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Abstract

Effect of matrine on reducing damage to bovine mammary epithelial cells induced by Staphylococcus aureus alpha-hemolysin

Introduction

Bovine mastitis caused by strains of Staphylococcus aureus is the most economically important disease affecting the dairy industry worldwide (Perez-Casal et al. 2006). It is known that milk contains multiple types of immune cells and bovine mammary epithelial cells (Sordillo. 2005). Infections with S. aureus which secrete α-hemolysin often result in tissue damage and a depletion of immune cells, including macrophages and T cells. Immune cell death helps S. aureus to evade the bovine’s innate immunity and adhere to BMECs. The co-action of S. aureus and α-hemolysin leads to the mass death of BMECs. The prevalence of methicillin-resistant S. aureus, which can tolerate multiple antibiotics is able to withstand the effect and presence of the antibiotic that previously killed them (Kumar et al. 2010). The term antibiotic resistance refers to situations where antibiotics that normally inhibit certain types of bacteria no longer have the desired effect. Antibiotic residues in milk can affect consumers health and also cause financial losses in the dairy industry (Pogurschi et al. 2015). Thus taking bacterial virulence factors as targets is a new therapy for the development of antimicrobial.

In past years, several studies investigated whether effective antibacterial ingredients extracted from traditional Chinese medicine could eliminate the virulence factors secreted by S. aureus (Qiu et al. 2011, Liu et al. 2015). It has been reported in preliminary rease-
arch that the antimicrobial activity of matrine, an alkaloid found in plants from the Sophora genus against S. aureus and Staphylococcus epidermidis (SE) has a inhibitory effect on the expression of virulence factors which are related to the ability of SE trapping resistance genes in biofilm cells (Yong et al. 2015, Li et al. 2016). Therefore, the aim of this study was to investigate the effect of matrine on α-hemolysin production of S. aureus and protection against α-hemolysin and S. aureus-induced BMECs injury in the co-culture system. The minimal inhibitory concentrations (MIC) of matrine against S. aureus in TSB were evaluated in triplicate by a broth microdilution method as recommended by the Laboratory Standards Institute (Ku et al. 2015).

### Materials and Methods

BMECs were isolated and identified and cultured in DMEM/F12 (Gibco BRL) supplemented with 20% (v/v) fetal bovine serum, 5 μg/ml insulin (SIGMAAALDRICH Chemie GmbH, USA), 1 μg/ml hydrocortisone, 1μg/ml corporin (SIGMAAALDRICH Chemie GmbH, USA), 5 μg/ml transferrin (SIGMAAALDRICH Chemie GmbH, USA), and antibiotics (100 μg/ml gentamicin and 100 μg/ml penicillin-streptomycin) under a 5 % CO₂ atmosphere. Cytotoxicity (LIVE/DEAD) assay was measured by using the LIVE/DEAD reagent (KeyGEN BioTECH, China) and Microscopic images of the stained cells were acquired by using an inverted fluorescence microscope (Olympus, Japan). Cell proliferation was determined using the CCK-8 assay. Hemo-

<table>
<thead>
<tr>
<th>Hemolysis (%) of rabbit erythrocytes by culture supernatants</th>
<th>0 µg/ml</th>
<th>16 µg/ml</th>
<th>64 µg/ml</th>
<th>256 µg/ml</th>
<th>625 µg/ml</th>
<th>1250 µg/ml</th>
<th>2500 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>8325-4</td>
<td>100</td>
<td>99.98±1.23</td>
<td>99.89±1.21</td>
<td>99.86±0.98</td>
<td>99.88±1.12</td>
<td>99.89±1.14</td>
<td>99.89±0.87</td>
</tr>
<tr>
<td>USA300</td>
<td>100</td>
<td>99.87±1.25</td>
<td>99.91±1.38</td>
<td>99.94±0.52</td>
<td>99.94±1.23</td>
<td>99.92±0.89</td>
<td>99.95±1.10</td>
</tr>
<tr>
<td>ATCC43300</td>
<td>100</td>
<td>99.89±1.67</td>
<td>99.85±1.33</td>
<td>99.68±1.19</td>
<td>99.92±0.96</td>
<td>99.97±0.25</td>
<td>99.90±1.13</td>
</tr>
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Hemolytic activity of the drug-free group served as the 100% hemolysis control.
lytic activity assay was determined according to the method of Rowe and Welch (1994). Based on the hemolysis assay results, western blot assays (Zhou et al. 2015) and Semi-quantitative RT-PCR (Liu et al. 2015) were performed with the culture supernatants described above to detect whether the reduced hemolytic activity in the \( S\). aureus culture supernatants was due to a decrease in hla expression.

### Results and Discussion

The MIC values for matrine against \( S.\) aureus USA 300 (KWIKSTIK™, Microbiologics, USA) and 8325-4 were 5 mg/ml, 2.5 mg/ml respectively. The MIC values were greater than 256 µg/ml, which means that matrine at levels under 256 µg/ml had no significant effect on the growth of \( S.\) aureus. The hemolytic activity of \( S.\) aureus \( \alpha\)-hemolysin is shown in Table 1.

The results indicated that matrine did not inhibit the hemolysin and at a concentration of 1-256 µg/ml, matrine did not directly cause hemolysis of rabbit erythrocytes directly. Therefore, the effect of matrine on the transcriptional levels of \( \alpha\)-hemolysin was evaluated, which was encoded by the hla gene and agrA. In accordance with the results of western blot assay (Fig. 1A), the transcriptional levels of both genes were reduced significantly (\( p<0.01\)) when treated with matrine (Fig. 1B).

Cells were treated with varying doses (0, 30, 60, 90, 120 ng/ml) of \( \alpha\)-hemolysin for 8 hrs. The \( \alpha\)-hemolysin treatment induced BMECs death in a dose-dependent manner (\( p<0.01\)) (Fig. 2A). Photomicroscope analysis also supports these results (Fig. 2B).

Statistically significant inhibition of cell viability of BMECs co-treated with \( S.\) aureus which can secrete \( \alpha\)-hemolysin. Matrine(100 µg/ml) was incubated with BMECs in one group and the other group untreated with matrine prior to stimulation with \( S.\) aureus (8325-4). The cell morphology clearly indicated the effects of matrine on cell death (Fig. 3).

Similar to other Gram-positive bacteria, the pathogenicity of \( S.\) aureus is, to a great extent, dependent upon the secretion of numerous extracellular virulence factors (Qiu et al. 2010). Therefore, the clinical perfor-
formance of antimicrobial agents used for the treatment of S. aureus infections not only depends on the respective bacteriostatic or bactericidal effects, but also on the ability to prevent the release of virulence factors by dying or stressed bacteria (Mun et al. 2016). Recently, plant extracts have garnered great interest for their potent antimicrobial properties against a broad spectrum of microorganisms. In our research, it was demonstrated that matrine is active against both MSSA and MRSA, these results are in accordance with the findings of other investigators (Fu et al. 2012). Hla is one of the most prominent virulence factors secreted by S. aureus that contributes to host colonization and diseases. Inhibition of α-hemolysin synthesis involves neutralizing toxins and the inhibition of DNA or RNA synthesis. Subinhibitory concentrations of matrine cannot inhibit hemolytic activity but can inhibit S. aureus secretion of α-hemolysin. The result just like Diosmetin or Eugenol perform the inhibitory effect on α-hemolysin synthesis through inhibition of agrA transcription and directly inhibits expression of hla-gene encoding α-hemolysin synthesis, then production of α-hemolysin was reduced (Liu et al. 2015, Qiu et al. 2010). In this study, it was determined that α-hemolysin induced inflammation injury of BMECs and these effects could be weakened by matrine. The precise mechanism has not been clarified and further studies are necessary to elucidate the precise mechanisms by which matrine exhibited a protective effect on S. aureus-induced BMECs injury.

Acknowledgments

This study was supported by the National Natural Science Foundation of China (No. 31460676) and Major Innovation Projects for Building First-Class Universities in China’s Western Region (ZKZD2017001).

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