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Sonodynamic action of chlorin e6 on *Staphylococcus aureus* and *Escherichia coli*

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ABSTRACT

Bacteria remain a great threat to human health. In the present study, we examined whether sonodynamic action of chlorin e6 had antibacterial activity on gram-positive bacterial strain *Staphylococcus aureus* (*S. aureus*) and gram-negative bacterial strain *Escherichia coli* (*E. coli*). Colony forming unit (CFU) assay showed that sonodynamic treatment of chlorin e6 induced a 2-log reduction in CFU of *E. coli* cells, 7-log reduction in CFU of *S. aureus*. Fluorescent microscopy observed that dead cells remarkably increased whereas live cells decreased after sonodynamic treatment of chlorin e6 on *S. aureus* cells. We first demonstrated that sonodynamic action of chlorin e6 has antibacterial effect on both gram-positive and negative bacteria, more powerful on gram-positive bacteria.

1. Introduction

Sonodynamic therapy (SDT) is a recently-developed technique on the basis of photodynamic therapy (PDT) [1]. SDT functions by activating a sonosensitizer to generating reactive oxygen species (ROS), inducing death of cancer cells directly or indirectly [2]. Recent observations on cancer cells indicated that SDT in vitro inhibited cancer growth [3–10]. Notably, in vivo study on animal reported that SDT delayed the tumor growth and the inhibitory effect of ROS scavenger supported the ROS-mediated mechanism of SDT [11]. However, most explorations on SDT were focused on cancer research. Limited investigation was performed on infection disease.

Effective sonosensitizer is essential for sonodynamic therapy. Chlorin e6 is a well-established photosensitizer, showing high affinity with tumor tissue and no damage to normal tissue. Studies on animal and clinical patients reported beneficial effects of chlorin e6-mediated PDT against angiosarcoma, skin cancer and skin metastases of melanoma [12,13]. Owing to sonochemical property of the chlorin e6, it can also be triggered by ultrasound to cause ROS production, which can in turn lead to cell death. Recent researches demonstrated that sonodynamic action of chlorin e6 inhibited cancer growth in tumor-bearing mice, showing valuable evidence for SDT of chlorin e6 [14,15]. However, the effect of SDT with chlorin e6 on bacterial cells remains unknown. Thus, the present study was designed to explore whether sonodynamic action of chlorin e6 had antibacterial effect on two common pathogens *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*).

2. Materials and methods

2.1. Bacteria culture

Bacterial strains *S. aureus* (ATCC 25923) and *E. coli* (ATCC 35218) used in the study were provided by the Microbiology Department of the Chinese University of Hong Kong. After growing overnight at 37 °C in nutrient broth medium (Difco, Franklin Lakes, NJ, USA), the bacteria were shaked for 3 h at 37 °C in broth medium. Bacterial suspension was centrifuged at 4500 revolutions per minute (RPM) for 15 min at room temperature, the supernatant discarded and the cell pellet was resuspended in normal saline to a final concentration of 10^6 bacteria/ml.

2.2. Sonodynamic treatment

An inoculum of 10^6 bacterial/ml was transferred to each of wells in a 24-well plate and assigned to different treatments: sham control, ultrasound alone (US), chlorin e6 (Ce6, purchased from frontier scientific Inc (Utah, USA), chlorin e6 + ultrasound (Ce6 + US). The final chlorin e6 concentration was 1.25 μM, 2.5 μM, 5 μM, 10 μM and 20 μM respectively for investigating sonodynamic action of chlorin e6 on *S. aureus*, 5 μM, 10 μM, 20 μM, 40 μM and 80 μM for *E. coli* study. The cells were incubated for

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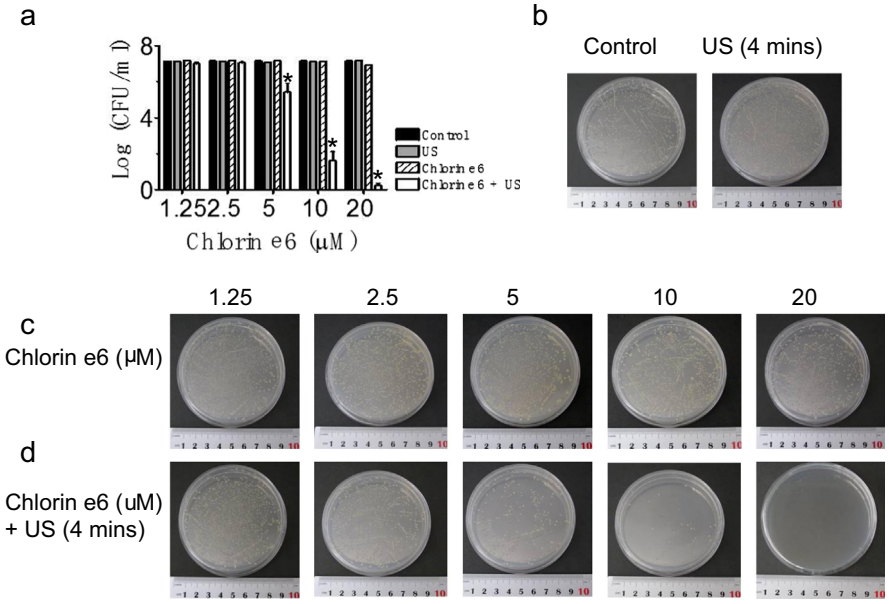

Fig. 1. Chlorin e6 with ultrasound treatment inhibited bacterial growth of *S. aureus* in a dose-dependent manner. (a) Combined treatment of ultrasound with chlorin e6 inhibited bacterial growth in a dose-dependent fashion, with 7 log reduction at 20 μM. (b and c) Ultrasound or chlorin e6 alone use had no effect on bacterial growth. (d) Maximal antibacterial activity was obtained with 20 μM. Data are mean ± SEM of 3 experiments. $^{*}P < 0.05$.

15 min in darkness before ultrasonic treatment. A 1-cm diameter transducer with a central frequency of 1.0 MHz and intensity of 1.56 W/cm^2 was employed to produce continuous ultrasound energy at various exposure times as described by Wang et al [16]. The plane transducer was placed in a water tank filled with degassed water and the temperature was kept at 37 °C during ultrasonic exposure. The ultrasound parameters were measured as described in our previous studies [17].

2.3. Colony forming unit (CFU) assay

Enumeration of the bacterial counts was performed by plating serially diluted bacterial suspension on nutrient agar after ultrasonic exposure. Plates were incubated at 37 °C for 24 h and colonies were counted.

2.4. Bacterial viability assay

After sonodynamic treatment, one milliliter of bacterial suspension from each group was prepared for bacterial viability assay. Each bacterial suspension sample was incubated with the mixture of SYTO 9 and PI (Live/Dead Bacterial Viability Kits 7012, Invitrogen, CA, USA) in darkness for 15 min at room temperature. 10 μL of stained suspension was spotted onto a microscope slide, air-dried for 5 min, heat fixed for approximately 1–2 s, and sealed for microscopic analysis. The stained bacterial suspension was

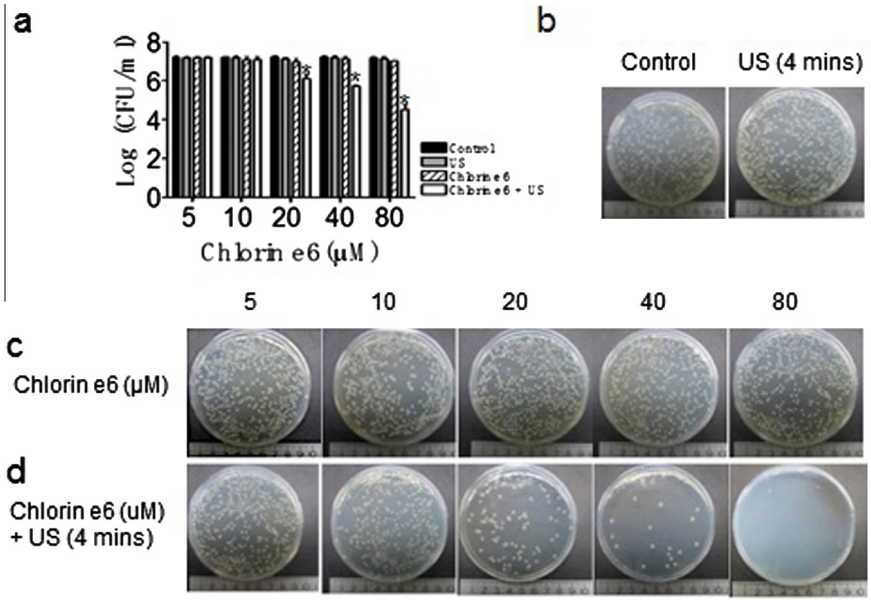

Fig. 2. Ultrasound, together chlorin e6 inhibited bacterial growth of *E. coli* in a dose-dependent manner. (a) Combined ultrasound treatment with chlorin e6 revealed a dose-dependent inhibitory activity, with only 2-log bacterial reduction at 80 μM. (b and c) Ultrasound or chlorin e6 alone had no effect on bacterial growth. (d) Antibacterial activity was obtained with ultrasound and chlorin e6 at concentration of 80 μM. Data are mean ± SEM of 3 experiments. $^{*}P < 0.05$ versus control.