivery system (chitosan as carrier). In general, chitosan possesses enhanced drug absorption and retention at the target site, as well as stability and controlled release ability.[19] Cinnamaldehyde was used to enhance the stability of chitosan.[20] Nanoparticles were prepared via a one-step reaction, and baicalein was released successfully under physiological conditions. *S. aureus* was eliminated with this delivery system. In vitro and in vivo results showed that the nanodelivery system had excellent anti-inflammatory and anti-lung-infection performance, which can be attributed the successful release of baicalein and the low minimum inhibitory concentration (MIC; ~15 μg/mL) of nanoparticles against *S. aureus*. In addition, the nanodelivery system exhibited high biocompatibility, which is critical for the treatment of lung infection due to the fact that the systemic inflammation may be induced by lung biological toxicity. Our drug-delivery system could be an efficient platform for promoting the remission of bacterium-induced lung infection.

Materials and Methods

Materials

Baicalein (CAS 491–67-8, catalogue HY-N0196), lipopolysaccharide (LPS; catalogue HY-D1056), and 2-nitrophenyl-β-D-galactopyranoside (ONPG, CAS 369–07-3, catalogue HY-15926) were purchased from MedChemExpress. Chitosan (catalogue 448869, molar mass 50–190 kDa, based on viscosity), cresyl violet acetate (CAS 10510–54-0, catalogue 506903), and glacial acetic acid (ACS reagent, CAS 64–19-7, catalogue 695092,) were purchased from Millipore Sigma. A living/dead bacterial (DMAO and EthD-III) staining kit (catalogue 40274ES60) was purchased from Yeasen Biotechnology. A reactive oxygen species (ROS) assay kit (catalogue S0033S), Cell Counting Kit 8 (CCK-8, catalogue C0038), and Hoechst 33342 (100 ×, catalogue C1029) were purchased from Beyotime. A superoxide dismutase (SOD) activity assay kit (WST1 method, catalogue BC5165) was purchased from Solarbio. Mouse interleukin 1β (IL1β, catalogue KE10003), mouse IL6 (catalogue KE10007), mouse IL10 (catalogue KE10103), and mouse tumor necrosis factor α (TNFα, catalogue KE10002) enzyme-linked immunosorbent assay (ELISA) kits, anti-TNFα polyclonal antibody (catalogue 17590-1-AP), anti-phospho-NFκB p65 (Ser468) recombinant antibody (p-p65, catalogue 82335-1-RR), and anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) polyclonal antibody (catalogue 10494-1-AP) were purchased from Proteintech. *S. aureus* (ATCC 29213) were obtained from clinical laboratory of the First Affiliated Hospital of Xi'an Jiaotong University. All other reagents and experimental consumables were local.

Methods

Baicalein-Loaded Nanoparticles

Baicalein-loaded nanoparticles were constructed as per a previously reported method.[21,22] Briefly, baicalein (10 mg) was dissolved in ethanol (20 mL, 0.5 mg/mL). Chitosan (50 mg) was dissolved in 6 mL of 1% acetic acid solution, and 0.35 mL of cinnamaldehyde was added. Then, 5 mL aqueous phase (chitosan in acetic acid solution) was poured into 5 mL oil phase (baicalein in ethanol, final concentration of baicalein about 0.25 mg/mL). Finally, the whole mixture was washed with water to remove possible impurities and centrifuged at 12,000 rpm for 45 min under room temperature, and then the nanoparticles were lyophilized (EYELA-FDU2110, lab temperature at room temperature, overnight). About

4 mg (0.2 mg/mL) of baicalein was successfully loaded (encapsulation efficiency about 40%) according to the standard curve (Figure S1).

Atomic Force Microscopy

Atomic force microscopy (AFM) images were obtained with a Shimadzu SPM-9700HT using a tap model. The nanoparticles were redispersed into water, and 10 μL of solution was dropped onto freshly cleaved mica surfaces. After 10 min, the solution was removed quickly and the silicon surface was then dried overnight under room temperature.

Transmission Electron Microscopy

Transmission electron microscopy (TEM) images were obtained using a field-emission microscope (Talos-F200X, Thermo Fisher Scientific, USA). The nanoparticles were redispersed into water or cell culture medium, and then 5 μL of solution was dropped onto an ultrathin carbon film. After 10 min, the solution was removed quickly and the film then dried overnight under room temperature.

Dynamic Light Scattering and Zeta Potential

Dynamic light scattering (DLS) and zeta-potential results were obtained with a Zetasizer Nano ZSE.

Baicalein Release

Baicalein release was evaluated in physiological pH (~7.4) and also in bronchial pH (~6.5)[22] at 37°C or 25°C. Briefly, the nanoparticles were redispersed in 10 mL of phosphate-buffered saline (PBS; pH = 7.4 or 6.5), and then poured into dialysis bags (100–500Da, catalogue: MP1732-0.5M). The bags were incubated at 37°C. At regular intervals (4 hours), samples were removed and the ultraviolet absorption was measured (270 nm, UV-1780 UV-Visible spectrophotometer). The accumulative release-time curve was described according to the standard curve (Figure S1).

Antibacterial Properties of Nanoparticles In Vitro

Similar antibacterial performance evaluation methods have been reported.[23] iIn short, 50 μL of *S. aureus* suspension (6 × 10^5 CFU/ mL) and 50 μL of nanoparticle solutions (final concentrations 5 μg/mL, 15 μg/mL, and 30 μg/mL) or PBS were added to a 96-well plate. The plate was incubated for 24 hours at 37°C. After that, absorbance values at 630 nm (OD_{630}) were recorded by a BioTek Synergy LX multimode microplate reader (Agilent Technologies, USA). Bacterial suspension (50 μL, not diluted) was spread on the surface of LB agar medium (not diluted) and the media were incubated at 37°C for 24 hours under aerobic conditions and photographed.

Crystal Violet Assays

A 96-well plate containing *S. aureus* without nanoparticles or treated with nanoparticles was prepared according to the methods above. After being washed with PBS, each well of the plate was added to 50 μL of 1% crystal violet solution, and then the plate was incubated for 45 min at room temperature. The well was washed with PBS, photographed, and 100 μL of ethanol added. Absorbance at 590 nm (OD_{590}) was measured using the BioTek Synergy LX multimode microplate reader.

ONPG Assays

The 96-well plate including *S. aureus* without nanoparticles or treated with nanoparticles was prepared according to the method above. After that, the suspensions (100 μL) were added to 100 μL of ONPG solution (final concentration 250 μM) and incubated for 4 hours at 37°C. Absorbance at 405 nm (OD_{405}) was measured using the BioTek Synergy LX multimode microplate reader.

Living/Dead Bacterial Staining

S. aureus without nanoparticles or treated with nanoparticles was prepared according to the method above. After that, 50 μL of suspensions were added into 1 μL of 100 × dye solutions (solutions containing one volume of DMAO, two volumes of EthD-III, and eight volumes of 0.9% NaCl) and incubated on the glass surface for 15 min without light under room temperature. Fluorescence images were collected through laser confocal microscopy (Leica, TCS SP5, Germany).

 https://doi.org/10.2147/IJN.S534294

Cell-Viability Assays

CCK-8 assays were performed to evaluate the biocompatibility of nanoparticles towards RAW 264.7 or A547 cells. Cells (1.0×10^5 cells/mL) were seeded into a 96-well plate. After 24 hours, the culture media were discarded and fresh media containing different concentrations (0–40 μg/mL) of nanoparticles were added. After 24 hours, the culture media was discarded and an aliquot of 10 μL of CCK-8 solution was added to 100 μL of fresh medium in each well. The plate was incubated for 1–2 hours at 37°C. Finally, the absorbance at 450 nm (OD_{450}) was recorded using a BioTek Synergy LX multimode microplate reader. Cell viability was calculated according to the following formula:

$$\text{Cell viability}(\%) = \frac{OD450_{\text{particles}}}{OD450_{\text{control}}} \times 100 \tag{1}$$

where $OD450_{\text{particles}}$ means the absorbance value at 450 nm of suspension containing cells treated with different concentrations of nanoparticles, and $OD450_{\text{control}}$ means the absorbance value at 450 nm of suspension containing cells only.

ROS-Scavenging Properties of Nanoparticles

RAW 264.7 cells (1.5×10^5 cells/mL) were treated with LPS (1.5 μg/mL) or both LPS (1.5 μg/mL) and nanoparticles (concentrations 0, 15, and 30 μg/mL) for 24 hours. After that, cells were examined with an ROS assay kit according to the protocols and then the fluorescence images were collected using laser confocal microscopy (Leica, TCS SP5, Germany). Fluorescence intensities were recorded with a NovoCyte flow cytometer (emission wavelength: FITC) and SOD activity was assessed with an SOD assay kit according to the protocols.

WB Assays

RAW 264.7 cells (1.5×10^5 cells/mL) were treated according to the above protocols. Cells were then lysed and the protein mixtures collected for WB assays.

Animal Experiments

The animal experiments were permitted by the institutional animal ethics committee of Xi'an Jiaotong University (approval XJTUIAF2021LSK-487). Reduction, replacement, and refinement principles were the guidelines followed for the welfare of the laboratory animals. C57BL/6 mice (healthy, female, 6–8 weeks) were randomly divided into two groups (N = 5): a control group and nanoparticle group. In the drug-treated group (nanoparticle group), mice were intraperitoneally injected with nanoparticles (150 μg/kg or 300 μg/kg) three times on day 1, day 4, and day 7. In the control group, mice were intraperitoneally injected with equal volumes of PBS (100 μL) on day 1, day 4, and day 7. On day 9, the mice were then euthanized, the organs (heart, liver, lung, spleen, and kidney) sliced for hematoxylin and eosin (H&E)-staining assays, and sera were selected for biomarker tests at Lilai Biomedicine. Other C57BL/6 mice (healthy, female, 6–8 weeks) were randomly divided into four groups (N = 5): a control group, *S. aureus* group, free baicalein group, and nanoparticle group. Mice were anesthetized and *S. aureus* suspensions (8×10^6/mouse) were injected through endotracheal intubation in the *S. aureus* group. Mice were anesthetized and injected with *S. aureus* suspensions (8×10^6/mouse) through endotracheal intubation and then free baicalein (150 μg/kg in PBS) or nanoparticles (150 μg/kg in PBS) three times at 8, 24, and 36 hours via endotracheal intubation after *S. aureus* injection in the nanoparticle group. All mice were euthanized at 48 hours after *S. aureus* injection, and parts of lungs were collected or sliced for ELISA tests and H&E staining.

Results

Morphology and Baicalein Release of Nanoparticles

We synthesized the baicalein- loaded nanoparticles as per a previous method.[20,21] A schematic diagram of the nanoparticle-formation process is shown in Figure 1A. In order to characterize the morphologies of the nanoparticles obtained, AFM, DLS, and TEM assays were performed. As shown in Figure 1B, the size of the nanoparticles was uniform (~200 nm), which was confirmed by DLS (PDI 0.144). The zeta potential of the nanoparticles was about 18.5 ±

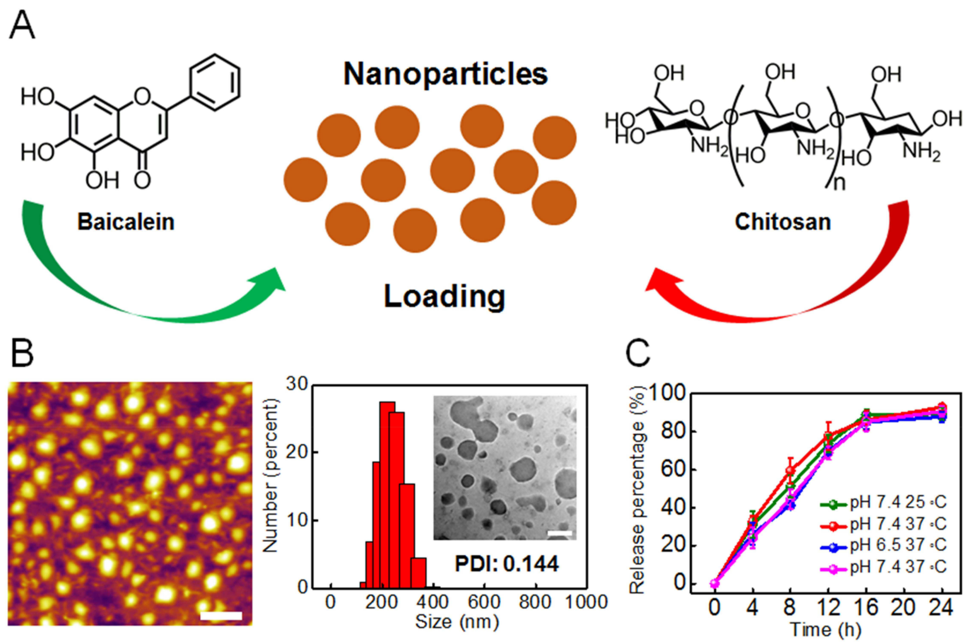

Figure 1 (**A**) Schematic diagram of nanoparticle formation. (**B**) Representative AFM and TEM images and DLS results for nanoparticles. AFM scale bar 1 μm. TEM scale bar 500 nm. (**C**) Effects of time on the release percentages of baicalein under different pH.

0.3 mV. Then, the baicalein-release efficiency of loaded nanoparticles was studied. As shown in Figure 1C, the release of baicalein was >80% under different temperatures and pH (including physiological and bronchial conditions), close to reported values,[22] suggesting the delivery system has potential in the treatment of bacterium-induced infection. Baicalein was released from the nanoparticles within 24 hours, suggesting the stability of nanoparticles in solution may be unsatisfactory. However, as shown in Figure S2, the dry nanoparticles (2 months after being prepared) were well dispersed in the DMEM, demonstrating its practicality.

Antibacterial Properties of the Nanoparticles

Next, the antibacterial performance of the nanoparticles was investigated. We chose *S. aureus* (ATCC 29213, one kind of representative bacterium) as the model bacterium. *S. aureus* is a Gram-positive bacterium that can cause a wide variety of infections, such as those of the lung, skin, and blood. As shown in Figure 2A, the colonies have disappeared following treatment with 15 µg/mL nanoparticles, which was determined by the decrease in absorbance values at 630 nm (OD_{630}, $p < 0.005$) of suspensions (Figure 2A and Figure S3). As such, the MIC of our delivery system toward *S. aureus* is about 15 µg/mL. It is known that physical damage to membranes by external stimuli is the main reason for bacterial -elimination,[24–26] and the results of the ONPG assays showed that the content of β-galactosidase leaked from *S. aureus* in suspensions increased upon treatment with 30 µg/mL (2 MIC) of nanoparticles ($p < 0.005$, Figure 2B), indicating that the membranes of *S. aureus* were destroyed by the baicalein. The formation of biofilm (determined by crystal violet staining[27]) of *S. aureus* was also studied. The OD_{590} of suspensions containing *S. aureus* treated with 30 µg/mL (2 MIC) of nanoparticles decreased ($p < 0.005$, Figure 2C), meaning that biofilm was curbed. This may be solely attributed to the antibacterial activity of the nanoparticles. Furthermore, the results of live/dead bacteria staining (redder fluorescence means more dead bacteria induced by membrane destruction[27]) further confirmed that the membranes of *S. aureus* were damaged (Figure 2D). The results show that our nanoparticles (delivery system) are powerful tools for sterilization.

Anti-Inflammatory Properties of the Nanoparticles

The anti-inflammatory properties of baicalein were studied. The cytotoxicity of the nanoparticles (delivery system) was investigated first. As shown in Figure S4, the cell viability of RAW 264.7 and A547 cells is unaffected by the nanoparticles, demonstrating that the nanoparticles are biocompatible. Next, ROS-scavenging performance was

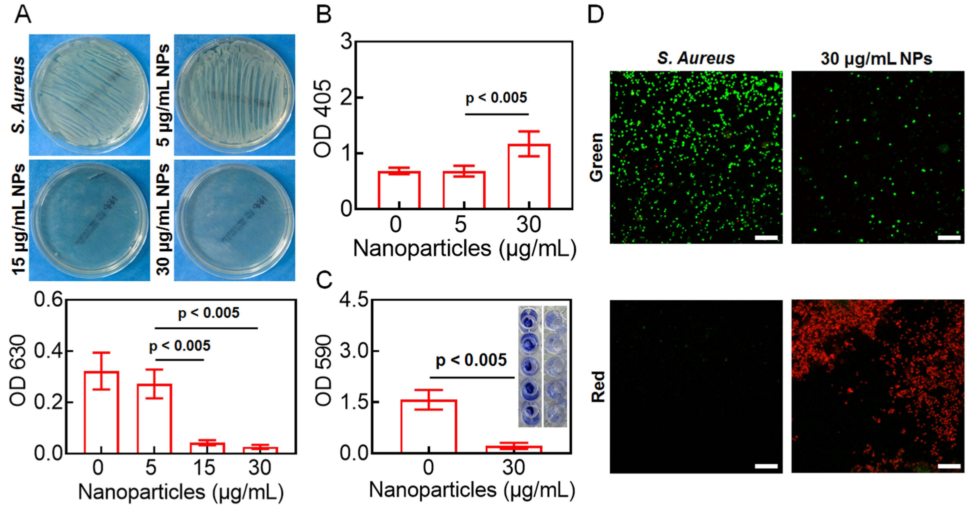

Figure 2 (**A**) Upper: Representative photos of *S. aureus* treated with different concentrations of nanoparticles. Lower: Absorbance values at 630 nm of different wells from different groups. N = 5, ANOVA. (**B**) Absorbance values at 405 nm of different wells from different groups. N = 5, ANOVA. (**C**) Absorbance values at 590 nm of different wells from different groups. Inset shows the photos of each well. N = 5, ANOVA. (**D**) Representative fluorescence images of *S. aureus* from different groups stained with DMAO (green) and EthD-III (red). Scale bar 50 μm.

examined. As shown in Figure 3A, large amounts of ROS are produced in RAW 264.7 cells upon the treatment of LPS (fluorescence images and flow fluorescence intensity). After being incubated with 30 μg/mL nanoparticles, ROS content decreased in cells (fluorescence images and flow fluorescence intensity, Figure 3A), meaning the nanoparticles were able to eliminate ROS during the inflammatory process. At the same time, the recovery of SOD activities in the nanoparticle-

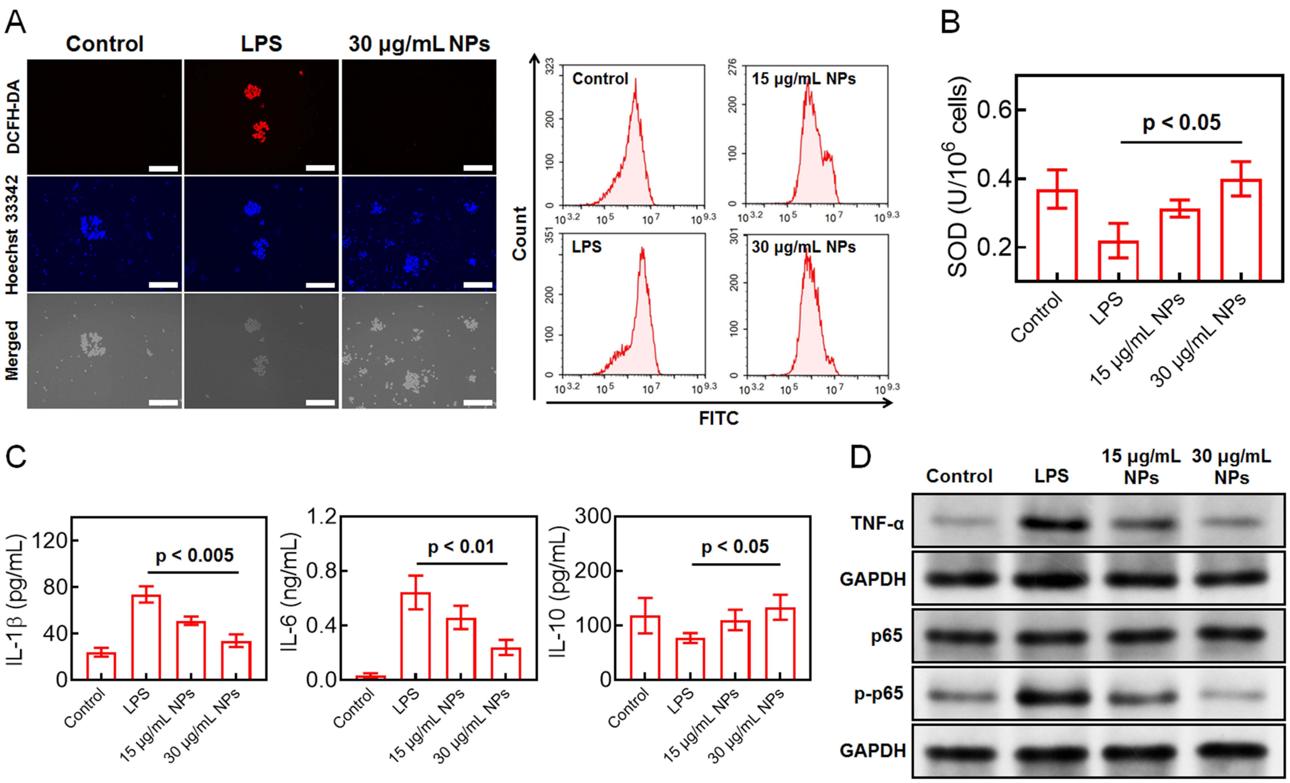

Figure 3 (**A**) Left: Flow fluorescence intensity of RAW 267.7 cells stained with DCFH-DA (50 μM). Right: Representative fluorescence images of RAW 267.7 cells stained with DCFH-DA (50 μM) and Hoechst 33342 (1 ×) under different conditions. Scale bar 50 μm. (**B**) SOD activity of RAW 267.7 cells from different groups. N = 3, ANOVA. (**C**) Concentrations of IL1β, IL6, and IL10 of different groups. N = 3, ANOVA. (**D**) Western blot of different proteins from different groups.

treated group confirms the above point ($p < 0.05$, Figure 3B). Then, the concentrations of several main cytokines secreted from RAW 264.7 cells were studied. The concentrations of IL1β and IL6 increased in the LPS group compared with the control group ($p < 0.005$ for IL1β comparison, $p < 0.01$ for IL6 comparison), but decreased in the nanoparticle-treated group ($p < 0.05$, Figure 3C). On the other hand, the tendency of IL10 was the opposite ($p < 0.05$, Figure 3D). Furthermore, the expression of TNFα and p-p65 (two important cytokines) was inhibited by the treatment with nanoparticles (the weaker protein bands). All these results demonstrate that these nanoparticles have excellent anti-inflammatory properties, and their antibacterial and anti-inflammatory performance give our delivery system the capability of fighting lung infection.

Anti-Lung-Infection Performance of the Nanoparticles

Lastly, the anti-lung-infection performance of the nanoparticles (delivery system) was studied. Before this, the biosafety of the nanoparticles was tested. As shown in Figure S5, H&E-staining results show that the tissue structures of the heart, liver, spleen, lung, and kidney remain complete and no inflammatory damage is observed. It should be noted that the organs were damaged under treatment with 300 μg/kg nanoparticles, suggesting the maximum tolerable dose (MTD) in vivo is 300 μg/kg. Therefore, the dosage (150 μg/kg: MTD/2) herein is reasonable. Meanwhile, the concentrations of alkaline phosphatase (ALP) and aspartate transaminase (AST) were unaffected by the nanoparticles (MTD/2, Figure S6), confirming the nanoparticles' excellent biocompatibility. After confirmation of biocompatibility, we assessed the anti-lung-infection performance of the nanoparticles. As shown in Figure 4A, there was much redness and swelling in lungs from the *S. aureus*-infected group, but these symptoms almost disappeared after treatment with the nanoparticles. Moreover, the redness and swelling in lungs were slighter than those of mice treated with free baicalein (Figure S7). In addition, the concentrations of IL1β, IL6, and TNFα secreted from lung tissue were reduced ($p < 0.01$ for IL1β comparison, $p < 0.01$ for IL6 comparison, and $p < 0.005$ for TNFα comparison, Figure 4B), accompanied by an increase in IL10 (and $p < 0.01$), suggesting a protective effect of the nanoparticles against the lung infection process. These results indicate that the delivery system could provide us a direction for the remission of bacterium-induced lung infection.

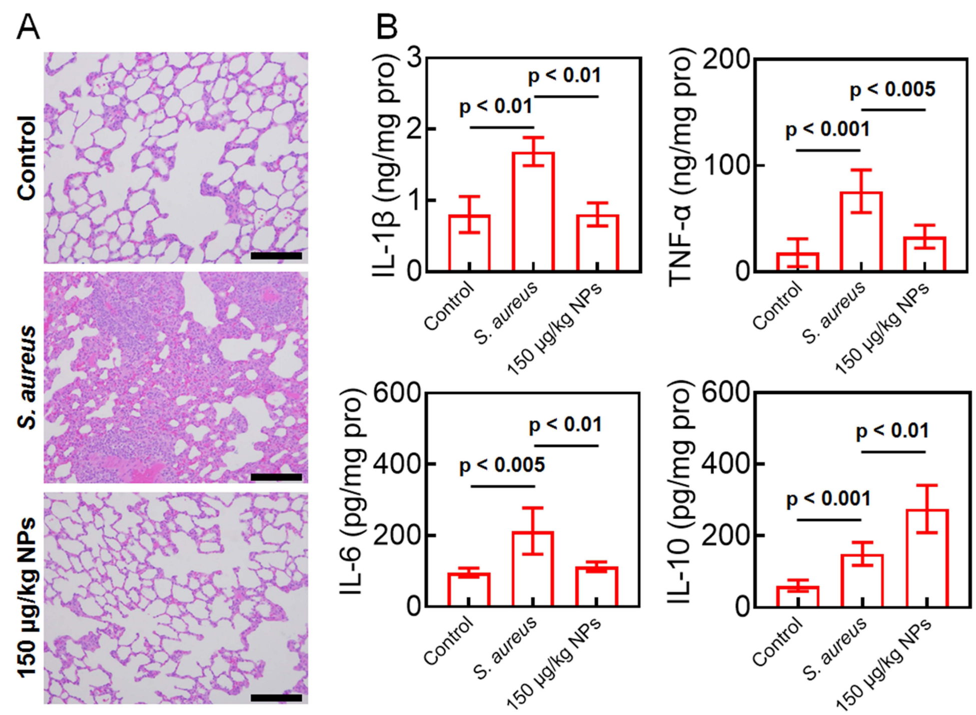

Figure 4 (**A**) Representative H&E-stained images of lung tissue from different groups. Scale bar 20 μm. (**B**) Concentrations of IL1β, TNFα, IL6, and IL10 in lung tissue from different groups. N = 5, ANOVA.

Discussion

Despite several nanoparticle types having been developed for infection-caused lung damage,[17,18] traditional Chinese medicines such as baicalein have huge clinical translation potential. Baicalein has cell-protective and anti-inflammatory effects. However, its poor water solubility and side effects limit its broad applications.[28,29] As such, developing an efficient delivery system for baicalein is needed. Given chitosan's advantages of atoxicity, biocompatibility, and biodegradability,[30] baicalein-loaded chitosan nanoparticles (delivery system) were successfully obtained (Figures 1A and B). A previously described method[21,22] was used for the baicalein- loaded nanoparticles due to its convenience and greenness.

The successful release of baicalein (Figure 1C) provides us a platform for antibacterial, and without doubt the *S. aureus* (one kind of representative bacterium) was eliminated upon treatment with the nanoparticles (15–30 μg/mL, Figure 2A). The MIC value of the nanodelivery system was about 15 μg/mL, which is lower or comparable to previous results,[31] indicating the effectiveness of our system. It has been reported that inner and outer membranes of bacteria can be disrupted by baicalein.[32] Interestingly, our results (Figures 2B and D) further confirm that the destruction of membranes of bacteria induced by baicalein is the main reason for the antibacterial activity of the delivery system. In order to ensure efficiency, a high nanoparticle concentration (30 μg/mL, about 2 MIC) was chosen for the following experiments. Without doubt, the membranes of *S. aureus* were destroyed by the nanoparticles (Figures 2B and D), which is the main mechanism of *S. aureus* elimination. On the other hand, biofilm is necessary for bacterial growth, and containment thereof can inhibit the formation of colonies.[33,34] The results showed that biofilm was destroyed by the nanoparticles (Figure 2C), which is another reason for bacterial elimination. Moreover, it has been reported that FmtA and lipophilic membrane protein play key roles in biofilm formation, antibiotic resistance, and the bacterial lysis rate of *S. aureus*,[35,36] and several compounds inhibiting these, such as ofloxacin, roflumilast, furazolidone, ZINC000072380005, ZINC000257219974, ZINC000176045471, ZINC000035296288, and ZINC000008789934, are candidates against *S. aureus*.[34,35] The interactions between nanoparticles or baicalein and FmtA or lipophilic membrane protein may provide us further insights into the mechanisms of *S. aureus* elimination, which will be studied in future work.

Given the benefits of baicalein, the anti-inflammatory and anti-lung-infection performances of the nanoparticles was systematically studied. The results show that the nanoparticles have ROS-scavenging capability (Figures 3A and B), which may be the reason for downregulation of IL1β, IL6, TNFα, and p-p65 and upregulation of IL10 (Figures 3C and D). This conclusion is backed by other reported results.[37] Lastly, considering the excellent antibacterial and anti-inflammatory properties of the nanoparticles, their anti-lung-infection effects were studied. As expected, our delivery system exhibits an appropriate anti-lung-infection curative effect (Figure 4). Moreover, the redness and swelling in lungs were slighter than those of mice treated with free baicalein (Figure S7), which may be attributed to its inefficiency caused by poor water solubility. In addition, its efficacy can be improved through endotracheal intubation in low dosages, meaning that the process of nanoparticle administration via inhalable aerosols could improve its clinical value. However, some limitations still exist. Firstly, the repair of lung tissue damage wrought by other common lung pathogens, such as *Pseudomonas aeruginosa* or *Klebsiella pneumonia*, was not studied here. Secondly, the long-term studies on efficacy, potential for recurrence, and chronic toxicity of our nanoparticles will be important for clinical translation. These two directions will be pursued in our future work.

Conclusion

Baicalein-loaded chitosan nanoparticles (delivery system) were prepared and baicalein released successfully under physiological conditions. *S. aureus* was eliminated by the delivery system. In vitro and in vivo results showed that the delivery system had excellent anti-inflammatory and anti-lung-infection performance, which could be attributed the successful release of baicalein and the low MIC (~15 μg/mL) of the nanoparticles against *S. aureus*. Our drug-delivery system could be an efficient platform for the remission of bacterium-induced lung infection.

Abbreviations

AFM, atomic force microscopy; TEM, transmission electron microscopy; ONPG, *O*-nitrophenyl-β-**d**-galactopyranoside; ELISA, enzyme-linked immunosorbent assay; WB, Western blot; H&E, hematoxylin and eosin; AMPs, antimicrobial peptides; IL, interleukin; TNF, tumor necrosis factor; MRSA, methicillin-resistant *Staphylococcus aureus*; Fe_3O_4, ferrosoferric oxide; ICG, indocyanine green; LPS, lipopolysaccharide; ROS, reactive oxygen species; CCK-8, Cell Counting Kit 8; SOD, superoxide dismutase; p-p65, phospho-NFκB p65; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PBS, phosphate-buffered saline; MIC, minimum inhibitory concentration; MTD, maximum tolerable dose.

Acknowledgments

The authors express their thanks for the funding support from the National Natural Science Foundation (82102976, 82202948) and Clinical Fundamental Integrated Project of Xi'an Jiaotong University (YXJLRH2022002) and for the support from the Translational Medicine Center of the First Affiliated Hospital of Xi'an Jiaotong University.

Author Contributions

All authors made a significant contribution to the work reported, whether in the conception, study design, execution, acquisition of data, analysis, interpretation, or all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval to the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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