Sonodynamic bactericidal efficacy of hypocrellin A and B against methicillin-resistant *Staphylococcus aureus*

AWN Leung *, M Ip, CS Xu, XN Wang, PT Yung, HY Hua

KEY MESSAGES

1. Hypocrellin A and B have significant bactericidal activity against methicillin-resistant *Staphylococcus aureus* (MRSA).
2. Sonodynamic treatment of hypocrellin A/B inhibits protein synthesis of MRSA.
3. Hypocrellin A/B–mediated sonodynamic action causes notable damage to membrane integrity, membrane potential, and ultrastructure of MRSA.

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[1] AWN Leung, [2] M Ip, [1] CS Xu, [1] XN Wang, [3] PT Yung, [1] HY Hua

[1] *School of Chinese Medicine, The Chinese University of Hong Kong*
[2] *Department of Microbiology, The Chinese University of Hong Kong*
[3] *Department of Electronic Engineering, The Chinese University of Hong Kong*

* Principal applicant and corresponding author: awnleung@cuhk.edu.hk

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a public health problem. Current antibiotics have limited efficacy against MRSA and may cause multi-drug resistance.[1] There is a need to develop novel strategies to combat MRSA. This study aimed to evaluate the sonodynamic bactericidal efficacy of hypocrellin A and hypocrellin B against MRSA.

Methods

This study was conducted from April 2012 to September 2014. The MRSA (ATCC BAA-43) strains were provided by the Department of Microbiology, The Chinese University of Hong Kong.

Bacterial suspensions were incubated with hypocrellin A/B at a series of concentrations in the dark at room temperature for different lengths of time. Cell suspensions were spread on Mueller-Hinton agar and cultured for further 24 hours at 37°C. Growth of MRSA was evaluated using colony forming unit (CFU) assay and expressed as CFU/mL ($\log_{10}$).

To measure the uptake of hypocrellin A/B in MRSA, bacterial suspensions were incubated with hypocrellin A/B (50 μM). Fluorescence of samples was measured every 10-minutes after addition of sensitisers. The fluorescence of hypocrellin A/B in MRSA was measured using fluorescent analysis on a microplate reader.

To evaluate the sonodynamic bactericidal efficacy of hypocrellin A/B against MRSA, the treated bacteria were spread on Mueller-Hinton agar and incubated for 24 hours. The growth of bacteria was evaluated using the CFU assay and described as dark toxicity. The ultrasound exposure system has been described in our previous study.[2]

To investigate mechanisms of sonodynamic bactericidal activity of hypocrellin A/B against MRSA, the ultrastructural morphology of the treated bacteria was observed using a transmission electron microscope. Bacterial DNA fragmentation was measured using pulsed-field gel electrophoresis with CHEF DR-III apparatus; bacterial DNA and RNA synthesis was measured using a microplate reader in combination with chemiluminescent bromodeoxyuridine enzyme–linked immunosorbent assay and flow cytometry with Click-iT RNA Alexa Fluor 488 HCS Assay, respectively; bacterial protein synthesis was measured using a flow cytometer with Click-iT Plus OPP Protein Synthesis Assay Kit; and bacterial membrane integrity and membrane potential were measured using a flow cytometer with propidium iodide and carbocyanine dye 3,3'-diethyloxacarbocyanine iodide, respectively.

Results

Hypocrellin A/B treatment alone had no significant effect on the growth of MRSA in terms of dark toxicity. The uptake of hypocrellin A/B increased with prolonged incubation time and reached a peak after 50 minutes. The growth of MRSA was inhibited after ultrasound sonication in the presence of 80 μM hypocrellin A ($P<0.0001$) or 20 μM hypocrellin B ($P<0.0001$). There was a 5-log reduction in CFUs following ultrasound treatment in the presence of 40 μM hypocrellin B. Sonodynamic treatment of hypocrellin A/B caused notable damage to the ultrastructure of MRSA, including septal deformations, loss of the septal midline, and cell lysis.

An incomplete cell envelope was found in bacteria treated by hypocrellin B and ultrasound together. There was no significant change to chromosomal DNA in any group. There was no significant change to DNA synthesis in the combined treatment groups versus control group. DNA and RNA synthesis was comparable among the ultrasound treatment alone group, hypocrellin A treatment alone group, hypocrellin B treatment alone group, and control group. For protein synthesis, combined treatment with ultrasound and hypocrellin had more effect than ultrasound treatment alone or hypocrellin treatment alone ($P<0.05$). Sonodynamic treatment of hypocrellin A/B had more effect on membrane integrity than ultrasound treatment alone ($P<0.05$). Sonodynamic treatment of hypocrellin A/B had more effect on bacterial membrane potential than ultrasound treatment alone and hypocrellin treatment alone ($P<0.05$).

Conclusion

Sonodynamic treatment of hypocrellin A/B had significant bactericidal activity against MRSA. There was notable damage to the bacterial membrane caused by the sonodynamic antibacterial action of hypocrellin A/B.

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