Austin Journal of Tropical Medicine & Hygiene

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Austin Publishing Group

Short Communication

Antibacterial Activity of Berberine against Methicillin-Resistant *Staphylococcus Aureus* Planktonic and Biofilm Cells

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Received: December 19, 2014; **Accepted:** February 17, 2015; **Published:** February 19, 2015

Abstract

Methicillin-Resistant *Staphylococcus Aureus* (MRSA) is a pathogenic bacterium which may cause serious infections. Biofilm formation is an important factor involved in difficult eradication of bacteria. Natural products may be an alternative for therapeutic development and berberine has diverse biological effects including antibacterial. In this study, activity of berberine against planktonic and biofilm cells of MRSA were determined. MICs values ranged from 62.5 to 250 µg/ml and MBC values were the same or two fold above the MIC. Biofilms of half of the strains were also inhibited by berberine at concentrations of 125 to 1000 µg/ml. Further studies are necessary; however there is a potential application of berberine in the development of strategies against bacteria.

Keywords: Berberine; Antibacterial; MRSA; Biofilm

Introduction

Methicillin-Resistant *Staphylococcus Aureus* (MRSA) is a Gram-positive bacterium which causes community- and hospital-acquired infections. Biofilm formation is one defense mechanism, since bacteria embedded in biofilms are more difficult to eradicate than planktonic cells. Single-drug treatment for MRSA related infections is becoming less effective and natural products may be an alternative for future antibacterial medicine development [1-3]. Berberine is a plant alkaloid, important in Chinese medicine and characterized by diverse biological activities such as antimicrobial, antihypertensive, anti-inflammatory, anti-oxidant, anti-depressant, anti-cancer, anti-diarrheal, cholagouge, hepato protective, and anti-diabetic activities [4,5]. In this short communication we relate activity of berberine against planktonic and biofilm cells of MRSA and MSSA.

Materials and Methods

Strains and growth conditions

Bacterial strains were *Staphylococcus aureus* ATCC 29213 and 13 clinical isolates, 10 MRSA (methicillin-resistant strains 72,73,74,76,77,78,79,81,83,90) and 3 MSSA (methicillin-sensitive strains 97,170,212). They were maintained in Mueller Hinton Agar (AMH-Difco) at 4°C and cultured in Mueller Hinton Broth (MHB-Difco) before each experiment.

Microdilution MIC determination

Antibacterial and antifungal assays were performed by micro dilution method in sterile flat-bottom microplates according to CLSI [6]. Each well contained appropriate test samples, culture medium, and approximately 10^5 cells/ml for bacteria. Serial two-fold dilutions of berberine were done in a micro dilution plate (96 wells) containing 100 µl of culture medium. Next, the inoculum was added to each well. The microplates were incubated at 37°C during 24h. The MIC was defined as the lowest concentration which resulted in inhibition of visual growth. Minimal Bactericidal Concentrations (MBC) was determined by sub culturing 10 µl of the culture from each negative well and from the positive control.

Biofilm

S. aureus biofilm was formed in a polystyrene 96-well micro titer plates. Assays were done twice in triplicate. 100 µl of a suspension containing 1 x 10^8 cells/ml TSB medium supplemented with 1% glucose were seeded in wells and incubated at 37°C for 24 h. Well content was discharged, wells were washed with Phosphate Buffered Saline (PBS) and dilutions of berberine ranging from 15.6 to 1000 µg/ml were added to each well. After incubation at 37°C for 24h, wells were rinsed with PBS. For MTT reduction assay, slight modifications on method utilized by Schillaci et al. [7] were done. 20 µl of MTT solution (5 mg/ml in PBS) was added to each well and plates were incubated at 37°C for 2 h. After staining, MTT solution was removed from each well and 100 µl of DMSO was added to dissolve MTT formazan product. 100 µl of DMSO was transferred to a new plate and optical density was measured at 570 nm using a microplate reading. BIC50 was the concentration enough to inhibit viability of biofilm in 50%.

Results and Discussion

Berberine is a plant alkaloid with historical medicinal use in Chinese medicine; it has demonstrated antimicrobial activity against bacteria, fungi, and viruses, protozoans, helminthes and chlamydia [4]. In this study, MIC values for berberine ranged from 62.5 to 250 µg/ml against MRSA strains, MBC values were the same or two fold above the MIC. To Methicillin-sensitive *S. aureus* (MSSA) strains 97,170 and 212, berberine MIC values were 125 and 500 µg/ml. Inhibitory concentration for biofilm (BIC50) were 1000 µg/ml for four strains, 500 µg/ml for two strains and 125 µg/ml for just one strain. BIC50 could not be determined to half of all strains because the maximum concentration tested was 1000 µg/ml [Table 1].

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Citation: Endo EH, Filho D and BP. Antibacterial Activity of Berberine against Methicillin-Resistant *Staphylococcus Aureus* Planktonic and Biofilm Cells. Austin J Trop Med & Hyg. 2015;1(1): 1005.

Table 1: MIC, MBC and BIC50 values for berberine against *S. aureus* strains.

S. aureus strains	MIC	MBC	BIC50
ATCC 29213	125	125	-
72	125	250	-
73	125	125	1000
74	125	250	-
76	125	125	-
77	125	125	-
78	250	250	500
79	62.5	125	125
81	125	250	-
83	125	125	1000
90	125	250	1000
97	125	250	-
170	125	250	1000
212	500	1000	500

Our MIC results are according to other authors who showed antibacterial activity of berberine. Yu et al. [8] related MICs of berberine against MRSA between 32 and 128 µg/ml and a synergistic effect when associated with oxacillin. Wang et al. [9] related the effect of berberine on *Staphylococcus epidermidis* biofilm formation. Wojtyczka et al. [10] showed MICs of berberine chloride against coagulase-negative *Staphylococcus* between 16 and 512 µg/ml with synergistic effect in combination with linezolid, cefoxitin and erythromycin. Liang et al. [11] showed synergistic effect of berberine in association with fusidic acid against MRSA clinical isolates. However, the mechanism involved in antibacterial action of berberine has not been extensively studied. Possible action would be binding to DNA, which contributes to antimicrobial effect, and binding proteins in biofilm, interrupting its stability and thus enhance the antibacterial activity of antibiotic [12,13].

Conclusion

These results showed antibacterial activity of berberine against planktonic and biofilm cells of MRSA and MSSA. Further studies are necessary to assess synergistic effects of berberine in combination with antibiotics against these strains. However, these data indicated a potential application of berberine in the development of strategies for *staphylococcal* infections.

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Austin J Trop Med & Hyg - Volume 1 Issue 1 - 2015
ISSN: 2472-3681 | www.austinpublishinggroup.com
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