Research Article

Photodynamic Action of LED-Activated Curcumin against Staphylococcus aureus Involving Intracellular ROS Increase and Membrane Damage

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Aim. To investigate the effect of photodynamic action of LED-activated curcumin on cell viability, membrane permeability, and intracellular reactive oxygen species of Staphylococcus aureus.

Methods. Staphylococcus aureus was incubated with the different concentrations of curcumin for 60 min and then irradiated by blue light with the wavelength of 470 nm and with light dose of 3 J/cm². The colony forming unit assay was used to investigate photocytotoxicity of curcumin on Staphylococcus aureus, confocal laser scanning microscopy (CLSM) and flow cytometry (FCM) for assaying membrane permeability, FCM analysis with DCFH-DA staining for measuring the intracellular ROS level, and transmission electron microscopy (TEM) for observing morphology and structure.

Results. Blue light-activated curcumin significantly killed Staphylococcus aureus in a curcumin dose-dependent manner. TEM observed remarkable structural damages in S. aureus after light-activated curcumin. More red fluorescence of PI dye was found in S. aureus treated by blue light-activated curcumin than in those of the controlled bacterial cells. Intracellular ROS increase was observed after light-activated curcumin.

Conclusion. Blue light-activated curcumin markedly damaged membrane permeability, resulting in cell death of Staphylococcus aureus and highlighted that intracellular ROS increase might be an important event in photodynamic killing of Staphylococcus aureus in the presence of curcumin.

1. Introduction

Staphylococcus aureus is one of the commonest opportunistic pathogens in veterinary medicine, which usually causes skin and soft tissue infections as well as respiratory infection diseases and bacteremia [1–4]. Antibiotics are the commonest drugs currently used in the clinical settings. However, prolonged antibiotic treatment easily resulted in the emergency of antibiotic-resistant bacterial strains, which affect and lower the therapeutic efficacy of antibiotics. Therefore, there is an urgent need in exploring novel and more efficient strategies for eradicating S. aureus.

Photodynamic therapy (PDT) is an alternative way to eradicate fast growing cells and tissues. In PDT, nontoxic dyes, also called photosensitizers, are activated by a harmless visible light to produce cytotoxic reactive oxygen species (ROS), which induce fatal damages on target cells and tissues [5–8]. PDT as an alternative regime has been approved by many countries to treat the patients with malignant tumors as well as age-related macular degeneration (AMD). Recently, growing data showed that photodynamic action could effectively kill pathogenic microorganisms, termed photodynamic inactivation (PDI) and photodynamic antimicrobial chemotherapy (PACT) [5–7, 9–11]. In comparison to conventional antibiotic treatment, PDI has unique advantages of dual specificity in targeting eradication of pathogenic microorganisms: preferential absorption of pathogenic microorganisms and target lesion irradiated from laser or visible light. Furthermore, the resistance of bacteria
to PDI is very unlikely because of the nonspecific damage of PDI on bacteria [12]. So, PDI has shown potential promise in eradicating pathogenic microorganisms and treating infectious diseases.

Curcumin (CUR), isolated from the rhizomes of Curcuma longa, is a naturally occurring polyphenolic compound. Many investigations have found various biological activities of curcumin including antiproliferative, antimicrobial, and antioxidant activity [13] and the activities of curcumin could be enhanced in the presence of visible light irradiation at the wavelength of around 400–500 nm [14]. These data also demonstrate that curcumin is a kind of effectively natural photosensitizer. Therefore, in the present study our aim is to investigate the effect of photodynamic action of LED-activated curcumin on cell viability, membrane permeability, and intracellular reactive oxygen species (ROS) of Staphylococcus aureus.

2. Materials and Methods

2.1. Photosensitizer. Curcumin was used as a photosensitizer in the present study from Sigma (America). A stock solution was made in dimethyl sulfoxide (DMSO) at a concentration of 100 mM and kept in the dark at ~20°C.

2.2. Bacterial Strain. S. aureus strain was gifted by Mr. Liangkui Wong from the Second Affiliated Hospital, Chongqing Medical University, China. The strain was originally isolated from a patient with S. aureus pneumonia and identified by CHROM agar technique. The bacterial cells were grown overnight at 37°C in Luria-Bertani (LB) medium with shaking. And Bacterial suspension was then spread over LB-Agar plates and cultured aerobically as described by our previous report [15].

2.3. Photocytotoxicity Assay of Curcumin on Bacteria. The bacterial cells of S. aureus grown in exponential phase in LB medium were harvested by centrifugation at 4000 rpm for 5 min. Bacterial suspension (10⁶ cfu/mL) was prepared and incubated with different concentrations of curcumin (0, 0.5, 1, 1.5, 2, and 2.5 μM) in a 6-well plate at room temperature for 60 min in the dark and then irradiated by blue light emitted from a LED light source with the wavelength of 470 nm and the power density of 60 mW/cm² described by liang et al. [16].

All experiments were randomly divided into four groups as follows.

(1) Group 1 (sham control): the bacterial cells in the sham control were treated by neither curcumin nor blue light.

(2) Group 2 (curcumin treatment alone): the cells in the curcumin treatment alone group were treated by curcumin without light irradiation.

(3) Group 3 (blue light irradiation alone): the cells in the light irradiation alone group were irradiated by blue light with the dose of 3 J/cm² without curcumin treatment.

After light-activated curcumin, bacteria were serially diluted 10-fold in phosphate buffered saline (PBS) to obtain dilutions of 10⁻¹ ~ 10⁻⁶ times of the original concentration. 50 μL of each dilution was then spread on LB-Agar plates and cultured aerobically in the dark for 16 h at 37°C. The viability of bacteria was investigated through counting the colony forming units.

2.4. Membrane Permeability Assay. Membrane permeability of bacterial cells was investigated using confocal laser scanning microscopy (CLSM) and flow cytometry (FCM). In brief, bacteria were firstly incubated with curcumin (2.5 μM) for 60 min in the dark at 37°C and then irradiated by blue light with the dose of 3 J/cm². After photodynamic treatment, bacteria were harvested (at 4000 rpm, 5 min) and propidium iodide (PI) (10 μg/mL) was then added into each well. After the incubation for 20 min in the dark, membrane permeability of the stained cells was observed immediately using a CLSM and the images were recorded using a colorful charge-coupled device camera. In addition, membrane permeability of the cells stained by PI was also analyzed using a FCM (SE, Becton Dickinson, USA) with the excitation of the light at the wavelength of 488 nm.
2.5. ROS Measurement. After bacterial cells were photosensitized in curcumin (2.5 μM, 3 J/cm²), bacterial cells were incubated with 1 μL 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA, 10 mM, Beyotime, Jiangsu, China) for 20 min in the dark at 37°C. ROS were analyzed using flow cytometry (FCM) (SE, Becton Dickinson, USA) with the excitation of the light at the wavelength of 488 nm and the signals were finally acquired at the FL-2 channel.

2.6. Ultrastructural Observation. After light-activated curcumin (2.5 μM, 3 J/cm²), bacterial cells were fixed for 1 day in 2% glutaraldehyde and postfixed with 2% OsO₄, dehydrated with graded alcohol and embedded with Epon 812 (Electron Microscopy Sciences, Fort Washington, PA, USA). Ultrathin sections (100 nm) of bacteria cells were stained in uranyl acetate and lead citrate and observed under a transmission electron microscopy (TEM) (H-600; Hitachi, Japan).

2.7. Statistical Analysis. All data were expressed as mean ± SD and statistically analyzed using SPSS 18.0 for Windows. One-way ANOVA (analysis of variance) was used to compare the differences between groups. A P value less than 0.05 was considered significant difference.

3. Results

3.1. Photocytotoxicity Assay. Photocytotoxicity of curcumin on S. aureus was measured using colony forming unit assay. Figure 1 showed that blue light-activated curcumin significantly inactivated S. aureus in a curcumin concentration-dependent manner. No significant inactivation activity was found in the controlled cells treated by curcumin alone or blue light irradiation alone (P > 0.05).

3.2. Membrane Permeability Assay. The membrane permeability of bacterial cells was measured using a CLSM after PI staining. More red fluorescence was found in S. aureus treated by blue light-activated curcumin than those of cells from sham control, curcumin treatment alone, and blue light irradiation alone. There was no remarkable difference between sham control, curcumin treatment alone, and blue light irradiation alone (Figure 2). Flow cytometry showed that the positive rate of bacteria stained by PI in sham control was 1.27%, 1.46% in curcumin treatment alone group, and 1.50% in blue light irradiation alone group, however, the positive rate of PI stained cells remarkably increased up to 28.31% after the combined treatment of curcumin and blue light irradiation (Figure 3).

3.3. ROS Production. After DCFH-DA staining, FCM investigation showed the spectral shift of the fluorescence curves to the right, indicating that the ROS level in S. aureus after light-activated curcumin was markedly higher than the controlled bacteria from sham control group, curcumin treatment alone group, and blue light irradiation alone group (Figure 4).

3.4. Ultrastructural Changes. TEM observed that the S. aureus cells from curcumin treatment alone group (2.5 μM)
Figure 3: Membrane permeability of *S. aureus* was measured using flow cytometry with PI staining after LED light-activated curcumin (2.5 μM, 3 J/cm^2). (a) Sham control; (b) curcumin treatment alone; (c) blue light irradiation alone; (d) blue light-activated curcumin.

Figure 4: The ROS level in *S. aureus* was analyzed using FCM with DCFH-DA staining after curcumin (2.5 μM) treatment and blue light irradiation (3 J/cm^2). (A) Sham control; (B) curcumin treatment alone; (C) blue light irradiation alone; (D) blue light-activated curcumin. and blue light irradiation alone group (3 J/cm^2) are intact and smooth (Figures 5(b) and 5(c)), similar to those of the sham control group (Figure 5(a)), whereas the electron density of the bacterial cells treated by blue light-activated curcumin was lower than those of the control groups (Figure 5(d)) and the vacuoles were observed in most *S. aureus* cells after LED-activated curcumin treatment. The results indicated that partial cytoplasm leakage occurred after the combined treatment of curcumin (2.5 μM) and blue light irradiation (3 J/cm^2).

4. Discussion

Medical plants are found to contain numerous active components killing pathogenic microorganisms [17]. Chinese herbs
are some specific medical plants, which are widely used in traditional medicine to combat a variety of clinical diseases [18]. Of which many traditional Chinese herbs such as cleaning hot and detoxification herbs have been widely used to treat infectious diseases. Curcumin is a naturally occurring pigment isolated from a traditional Chinese herb *Curcuma longa*. Numerous studies and clinical evidences have shown that curcumin has many medical activities including antioxidant, antitumor, antibacterial, and immunomodulation [13, 19–23]. Curcumin was found to induce bacterial FtsZ assembly and inhibit bacterial cytokinesis and even interrupt quorum sensing (QS) to reduce pathogenicity [13, 24]. Recent studies showed that curcumin inhibited Sortase A and *S. aureus* cell adhesion to fibronectin [25]. However, higher drug dose is often needed to achieve bactericidal efficacy of curcumin [26]. Interestingly, growing evidences showed that visible light irradiation could activate curcumin and enhance its antibacterial and antitumoral activity [8, 14]. In order to effectively activate curcumin we have successfully set up a blue light source using LED. In the present study, photodynamic action of blue light-activated curcumin against *S. aureus* was investigated using the CFU assay. We observed a significant reduction in viability of *S. aureus* after the combined treatment of curcumin and blue light irradiation and curcumin at lower doses significantly killed *S. aureus* upon blue light irradiation. Our TEM results also observed markedly ultrastructural damage in bacterial cells after the combined treatment of curcumin and blue light irradiation. The fact of PDI that it can kill pathogenic bacteria has been confirmed by us and other scientists [5–7, 9–11]. Curcumin, an active compound with multitarget activity from food and traditional Chinese herb, is a safe and effective antimicrobial agent and is also a naturally occurring photosensitizer [8, 13–16, 24, 25]. The combination of curcumin and light irradiation would have dual antibacterial effect, including direct antibacterial activity of curcumin itself and ROS-mediated antibacterial effect of curcumin upon blue light irradiation. Thus, blue light-activated curcumin might be a potential way to kill *S. aureus*.

Membrane integrity is a prerequisite of bacterial survival. PI is fluorescent nucleic acid binding dyes which is excluded by the intact cell membranes of both bacteria
and eukaryotes and is generally used as indicator of cell membrane permeability [27]. In the present study CLSM with PI staining observed more bacteria with red fluorescence after curcumin treatment in combination with blue light irradiation than that of the controls, including sham irradiation, curcumin treatment alone, and blue light irradiation alone. Flow cytometry also reinforced the observation of CLSM, demonstrating that blue light-activated curcumin markedly damaged the membrane of S. aureus. TEM found vacuoles in many bacterial cells, showing partial cytoplasm leakage after the combined treatment of curcumin and blue light irradiation. The observations indicated the damages of membrane integrity might directly lead to the loss of cytoplasm contents, subsequently resulting in bacterial death.

Excessive accumulation of intracellular ROS is a direct or indirect cause of cell death [15]. Excessive ROS can destruct cell membrane, cytoplasmic membrane, and nuclear membrane to directly cause cell death. Furthermore, ROS can indirectly cause fatal cell damage through oxidizing intracellular biomolecules such as nucleus acid and protein [6, 15, 28–31]. Our flow cytometry with DCFH-DA staining also observed that intracellular ROS level significantly increased after the combined treatment of curcumin and blue light irradiation, indicating that blue light-activated curcumin damaged membrane permeability probably through excessive accumulation of intracellular ROS to cause cell death of S. aureus. However, photodynamic action of curcumin is a complex cellular and molecular event; the exact mechanism will further be explored in our future investigations.

5. Conclusion

The rise of antibiotic-resistant bacteria and relatively slow development of new antibiotics are driving the researchers to seek novel antibacterial strategies. Our present data showed that blue light-activated curcumin damaged membrane permeability, causing cell death of S. aureus and highlighted that intracellular ROS increase might be an important cause to photodynamically kill S. aureus in the presence of curcumin.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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