Ultrasonics 54 (2014) 2109–2114

Contents lists available at ScienceDirect

Ultrasonics

journal homepage: www.elsevier.com/locate/ultras

Sonodynamic inactivation of methicillin-resistant *Staphylococcus aureus* in planktonic condition by curcumin under ultrasound sonication

Xinna Wang [a], Margaret Ip [b], Albert Wingnang Leung [a], Chuanshan Xu [a,c,*]

[a] School of Chinese Medicine, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, Hong Kong
[b] Department of Microbiology, Faculty of Medicine, The Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, Hong Kong
[c] Shenzhen Research Institute, The Chinese University of Hong Kong, Shenzhen, China

ARTICLE INFO

Article history:
Received 13 December 2013
Received in revised form 12 June 2014
Accepted 19 June 2014
Available online 10 July 2014

Keywords:
Sonodynamic inactivation
Sonodynamic chemotherapy
Curcumin
MRSA
Planktonic bacteria

ABSTRACT

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is an important cause of difficult-to-treat infections. The present study aims to investigate sonodynamic inactivation of MRSA in planktonic condition using curcumin under ultrasound sonication. Dark toxicity of curcumin to MRSA was investigated to choose the concentration range of curcumin used in the study. The uptake of curcumin in MRSA was observed before ultrasound sonication. After sonication colony forming units (CFUs) and bacterial viability were investigated using fluorescence assay. Additionally, chromosomal DNA fragmentation was also analyzed. Curcumin showed no dark toxicity to MRSA in the concentration range of $\leqslant$500 μM. The maximum uptake of curcumin in MRSA occurred in 50 min after curcumin incubation. Counting of CFUs showed that curcumin had significantly sonodynamic killing effect on MRSA in a curcumin dose-dependent manner, and 5-log reduction in CFU was observed after curcumin treatment (40 μM) at room temperature in the dark for 50 min followed by exposure to ultrasound with intensity of 1.56 W/cm^2 for 5 min. The ratio of green-fluorescent intensity to red-fluorescent intensity was obviously decreased after curcumin treatment under ultrasound sonication. No significant change in chromosomal DNA was found in the cultured MRSA after the combined treatment of curcumin and ultrasound. These results demonstrated that sonodynamic action of curcumin had significant inactivation of MRSA in planktonic condition.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is a Gram-positive bacterium which was first reported in 1961 [1]. Recently, MRSA has evolved from controllable nuisance into a serious public health concern and resulted in more than one-half of the nosocomial infections with *S. aureus* strains in most countries including China [2]. In the community, MRSA mostly causes skin and soft tissue infections [3,4]. In the hospital, MRSA is an important cause of nosocomial infections such as intravenous catheter associated infections, ventilator associated pneumonias and surgical wound infections [5,6]. Owing to the antibiotic resistance common β-lactam antibiotics including oxacillin, penicillin, and amoxicillin are ineffective. Although vancomycin, a glycopeptide, is considered to be an effective way to treat MRSA infections, there has also been increasing evidence of vancomycin-resistant *S. aureus* in the clinical setting [7,8]. Therefore, alternatives are urgently needed to treat MRSA infections.

Ultrasound has been extensively applied in medicine and biology over the past few years. Among these numerous applications, ultrasound therapeutic technology has attracted more and more attentions because of its many unique advantages such as safe and convenient operation, relative low-cost, easy focal and deep penetration in biological tissue [9,10]. Sonodynamic therapy (SDT) or sonodynamic chemotherapy (SDCT) or sonodynamically antibacterial chemotherapy (SDACT) is an interesting ultrasound therapeutic modality for treating malignancies [11–13]. SDT is based on the generation of reactive oxygen species (ROS) in situ to kill cancer cells via the activation of sonosensitive agent called sonosensitizer under ultrasound sonication [14,15]. Sonosensitive agent is an important component to develop sonodynamic therapy in clinical settings. Our previous study showed curcumin, a naturally occurring pigment from a traditional herb the rhizomes of *Curcuma longa*, had significant ROS production in cultured cancer cells and caused remarkably cellular damage under ultrasound

* Corresponding author at: School of Chinese Medicine, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, Hong Kong. Tel.: +852 3943 3769; fax: +852 3942 0972.
E-mail address: csxu@cuhk.edu.hk (C. Xu).

http://dx.doi.org/10.1016/j.ultras.2014.06.017
0041-624X/© 2014 Elsevier B.V. All rights reserved.

sonication [16,17]. In the present study, we extended our study to investigate sonodynamic killing of MRSA in planktonic condition using curcumin under ultrasound sonication.

2. Materials and methods

2.1. Bacterial strain and culture

Methicillin-resistant *Staphylococcus aureus* (MRSA) strain (ATCC BAA-43) was inoculated in 2 ml Mueller–Hinton (MH) broth (Oxoid, Thermo Fisher Scientific, MA, USA) and cultured aerobically at 37 °C overnight. An aliquot of 500 μL was transferred to 50 ml MH broth with shaking at 200 rpm for 3 h. After that, the cells were harvested by centrifugation at 4500 rpm for 15 min and resuspended in 0.85% saline to a turbidity of approx 1.0×10^6 CFU/ml.

2.2. Sensitizer

Curcumin was used as a sensitizer in the present study from Sigma–Aldrich (MO, USA). A stock solution (10 mM) was prepared in dimethyl sulfoxide (DMSO) and kept in the dark at −20 °C until used.

2.3. Dark toxicity test

Bacterial suspensions were incubated with curcumin at various concentrations (500, 50, 5, 0.5 μM) in the dark at room temperature for 0, 5, and 24 h. After that, 10 μL of cell suspension was spread on the Mueller–Hinton agar (MHA) in 10-fold serial dilutions. colony forming unit (CFU) was counted after incubation for 24 h at 37 °C.

2.4. Uptake of curcumin in MRSA

According to the method described by Tseng et al. [18], curcumin was added into the bacterial suspension to a final concentration of 50 μM. After addition of curcumin, 0.5 ml of each sample was removed at every 10-min interval. All samples were rinsed with 0.85% saline twice and resuspended in 1 ml of 0.1 M glycine hydrochloride (pH 3.0) for 17 h at RT. Samples were centrifuged at 5600 g for 10 min. Fluorescence of the supernatant was measured at an excitation wavelength 425 nm with emission wavelength at 500–700 nm on a microplate reader (Infinite M200, Tecan Group Ltd., Switzerland). The relative fluorescence intensity was represented as F_t (after adding curcumin at time t) minus F_0 (before adding curcumin).

2.5. Sonodynamic treatment

All experiments were randomly divided into 4 groups: the combined treatment of curcumin and ultrasound (bacteria were incubated with curcumin before ultrasound exposure), sham insonication alone (bacteria were not pretreated by curcumin but only exposed by ultrasound), curcumin treatment alone (bacteria were not exposed by ultrasound but only incubated with curcumin), and sham treatment (bacteria were treated by neither ultrasound nor curcumin). Briefly, aliquots of 500 μL cell suspension and 500 μL curcumin working solution of various concentrations were transferred to separate wells of 24-well flat bottom plate (Corning, MA, USA). After incubation at room temperature in the dark for 50 min, the 24-well plate was fixed on a platform in an acrylic water tank containing degassed water and a 1.6-cm-diameter plane transducer emitting plane waves was fixed at the bottom of the tank, thus the cells in each well were centered in the ultrasound beam at a distance of 1.0 cm from the transducer and exposed to ultrasound with the spatial average ultrasonic intensity of 1.56 W/cm^2 and a frequency of 1 MHz in continuous waves for 5 min.

2.6. Determination of bacterial counts in colony formation unit (CFU)

500 μL cell suspension (1×10^6 CFU/ml) was transferred to 24-well flat bottom plate containing 500 μl volume with final concentrations of curcumin at 2.5, 5, 10, 20, 40 μM. The plates were incubated in the dark at room temperature for 50 min followed by ultrasound exposure, and the bacterial viability was assessed using the colony counting method as described above.

2.7. Bacterial viability assay

Bacterial viability was evaluated with LIVE/DEAD BacLight™ Bacterial Viability Kit (L7012, Invitrogen, CA, USA). The kit provides two colour fluorescence assay of bacterial viability that is based on the mixture of SYTO 9 and propidium iodide (PI), which are the green-fluorescent nucleic acid stain and the red-fluorescent nucleic acid stain, respectively [19]. According to the protocol, bacteria suspensions were adjusted to 2.0×10^7 CFU/ml and then treated by curcumin (40 μM) together with ultrasound exposure at intensity of 1.56 W/cm^2 for 5 min. After that, 3 μL mixture of SYTO 9 and PI was added to each 1 ml bacterial suspension and incubated at room temperature in the dark for 15 min. The stained bacterial cells were visualized under the fluorescence microscope (ECLIPSE 80i, Nikon, Tokyo, Japan) with the FITC-TRITC dual band excitation filter combination for SYTO 9 and PI simultaneously. Meanwhile, the fluorescence intensity of the stained bacteria were measured at the excitation/emission about 485/530 nm (emission 1; green) for SYTO 9 and 485/630 nm (emission 2; red) for PI using the fluorescence microplate reader (DTX-880, Beckman Coulter, CA, USA). The percentage of live cells in each group was represented by dividing the fluorescence intensity at emission 1 by the fluorescence intensity at emission 2.

2.8. DNA fragmentation analysis

Firstly, bacterial suspensions (1.0×10^8 CFU/ml) were subjected to the sonodynamic treatment as described. Bacterial DNA was prepared by embedding the treated bacteria in the agarose plugs and analyzed by pulsed-field gel electrophoresis (PFGE) with a CHEF DR-III apparatus (Bio-Rad, CA, USA) using switch times of 5 s to 35 s for 30 h at 6.0 V/cm. Last, the gel was stained for 1 h with SYBR® safe DNA gel stain (Invitrogen, CA, USA) and visualized using UV light transilluminator.

2.9. Statistical analysis

The statistical analysis of the data was processed using a statistical package SPSS 13.0. Differences between groups were analyzed by the analysis of variance (ANOVA). A P-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Toxicity of curcumin on MRSA

In order to identify whether curcumin itself has killing effect on MRSA in the dark, we incubated the bacterial cells with various concentrations of curcumin at different incubation times. No bac-

Download English Version:

https://daneshyari.com/en/article/10690385

Download Persian Version:

https://daneshyari.com/article/10690385

Daneshyari.com