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Short communication

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

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Abstract

Taking bacterial virulence factors as targets is a new therapy for treating host bacterial infection. The aim of this study was to investigate the effect of matrine on α-hemolysin production of *Staphylococcus aureus* (*S. aureus*) and reducing the damage to bovine mammary epithelial cells (BMECs) induced by *S. aureus* α-hemolysin. Subinhibitory concentrations of matrine decreased the production of α-hemolysin in none dose-dependent manner and matrine exhibited a protective effect on *S. aureus-induced* BMECs injury. The results indicated that the structure of matrine may potentially be used as a basic structure for development of drugs aimed at curing and preventing dairy bovine mastitis.

Key words: α-hemolysin, BMECs, matrine, cell damage

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-

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Table 1. Hemolytic activity of culture supernatants treated with various concentrations of matrine.

	Hemolysis (%) of rabbit erythrocytes by culture supernatants						
	0 μg/ml	16 μg/ml	64 μg/ml	25 6μg/ml	625 μg/ml	1250 μg/ml	2500 μg/ml
8325-4	100	99.98±1.23	99.89±1.21	99.86±0.98	99.88±1.12	99.89±1.14	99.89±0.87
USA300	100	99.87±1.25	99.91±1.38	99.94±0.52	99.94±1.23	99.92±0.89	99.95±1.10
ATCC43300	100	99.89±1.67	99.85±1.33	99.68±1.19	99.92±0.96	99.97±0.25	99.90±1.13

Hemolytic activity of the drug-free group served as the 100% hemolysis control.

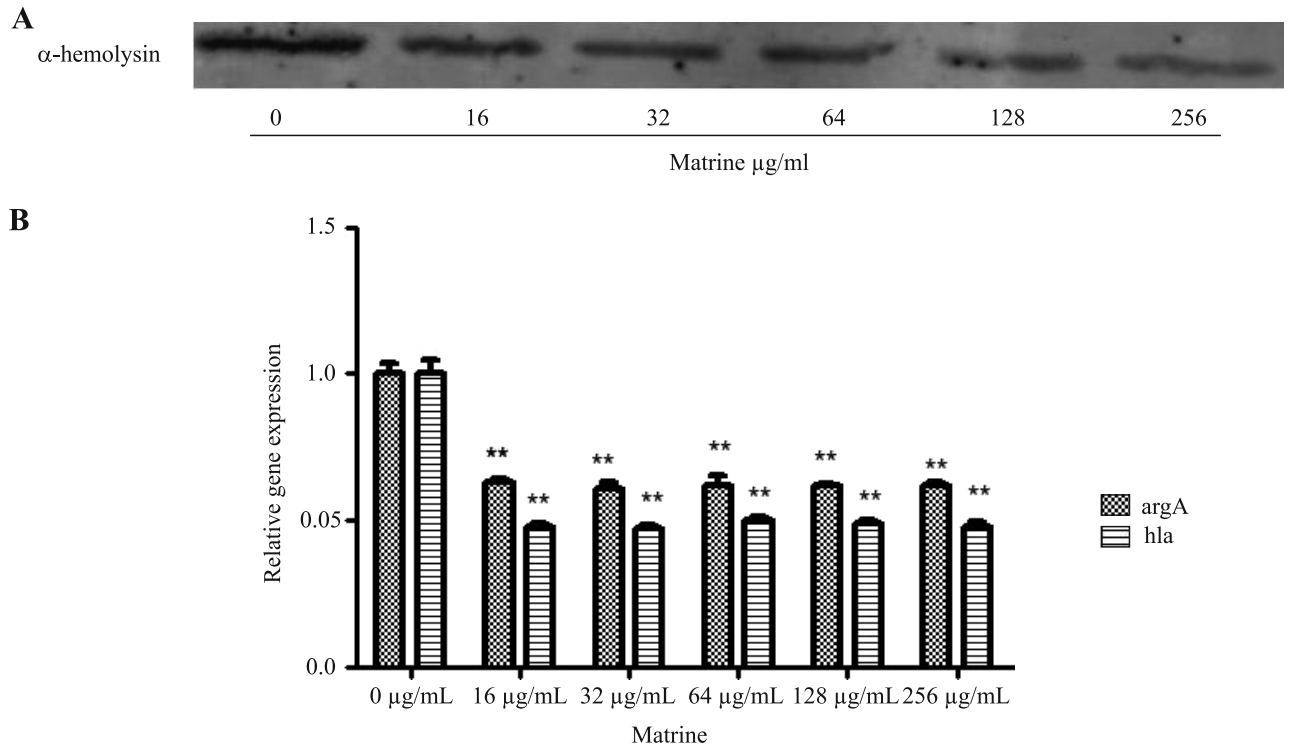

Fig. 1A. Western blot assays of α-hemolysin produced by *S. aureus* 8325-4 grown with increasing concentrations of matrine.
Fig. 1B. Relative gene expression of hla and agrA in *S. aureus* 8325-4 exposed to indicated concentrations of matrine.
The values are the averages of three independent experiments. **p<0.01 compared with the matrine-free samples.

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 (SIGMAALDRICH Chemie GmbH, USA), 1 μg/ml hydrocortisone, 1μg/ml corporin (SIGMAALDRICH Chemie GmbH, USA), 5 μg/ml transferrin (SIGMAALDRICH Chemie GmbH, USA), and antibiotics (100 μg/ml gentamicin and 100 μg/ml penicillin-streptomycin) under a 5 % CO_2 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-

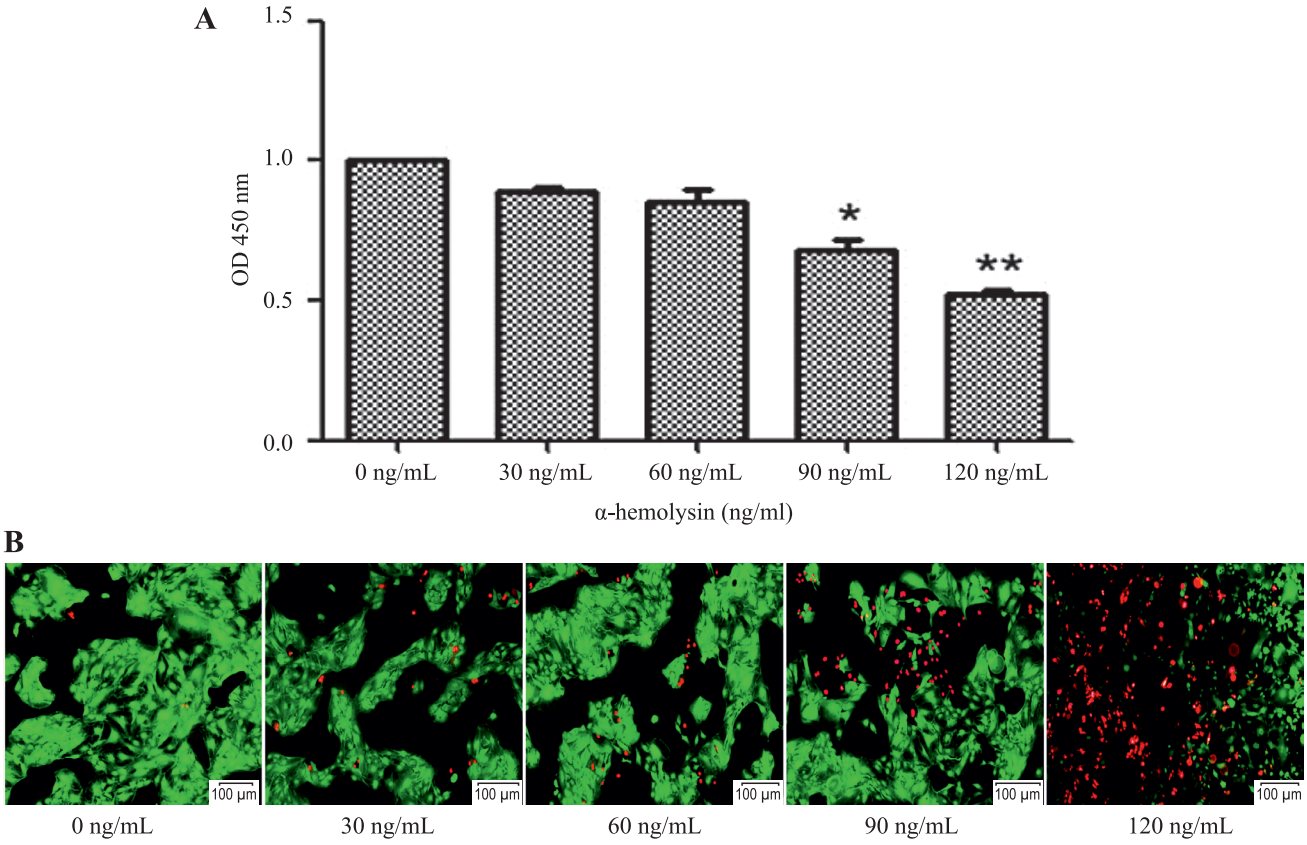

Fig. 2. The effect of α-hemolysin on primary cultured BMECs. (A) BMECs plated in a 6-well plate was treated with α-hemolysin (8 h) at the indicated doses. Cell viability was determined using the CCK-8 method. The viability of control cells was determined as 1; viability relative to the control was analyzed. The experiments were performed in triplicate at least three times. Bars indicate standard error. **p<0.01 vs. non-treated cells. (B) Live (green)/dead (red) stained BMECs were captured through using a inverted fluorescence microscope after co-cultured with different concentration of α-hemolysin (×200).

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* α-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 α-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 α-hemolysin for 8 hrs. The α-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 α-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-

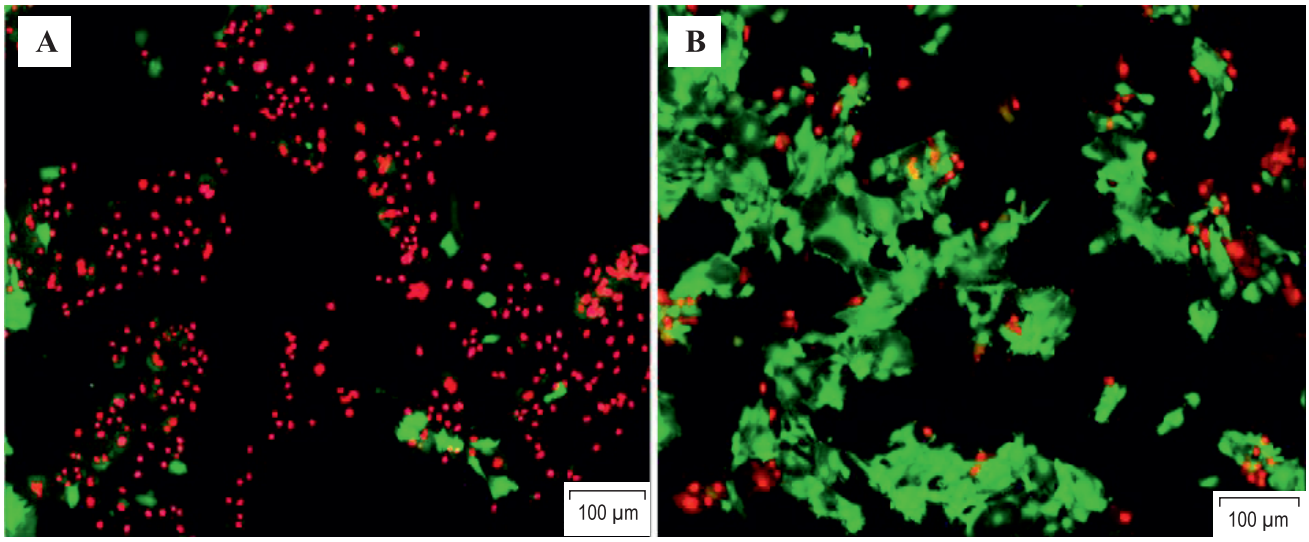

Fig. 3. Matrine protects BMECs from α-hemolysin-mediated injury. Live (green)/dead (red) stained BMECs were captured through using a inverted fluorescence microscope after infection with *S. aureus* 8325-4. Cells co-cultured with S. aureus 8325-4 without matrine (A) or with 100 μg/ml matrine (B) (×200).

mance 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 involvs neutralizing toxins and the inhibition of DNA or RNA synthesis. Subinhibitory concentrations of matrine cannot inhibit hemolytic activity but it can inhibit *S. aureus* secretion of α-hemolysin. The result just like Diosmetin or Eugenol perform the inhibitory effect on α-hemolysin synthesis through inhibiton 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.

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