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Matrine alleviates *Staphylococcus aureus* lipoteichoic acid-induced endometritis *via* suppression of TLR2-mediated NF-κB activation

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ABSTRACT

Endometritis is one of the main diseases that causes great economic losses in the dairy industry. Recent studies have shown that matrine extracted from the traditional Chinese herb *Sophora flavescens* is an alkaloid with a broad range of bioactivities. Here, we aimed to investigate the protective effects of matrine on *Staphylococcus aureus* lipoteichoic acid (LTA)-induced endometritis in mice and elucidate the possible molecular mechanisms *in vitro*. Histopathological changes showed that matrine remarkably attenuated the uterus injury in a mouse model of LTA-induced endometritis. qPCR and ELISA results showed that matrine dose-dependently reduced the expression of pro-inflammatory cytokines (TNF-α and IL-1β). To further elucidate the underlying mechanisms of this protective effect of matrine, LTA-stimulated bovine endometrial epithelial cells (bEECs) were employed in this study. The results demonstrated that TLR2 expression and its downstream nuclear factor (NF)-κB activation were both suppressed by matrine treatment. Furthermore, a small interference RNA targeting TLR2 gene mimicked matrine in its inhibition on LTA-induced activation of TLR2 and NF-κB. In conclusion, these findings suggest the protective effect of matrine against LTA-induced endometritis through negative regulation of TLR2-mediated NF-κB pathway.

1. Introduction

Endometritis is one of the most common reproductive system diseases in dairy cows, which causes great economic losses in the dairy industry [1,2]. Endometritis is a typical mucosal disease caused by infection with a variety of microbial pathogens during mating or delivery, such as Gram-positive bacteria *Staphylococcus aureus* (*S. aureus*) [3]. *S. aureus*, a typical opportunistic pathogen, is responsible for the prevalence of both clinical and subclinical endometritis. *S. aureus* can affect the host's immune system and colonize in the host cell to avoid being eliminated by some antibiotics [4,5]. Moreover, the abuse of antibiotics in the dairy industry aggravates the threat of antibiotic residues in food products and even leads to serious food safety problems [6]. Thus, it is necessary to develop new treatment strategies for endometritis in dairy cows.

Endometrial epithelial cells (EECs) are the first line of defense of the uterus against bacterial invasion and play a central role in the pathogenesis of *S. aureus* endometritis [7]. Lipoteichoic acid (LTA), a teichoic acid isolated from the *S. aureus* cell wall, is the main driving force of the host inflammatory response to *S. aureus* infection [8]. Studies have shown that toll-like receptors (TLRs) are critical for the innate immune system by recognizing specific pathogen-associated molecular patterns (PAMPs) of invading bacteria [9,10]. TLR2, as a key receptor for LTA, can induce the activation of extracellular signaling pathway, including NF-κB pathway [11], finally leading to the overexpression of pro-inflammatory cytokines, such as IL-1β and TNF-α.

Matrine (Fig. 1A), an alkaloid isolated from the traditional Chinese herb *Sophora flavescens* [12]. It has been reported to have a variety of biological activities, including anti-inflammatory, anti-tumor, anti-oxidative and anti-viral properties [13–15]. However, although there have been some reports on anti-inflammatory activities of matrine, no progress has been made in the study of endometritis elicited by *S. aureus*. Therefore, in the present study, we employed a mouse model of *S. aureus* LTA-induced endometritis, which closely mimics the inflammatory responses observed during *S. aureus* endometritis, to assess whether matrine could alleviate the endometritis and clarify the possible mechanisms *in vitro*.

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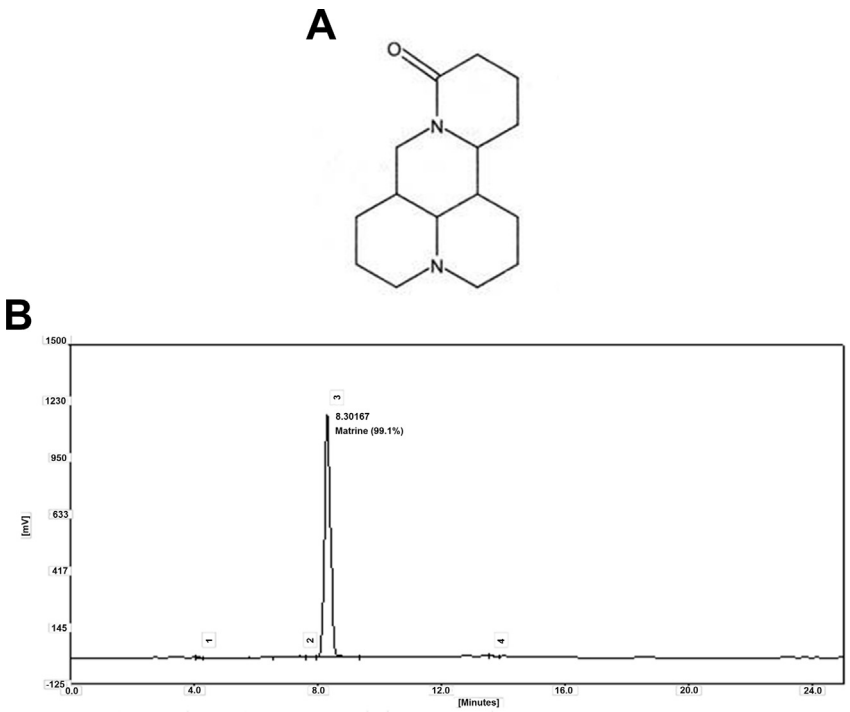

Fig. 1. (A) The chemical structure of matrine. (B) HPLC chromatogram of matrine.

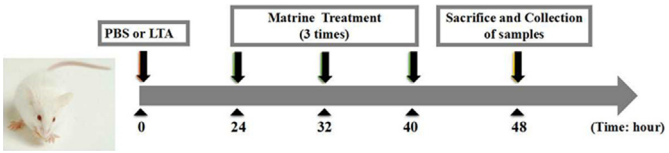

Fig. 2. Animal treatment protocols in this study.

2. Materials and methods

2.1. Reagents

Matrine was purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). The purity of matrine was tested with high performance liquid chromatography (HPLC), and the test was carried out on an EChrom2000 DAD data system (Elite, Dalian, China) following the manufacturer's instructions. In brief, the chromatographic separation was performed on a Hypersil ODS2-C18 analytical column (250 mm × 4.6 mm, 5 μm). The flow rate was 1.0 mL/min, and the detection wavelength was 203 nm. The specific purity of matrine was 99.1% (Fig. 1B). LTA from *S. aureus* was provided by Sigma-Aldrich Chemical Co. (Saint Louis, Missouri, USA). TNF-α and IL-1β enzyme-linked immunosorbent assay (ELISA) kits were purchased from BioLegend (Camino Santa Fe, CA, USA). Antibodies for β-actin, TLR2 and NF-κB were purchased from Cell Signaling Technology (Beverly, USA). All other chemical reagents conform to the reagent specification standard.

2.2. Animals and treatment

BALB/c mice (40 females, 8 weeks old) were provided by the Experimental Animal Centre of Huazhong Agricultural University (Wuhan, China). The mice were housed in individual cages, fed a standard diet under specific conditions at 24 °C ± 1 °C and 65% humidity, and kept on a 12 h of normal illumination for 1 week before the experiments. All animal experiments were carried out in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and approved by the Ethical Committee on Animal Research at Huazhong Agricultural University (HZAUMO-2015-12).

The mice were randomly divided into four groups (n = 10): the control group (CG), LTA group (LTA), and LTA + matrine (50 and 100 mg/kg) groups. The selection of doses was based on a previous report [16]. The murine model of LTA-induced endometritis was performed as previously described [17]. Briefly, each uterus was infused with equal amounts of LTA (5 mg/kg) to induce endometritis. 24 h after administration, matrine was intraperitoneally injected three times every 8 h at dosages of 50 and 100 mg/kg, respectively. The control group and LTA group received equal volumes of normal saline. The mice were euthanized at 8 h after the last matrine treatment, and then the uterus tissues were harvested and stored at −80 °C. A schematic diagram of the treatment schedule is shown in Fig. 2.

2.3. Histopathologic evaluation of the uterus tissue

The uterus tissues were collected and cut into small pieces of approximately 0.5 cm^3 and fixed in 10% formalin for subsequent histopathological analysis. Briefly, the uterus tissues were dehydrated by concentration gradient alcohol, embedded in paraffin, sectioned and stained with hematoxylin and eosin (H&E). Finally, the pathological changes were observed with an optical microscope (Olympus). Histology scoring was carried out according to injury degree such as the integrity of tissue structure and the numbers of infiltrated inflammatory cells.

2.4. Cell culture and treatment

Bovine endometrial epithelial cells (bEECs, BEND cells) were purchased from the American Type Culture Collection (ATCC® CRL-2398™), and cultured in DMEM medium with 10% fetal bovine serum at 37 °C with 5% CO_2 incubation. The cells were pretreated with various concentrations of matrine (200 and 400 μM) for 1 h and then stimulated with LTA for 6 h. Cells that were not given any treatment were used as the control group.

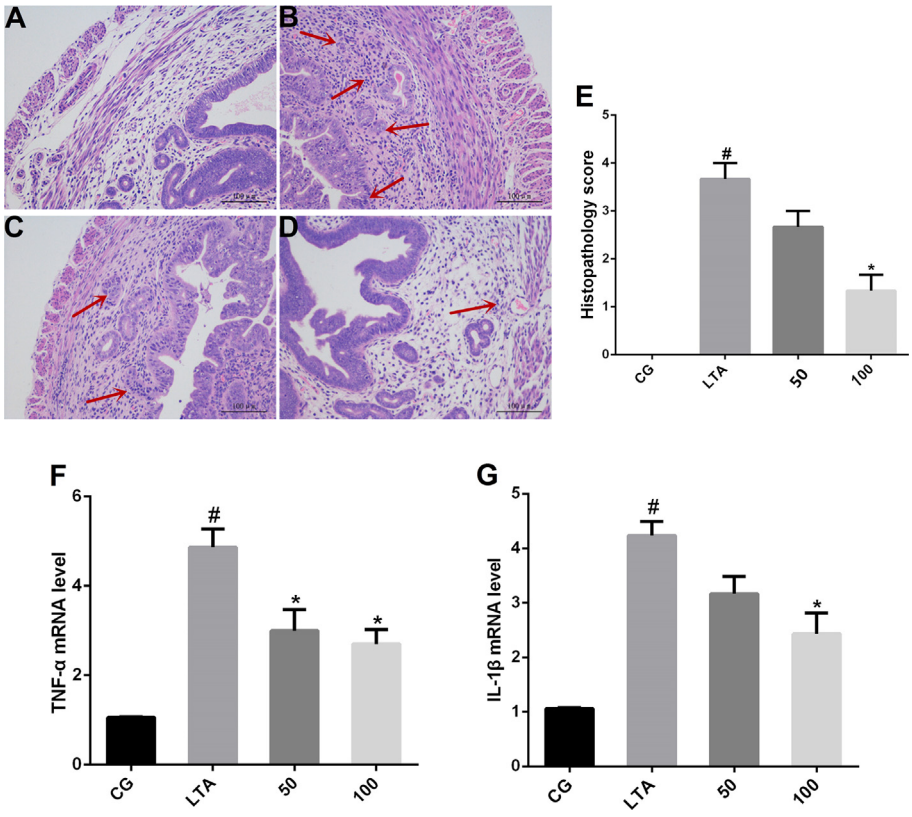

Fig. 3. The effects of matrine on LTA-induced uterus injury. Uterus tissues were harvested for H&E staining. Scale bar: 100 μm. (A) Control group, (B) LTA group, (C, D) matrine (50 and 100 mg/kg) groups. (E) Histopathology scores. (F, G) The expression of TNF-α and IL-1β in uterus tissues was examined by qPCR. GAPDH was used as an internal control. The red arrows indicate the tissue lesion areas and infiltrated inflammatory cells. CG is the control group; LTA is the LTA group; and 50 and 100 are the matrine treatment groups representing 50 mg/kg and 100 mg/kg per animal, respectively. Data represent the mean ± S.E.M. of three independent experiments. $^{\#}P < 0.05$ *vs.* the control group. $^{*}P < 0.05$ *vs.* the LTA group. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.5. Cell viability assay

Cell viability was determined by a Cell Counting Kit-8 (CCK-8) assay kit (Beyotime, China). Cells were plated in 96-well plates for 6 h, after which cells were treated with various concentrations of matrine for 24 h. Subsequently, 100 μL of DMEM medium containing 10% CCK-8 reagent was added in each well and cultured for 2 h at 37 °C. Finally, the optical density (OD) of the cells was measured at 450 nm with a microplate reader.

2.6. RNA extraction and qPCR assay

Total RNA was extracted using Trizol reagent (Invitrogen, United States) following the manufacturer's instructions, and then reverse transcribed into cDNA. The first-strand cDNA was used as a template for the qPCR reaction that was performed using a SYBR Green RT-PCR kit (Roche, Basel, Swiss) according to the manufacturer's recommendations. The expression levels of target genes were normalized to the corresponding GAPDH threshold cycle (CT) values using the $2^{-\Delta\Delta Ct}$ comparative method.

2.7. Western blot analysis

Total protein was harvested from uterus tissues and cells using RIPA lysis buffer supplemented with a protease inhibitor. The total protein concentration was measured with a BCA protein assay kit (Thermo Scientific, MA, USA). Next, equal amounts of protein were fractionated using 10% SDS-PAGE and then transferred onto polyvinylidene difluoride (PVDF) membranes. The membranes were blocked with 5% skim milk for 2 h and then incubated with the indicated primary antibodies at 4 °C overnight. After incubation with the horseradish peroxidase-conjugated second antibodies, the intensities of the proteins were quantified using a chemiluminescence detection system (ImageQuant LAS 4000mini, USA).

2.8. ELISA assay

The effects of matrine on the levels of cytokines were examined. After treatment, the protein levels of TNF-α and IL-1β in supernatants were detected using ELISA kits following the producer's directions.

2.9. Immunofluorescence staining

Immunofluorescence staining were prepared as previously described. Briefly, the sections or cells were permeabilized with PBS containing 0.3% Triton X-100, exposed to 10% BSA, and incubated with indicated antibodies at 4 °C overnight. Thereafter, the cells were incubated with FITC-labelled secondary antibodies for 1 h at 37 °C, and the nuclei were stained for 10 min with DAPI dye and visualized under a fluorescence microscope.

2.10. Small interfering RNA (siRNA) transfection

The siRNA of TLR2 (si-TLR2) and its negative control (si-NC) were synthesized by GenePharma (Shanghai, China). bEECs were cultured in 6-well plates for 24 h prior to transfection. The siRNAs were then transfected into cells using Lipofectamine 2000 (Invitrogen, Carlsbad, California, USA) following the manufacturer's instructions. At 24 h after transfection, the cells were stimulated with LTA for another 6 h and then subjected to further studies.

2.11. Statistical analysis

All data were presented as the means ± SEM. The comparisons between the different groups were performed by one-way ANOVA followed by Dunnett's multiple comparison test. A P value of < 0.05 was considered to be statistically significant.

3. Results

3.1. Matrine protects against LTA-induced endometritis in mice

The histological analysis were performed to assess the pathological changes in uterine tissues. We found that the administration of LTA resulted in severe injury, including hyperemia, hemorrhage and a large number of inflammatory cell infiltration (Fig. 3B). However, the LTA-induced pathological changes were dramatically improved by matrine at doses of 50 and 100 mg/kg (Fig. 3C and D). These findings were further confirmed by the subsequent histology scores (Fig. 3E). LTA can induce the activation of an inflammatory response followed by a notable increase in the secretion of pro-inflammatory cytokines [18]. To further confirm the protective effect of matrine on LTA-induced endometritis, the expression of TNF-α and IL-1β in uterus tissues was also measured. The qPCR results showed that the expression of TNF-α and IL-1β was decreased after matrine treatment (Fig. 3F and G). With increasing doses of matrine, the anti-inflammatory effect was more pronounced. Taken together, these results indicate that matrine effectively attenuates LTA-induced endometritis.

3.2. Matrine suppresses LTA-induced activation of TLR2 and NF-κB in vivo

It is generally accepted that LTA induces the immune response through TLR2 and its downstream NF-κB pathway [11]. NF-κB is a pivotal nuclear transcription factor and has been reported to regulate the production of a large number of inflammatory cytokines [19]. Western blot analysis showed an obvious increase in the levels of TLR2, p-p65 and p-IκBα that was induced by LTA and was decreased by matrine treatment in a dose-dependent manner (Fig. 4). To further validate these findings, paraffin-embedded uterus tissue sections were used to determine NF-κB p65 translocation to nucleus using immunofluorescence assay. We found that the nucleus NF-κB p65 intensity was markedly repressed upon matrine treatment (Fig. 5).

3.3. Effects of matrine on cell viability and cytokines production

The potential cytotoxicity effect of matrine on bEECs was detected using the CCK-8 assay. The CCK-8 assay results showed that matrine at the concentrations used (200 and 400 μM) had no cytotoxicity effect on bEECs (Fig. 6A). Meanwhile, the effects of matrine (400 μM) alone on the levels of TLR2, p-p65 and p-IκBα in cells were also determined, and the results further demonstrated that treatment with matrine alone had no effect on the cells (Fig. 6B). Subsequently, we measured the protein levels of TNF-α and IL-1β so as to explore whether matrine has a similar anti-inflammatory effect *in vitro*. Consistent with the *in vivo* results, the levels of IL-1β and TNF-α were dose-dependently decreased by matrine treatment (Fig. 6C and D).

3.4. Matrine blunts LTA-induced activation of TLR2 and NF-κB in vitro

As displayed in Fig. 7, the levels of TLR2, p-p65 and p-IκBα were noticeably increased in the LTA group relative to the control group. In contrast, their levels were greatly decreased in the matrine treatment group. Moreover, the inhibitory effect of matrine on NF-κB activation was further confirmed by an notable reduction in the nuclear translocation of NF-κB p65 (Fig. 8).

3.5. Effects of si-TLR2 transfection on LTA-induced inflammatory responses

Additionally, to confirm whether the anti-inflammatory effects of matrine were through the TLR2-mediated NF-κB pathway, bEECs were transfected with a small interference RNA (si-TLR2) and then stimulated with LTA for indicated time. The results showed that the TLR2 expression was significantly decreased by si-TLR2 transfection and matrine treatment (Fig. 9A and B). indicating that matrine functions as an inhibitor of TLR2. Furthermore, the NF-κB activation and cytokines production were also markedly repressed after TLR2 knockdown (Fig. 9C and D). Taken together, we speculated that the anti-inflammatory effects of matrine were mediated at least partially through the TLR2/NF-κB pathway.

4. Discussion

Endometritis is characterized by inflammation of the endometrium and has seriously hindered the development of dairy industry [20]. Although the clinical application of antibiotics in the treatment of endometritis has some efficacy, it has led to the emergence of drug-resistant strains. In recent years, traditional Chinese herbs have received great attention from researchers worldwide for their applications in health protection and disease control. *Sophora flavescens* is a kind of evergreen perennial shrub that grows mainly in China [21]. In traditional Chinese medicine (TCM), the root of *Sophora flavescens* has been employed to treat jaundice, fever and pyogenic infection [22]. Matrine is a major active component that is isolated from *Sophora flavescens*. Recent studies have found that matrine could alleviate focal cerebral chemic injury by improving antioxidant activity and inhibiting apoptosis in mice [23]. Besides, it also has been reported to have anti-tumor effects in many cancer, such as gastric cancer [24] and hepatoma [16]. More importantly, matrine has been demonstrated to inhibit pro-inflammatory cytokines production in LPS-activated mouse macrophages [25]. However, few studies have focused on the effects of matrine on

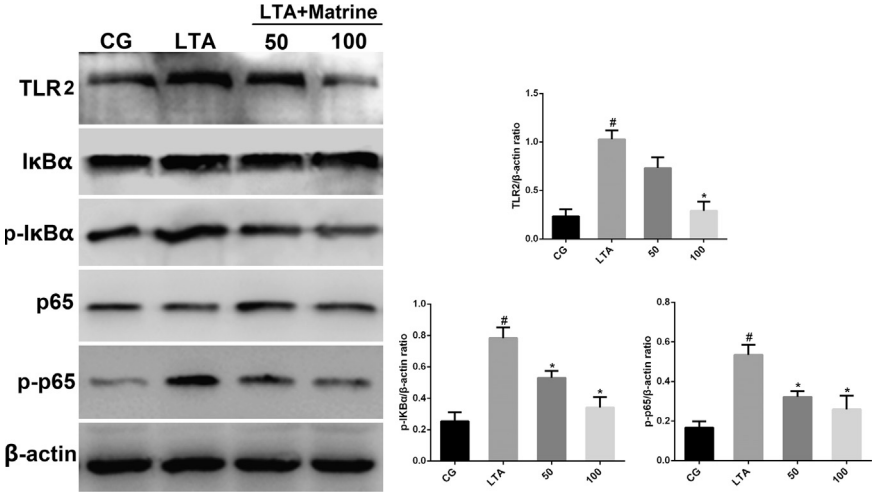

Fig. 4. The effects of matrine on LTA-induced activation of TLR2 and NF-κB *in vivo*. The protein levels of TLR2, p-p65 and p-IκBα in uterus tissues were measured by Western blotting. β-Actin served as the control. CG is the control group; LTA is the LTA group; and 50 and 100 are the matrine treatment groups representing 50 mg/kg and 100 mg/kg per animal, respectively. Data represent the mean ± S.E.M. of three independent experiments. $^{\#}P < 0.05$ *vs.* the control group. $^{*}P < 0.05$ *vs.* the LTA group.

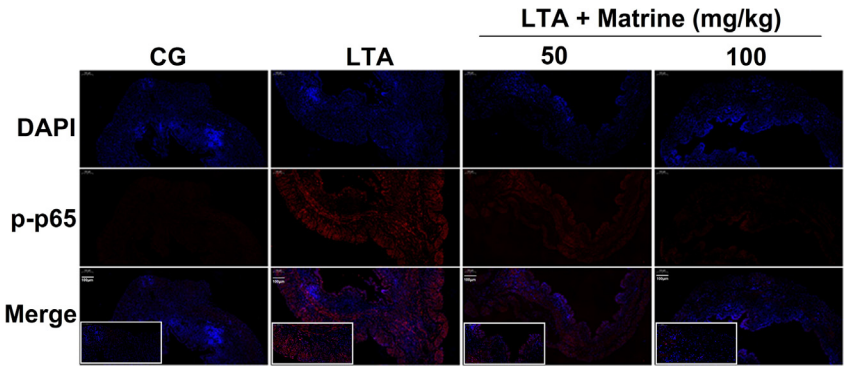

Fig. 5. The effects of matrine on NF-κB p65 translocation into the nucleus *in vivo*.
Paraffin-embedded uterine tissues were used to detect NF-κB p65 translocation to the nucleus by immunofluorescence staining. Scale bar: 100 μm. CG is the control group; LTA is the LTA group; and 50 and 100 are the matrine treatment groups representing 50 mg/kg and 100 mg/kg per animal, respectively.

endometritis. LTA, a specific component of the outer membrane of *S. aureus*, has been shown to induce the inflammatory response [26]. A mouse model of LTA-induced endometritis has been confirmed to be a suitable model for exploring the effective therapeutic strategies that can be used against endometritis [17]. In the present study, the histopathological findings of the excised uterus showed that treatment of matrine noticeably alleviated the pathological damage and reduced the infiltration of inflammatory cells, suggesting that matrine possesses a protective effect on LTA-induced endometritis.

Inflammation is a defensive reaction of the body to harmful stimuli, such as pathogenic microorganisms, damaged cells or irritants [27]. However, chronic inflammation or severe acute inflammation can cause damage to the body's tissue, which can be life-threatening in severe cases [28]. It has been well recognized that pro-inflammatory cytokines, including TNF-α and IL-1β, contribute to the development of endometritis and even cause severe uterus injury [29,30]. IL-1β and TNF-α are primary inflammatory cytokines produced by various types of cells, such as macrophages [9] and epithelial cells [4,31], and could promote the release of other inflammatory mediators. TNF-α and IL-1β stimulate the expression of specific cell adhesion molecules, which regulate leukocyte adhesion and migration to inflammatory sites [32]. Therefore, we speculated that reduction of TNF-α and IL-1β expression might improve the outcome of endometritis. To investigate whether matrine inhibits TNF-α and IL-1β production, we measured their levels in uterus. Intriguingly, matrine treatment significantly suppressed the expression of TNF-α and IL-1β. Epithelial tissue provides the first line of defense against bacterial invasion [33,34]. Hence, the bovine endometrial epithelial cells (bEECs) were used for *in vitro* studies. Consistent with the *in vivo* results, administration of matrine also decreased the levels of TNF-α and IL-1β in LTA-stimulated bEECs. Accordingly, the protective effect of matrine on LTA-induced endometritis may attribute to its suppression on pro-inflammatory cytokines TNF-α and IL-1β.

NF-κB, a pivotal nuclear transcription factor in many inflammatory diseases, has been reported to regulate the transcription of a large number of genes related to inflammation [19]. NF-κB generally exists in an inactive state in the cytoplasm by binding to its inhibitory protein IκBα; when inflammation stimulation occurs, the NF-κB p65 unit dissociates from IκBα and then translocates into the nucleus as an active form, activating gene expression [35,36]. To further unravel the potential mechanisms through which matrine exerts its anti-inflammatory effects, we measured the p-IκBα and p-p65 expression after treatment with matrine. The results showed that matrine markedly inhibited the levels of p-IκBα and p-p65 induced by LTA, suggesting that matrine decreased LTA-induced pro-inflammatory cytokines production through suppressing NF-κB activation. TLR2 is one of the TLR family and acts as a critical receptor for LTA from *S. aureus* [37]. It has been well established that LTA induces the immune response through TLR2 and its downstream NF-κB pathway [11]. In indeed, a previous study has clearly demonstrated that LTA from *S. aureus* induces cytokines production *via* activating the TLR2 pathway [38]. Upon activation of TLR2, adaptor protein MyD88 is recruited to Toll/IL-1R (TIR) domain and then triggers downstream intracellular signaling cascades, such as NF-κB [39]. As expected, the TLR2 expression that was up-regulated by LTA was also reduced by matrine treatment. Thus, we speculated that matrine restrained the NF-κB activation by suppressing the TLR2

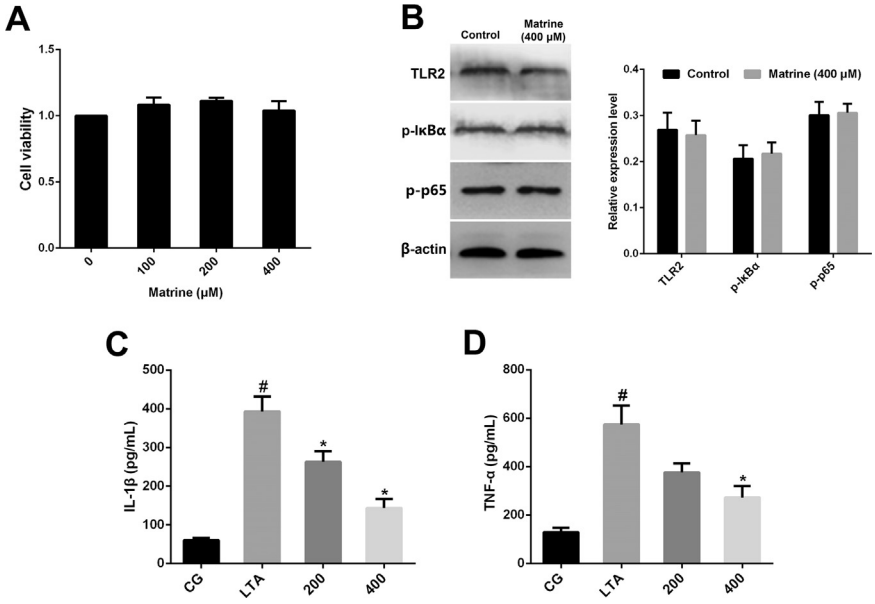

Fig. 6. The effects of matrine on cell viability and cytokines production. (A) The effects of matrine on the cell viability of bEECs. Cells were treated with the indicated concentration of matrine (0–400 μM) for 24 h, and the cell viability was assessed by CCK-8 kits. (B) The effects of matrine (400 μM) alone on the cultured cells. (C, D) The effects of matrine on the cytokines production *in vitro*. The protein levels of IL-1β and TNF-α were determined by ELISA. CG is the control group; LTA is the LTA group; and 200 and 400 are the matrine treatment groups representing 200 μM and 400 μM per cell plate, respectively. Data represent the mean ± S.E.M. of three independent experiments. $^{\#}P < 0.05$ *vs.* the control group. $^{*}P < 0.05$ *vs.* the LTA group.

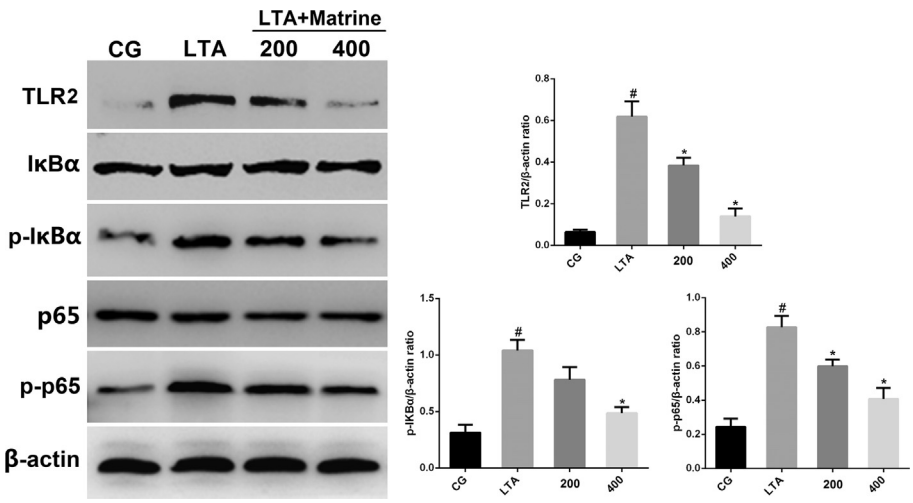

Fig. 7. The effects of matrine on LTA-induced activation of TLR2 and NF-κB *in vitro*. The protein levels of TLR2, p-p65 and p-IκBα in bEECs were measured by Western blotting. β-Actin served as the control. CG is the control group; LTA is the LTA group; and 200 and 400 are the matrine treatment groups representing 200 μM and 400 μM per cell plate, respectively. Data represent the mean ± S.E.M. of three independent experiments. $^{\#}P < 0.05$ *vs.* the control group. $^{*}P < 0.05$ *vs.* the LTA group.

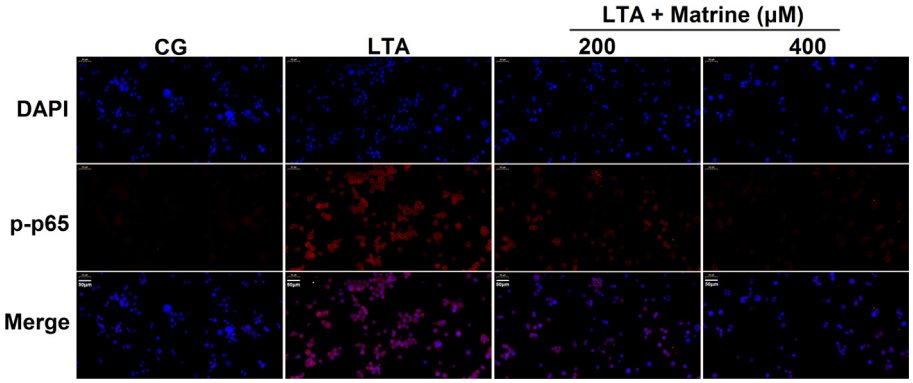

Fig. 8. The effects of matrine on NF-κB p65 translocation into the nucleus *in vitro*. Translocation of the NF-κB p65 subunit from the cytoplasm into the nucleus was assessed by immunofluorescence staining. Scale bar: 50 μm. Blue spots represent cell nuclei, and green spots indicate p-p65 staining. CG is the control group; LTA is the LTA group; and 200 and 400 are the matrine treatment groups representing 200 μM and 400 μM per cell plate, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

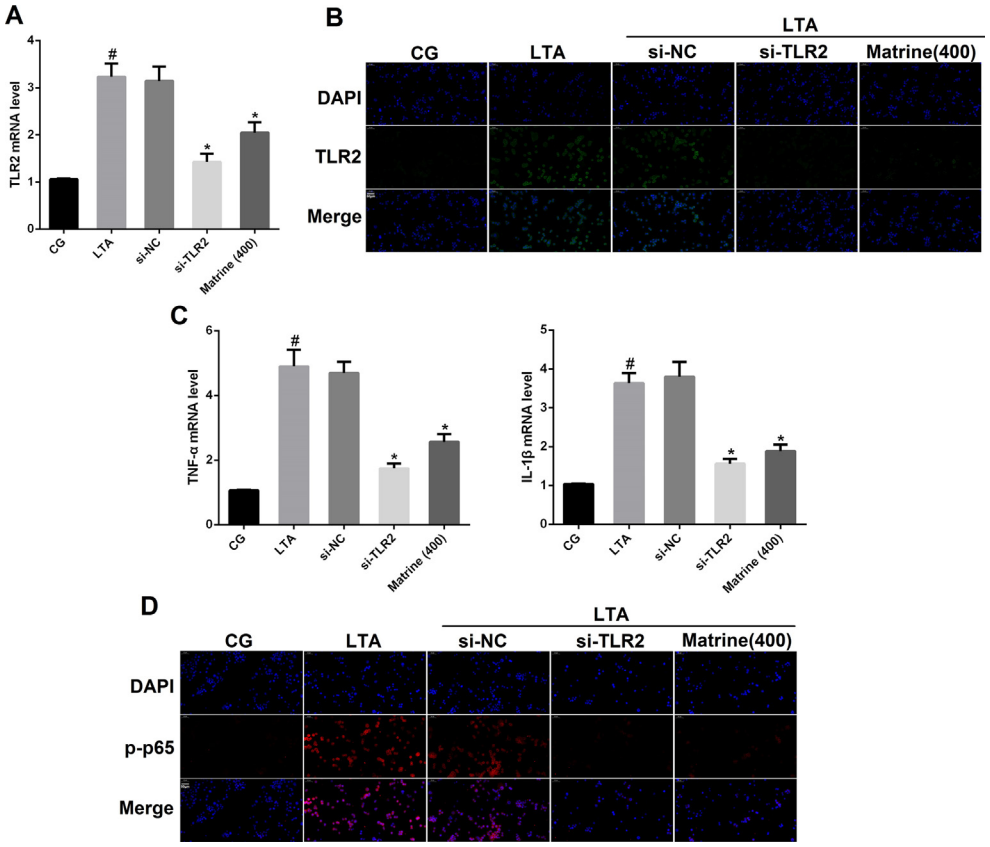

Fig. 9. Effects of si-TLR2 transfection on LTA-induced inflammatory responses. bEECs were transfected with a small interference RNA of TLR2 (si-TLR2) for 24 h, and then stimulated with LTA for 6 h. For the matrine group, cells were pretreated with matrine (400 μM) for 1 h followed by LTA stimulation. (A) The interfering efficiency of TLR2 siRNA was measured by qPCR. GAPDH was used as an internal control. (B) The protein expression of TLR2 were detected by immunofluorescence staining. Scale bar: 50 μm. (C) The expression of TNF-α and IL-1β was determined by qPCR. GAPDH was used as an internal control. (D) Translocation of the NF-κB p65 subunit from the cytoplasm into the nucleus was assessed by immunofluorescence staining. Scale bar: 50 μm. Blue spots represent cell nuclei, and green spots indicate p-p65 staining. CG is the control group; LTA is the LTA group; and 200 and 400 are the matrine treatment groups representing 200 μM and 400 μM per cell plate, respectively. Data represent the mean ± S.E.M. of three independent experiments. $^{\#}P < 0.05$ *vs.* the control group. $^{*}P < 0.05$ *vs.* the LTA group. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

expression. Furthermore, we also confirmed the vital role of TLR2 in *S. aureus* LTA-induced endometritis using a small interference RNA of TLR2; the results agreed with those for matrine and suggested that the anti-inflammatory effects of matrine were mediated at least partially through the TLR2/NF-κB pathway. In addition to the NF-κB pathway, TLR2 also activate the mitogen-activated protein kinase (MAPK) pathway, which is mediated by JNK, ERK and p38 protein kinases [40]. These protein kinases phosphorylate the transcription factor AP-1, leading to the secretion of pro-inflammatory cytokines [4]. Based on the above observation, we hypothesized that matrine could also decrease the expression of JNK, ERK and p38 *via* TLR2. However, the certain effect of matrine on MAPKs remains to be further explored in the future.

In summary, our data demonstrate that matrine exerts anti-inflammatory effects in *S. aureus* LTA-induced endometritis by inhibiting the TLR2-mediated activation of NF-κB signaling pathway. Accordingly, these findings indirectly reveal that matrine may serve as a potent anti-inflammatory agent in the treatment of inflammatory diseases, including *S. aureus* endometritis.

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Conflict of interest

The authors have declared no competing financial interest.

References

[1] W. Li, K. Fu, X. Lv, W. Yu, J. Wang, H. Li, et al., Lactoferrin suppresses lipopolysaccharide-induced endometritis in mice via down-regulation of the NF-κB pathway, Int. Immunopharmacol. 28 (2015) 695–699.
[2] A.M. Hussain, R.C.W. Daniel, Bovine endometritis: current and future alternative therapy, Transbound. Emerg. Dis. 38 (1991) 641–651.
[3] K. Jiang, X. Chen, G. Zhao, H. Wu, J. Mi, C. Qiu, et al., IFN-tau plays an anti-inflammatory role in Staphylococcus aureus-induced endometritis in mice through the suppression of NF-kappaB pathway and MMP9 expression, J. Interf. Cytokine Res. 37 (2017) 81–89.
[4] K.F. Jiang, G. Zhao, G.Z. Deng, H.C. Wu, N.N. Yin, X.Y. Chen, et al., Polydatin ameliorates Staphylococcus aureus-induced mastitis in mice via inhibiting TLR2-mediated activation of the p38 MAPK/NF-kappaB pathway, Acta Pharmacol. Sin. 38 (2017) 211–222.
[5] S.R. Clarke, R. Mohamed, L. Bian, A.F. Routh, J.F. Kokai-Kun, J.J. Mond, et al., The Staphylococcus aureus surface protein IsdA mediates resistance to innate defenses of human skin, Cell Host Microbe. 1 (2007) 199–212.
[6] Z. Ibraimi, A. Shehi, A. Murtezani, S. Krasniqi, Z. Agani, Kosovo's public health damage from abusive use of antibiotics in dairy cattle, Materia Socio Medica 27 (2015) 149–153.
[7] C. Piras, Y. Guo, A. Soggiu, M. Chanrot, V. Greco, A. Urbani, et al., Changes in protein expression profiles in bovine endometrial epithelial cells exposed to E. coli LPS challenge, Mol. BioSyst. 13 (2016) 392.
[8] K. Atanasova, S.V. Gucht, F. Barbé, L. Duchateau, K.V. Reeth, Lipoteichoic acid from Staphylococcus aureus exacerbates respiratory disease in porcine respiratory coronavirus-infected pigs, Vet. J. 188 (2011) 210–215.
[9] K. Jiang, S. Guo, T. Zhang, Y. Yang, G. Zhao, A. Shaukat, et al., Downregulation of TLR4 by miR-181a provides negative feedback regulation to lipopolysaccharide-induced inflammation, Front. Pharmacol. 9 (2018) 142.
[10] A.G. Hise, K. Daehnel, I. Gillette-Ferguson, E. Cho, H.F. Mcgarry, M.J. Taylor, et al., Innate immune responses to endosymbiotic Wolbachia bacteria in Brugia malayi and Onchocerca volvulus are dependent on TLR2, TLR6, MyD88, and Mal, but not TLR4, TRIF, or TRAM. J. Immunol. 178 (2007) 1068–1076.
[11] T. Matsuguchi, A. Takagi, T. Matsuzaki, M. Nagaoka, K. Ishikawa, T. Yokokura, et al., Lipoteichoic acids from Lactobacillus strains elicit strong tumor necrosis factor alpha-inducing activities in macrophages through Toll-like receptor 2, Clin. Diagn. Lab. Immunol. 10 (2003) 259.
[12] J. Huang, H. Xu, Matrine: bioactivities and structural modifications, Curr. Top. Med. Chem. 16 (2016).
[13] J. Yong, X. Wu, C. Lu, Anticancer advances of matrine and its derivatives, Curr. Pharm. Des. 21 (2015).
[14] H.F. Zhang, L.J. Shi, G.Y. Song, Z.G. Cai, C. Wang, R.J. An, Protective effects of matrine against progression of high-fructose diet-induced steatohepatitis by enhancing antioxidant and anti-inflammatory defences involving Nrf2 translocation, Food Chem. Toxicol. 55 (2013) 70–77.
[15] S. Na, P. Sun, H. Lv, Y. Sun, J. Guo, Z. Wang, et al., Matrine displayed antiviral activity in porcine alveolar macrophages co-infected by porcine reproductive and respiratory syndrome virus and porcine circovirus type 2, Sci. Rep. 6 (2016) 24401.
[16] J.Q. Zhang, Y.M. Li, T. Liu, W.T. He, Y.T. Chen, X.H. Chen, et al., Antitumor effect of matrine in human hepatoma G2 cells by inducing apoptosis and autophagy, World J. Gastroenterol. 16 (2010) 4281–4290.
[17] G. Zhao, K. Jiang, H. Wu, C. Qiu, G. Deng, X. Peng, Polydatin reduces Staphylococcus aureus lipoteichoic acid-induced injury by attenuating reactive oxygen species generation and TLR2-NFkappaB signalling, J. Cell. Mol. Med. 21 (2017) 2796–2808.
[18] C.H. Tang, C.J. Hsu, W.H. Yang, Y.C. Fong, Lipoteichoic acid enhances IL-6 production in human synovial fibroblasts via TLR2 receptor, PKCdelta and c-Src dependent pathways, Biochem. Pharmacol. 79 (2010) 1648–1657.
[19] P.P. Tak, G.S. Firestein, NF-κB: a key role in inflammatory diseases, J. Clin. Invest. 107 (2001) 7–11.
[20] K. Jiang, X. Chen, G. Zhao, H. Wu, J. Mi, C. Qiu, et al., IFN-tau plays an anti-inflammatory role in Staphylococcus aureus-induced endometritis in mice through the suppression of NF-kappaB pathway and MMP9 expression, J. Interf. Cytokine Res. 37 (2017) 81–89.
[21] M. Sun, H. Cao, L. Sun, S. Dong, Y. Bian, J. Han, et al., Antitumor activities of kushen: literature review, Evid. Based Complement. Alternat. Med. 2012 (2012) 373219.
[22] H. Kim, R.L. Mi, G.S. Lee, W.G. An, I.C. Su, Effect of Sophora flavescens Aiton extract on degranulation of mast cells and contact dermatitis induced by dinitrofluorobenzene in mice, J. Ethnopharmacol. 142 (2012) 253–258.
[23] P. Zhao, R.U. Zhou, X. Zhu, Y. Hao, L.I. Nan, J. Wang, et al., Matrine attenuates focal cerebral ischemic injury by improving antioxidant activity and inhibiting apoptosis in mice, Int. J. Mol. Med. 36 (2015) 633–644.
[24] C. Luo, Y. Zhu, T. Jiang, X. Lu, W. Zhang, Q. Jing, et al., Matrine induced gastric cancer MKN45 cells apoptosis via increasing pro-apoptotic molecules of Bcl-2 family, Toxicology 229 (2007) 245–252.
[25] Z. Bo, L. Zhong-Yang, L. Yan-Yan, L. Ying, L. Man-Ling, D. Hai-Ying, et al., Antiinflammatory effects of matrine in LPS-induced acute lung injury in mice, Eur. J. Pharm. Sci. 44 (2011) 573–579.
[26] P. Rainard, A. Fromageau, P. Cunha, F.B. Gilbert, Staphylococcus aureus lipoteichoic acid triggers inflammation in the lactating bovine mammary gland, Vet. Res. 39 (2008) 52.
[27] L. Ferrero-Miliani, O. Nielsen, P. Andersen, S. Girardin, Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1beta generation, Clin. Exp. Immunol. 147 (2007) 227–235.
[28] K.R. Martin, D. Ohayon, V. Witkosarsat, Promoting apoptosis of neutrophils and phagocytosis by macrophages: novel strategies in the resolution of inflammation, Swiss Med. Wkly. 145 (2015) w14056.
[29] H. Wu, A. Dai, X. Chen, X. Yang, X. Li, C. Huang, et al., Leonurine ameliorates the inflammatory responses in lipopolysaccharide-induced endometritis, Int. Immunopharmacol. 61 (2018) 156.
[30] T. Loyi, H. Kumar, S. Nandi, M.K. Patra, Expression of pathogen recognition receptors and pro-inflammatory cytokine transcripts in clinical and sub-clinical endometritis cows, Anim. Biotechnol. 26 (2015) 194–200.
[31] K. Jiang, X. Ma, S. Guo, T. Zhang, G. Zhao, H. Wu, et al., Anti-inflammatory Effects of Rosmarinic Acid in Lipopolysaccharide-induced Mastitis in Mice, 41 (2018) 437–448.
[32] A.M. Miller, A.R. Mcphaden, A. Preston, R.M. Wadsworth, C.L. Wainwright, TNFalpha increases the inflammatory response to vascular balloon injury without accelerating neointimal formation, Atherosclerosis 179 (2005) 51–59.
[33] Y.S. Choi, Y.C. Kim, S. Ji, Y. Choi, Increased bacterial invasion and differential expression of tight-junction proteins, growth factors, and growth factor receptors in periodontal lesions, J. Periodontol. 85 (2014) 313–322.
[34] G. Canny, O. Levy, G.T. Furuta, S. Narravula-Alipati, R.B. Sisson, C.N. Serhan, et al., Lipid mediator-induced expression of bactericidal/permeability-increasing protein (BPI) in human mucosal epithelia, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 3902–3907.
[35] C.C. Cao, X.Q. Ding, Z.L. Ou, C.F. Liu, P. Li, L. Wang, et al., In vivo transfection of NF-κB decoy oligodeoxynucleotides attenuate renal ischemia/reperfusion injury in rats, Kidney Int. 65 (2004) 834–845.
[36] Q. Li, I.M. Verma, NF-κB regulation in the immune system, Nat. Rev. Immunol. 2 (2002) 725–734.
[37] A. Ki Bum, J. Jun Ho, B. Jung Eun, P. Ok-Jin, K. Seok-Seong, Y. Cheol-Heui, et al., Muramyl dipeptide potentiates staphylococcal lipoteichoic acid induction of cyclooxygenase-2 expression in macrophages, Microbes Infect. 16 (2014) 153–160.
[38] E. Espen, M. Siegfried, F. Trude, S. Andra, H. Thomas, T. Christoph, et al., Induction of cytokine production in human T cells and monocytes by highly purified lipoteichoic acid: involvement of Toll-like receptors and CD14, Med. Sci. Monit. 8 (2002) BR149.
[39] I.T. Lee, S.W. Wang, C.W. Lee, C.C. Chang, C.C. Lin, S.F. Luo, et al., Lipoteichoic acid induces HO-1 expression via the TLR2/MyD88/c-Src/NADPH oxidase pathway and Nrf2 in human tracheal smooth muscle cells, J. Immunol. 181 (2008) 5098–5110.
[40] C.W. Lee, C.S. Chien, C.M. Yang, Lipoteichoic acid-stimulated p42/p44 MAPK activation via toll-like receptor 2 in tracheal smooth muscle cells, Am. J. Physiol. Lung Cell. Mol. Physiol. 286 (2004) 921–930.