Inflammation, Vol. 42, No. 1, February 2019 (© 2018)
DOI: 10.1007/s10753-018-0874-8

ORIGINAL ARTICLE

Oridonin Ameliorates Lipopolysaccharide-Induced Endometritis in Mice *via* Inhibition of the TLR-4/NF-κBpathway

Min Zhou,[1] Yinyi Yi,[1] and Li Hong[1,2]

Abstract— Endometritis is a health threat to both humans and animals and poses a huge economic burden. Oridonin (Ori) is a natural diterpenoid isolated from the traditional Chinese herb *Rabdosiarubescens* (*R. rubescens*) and has multiple health-promoting effects, including antioxidant, anti-inflammatory, and antitumor effects. There is little evidence showing that Ori can effectively treat endometritis, and the relevant mechanisms need to be further clarified. In this study, we investigated the effects of Ori on LPS-induced endometritis *in vivo*. Additionally, we examined the effects of Ori on LPS-stimulated mouse endometrial epithelial cells (mEECs). The results showed that Ori treatment significantly alleviated LPS-induced endometritis and reduced the activity of myeloperoxidase. ELISA and qPCR results indicated that Ori dose-dependently decreased the expression of TNF-α, IL-1β, and IL-6 both in tissues and in mEECs. In addition, Ori was found to inhibit LPS-induced TLR4/NF-κB signaling pathway activation. These results suggest that Ori effectively attenuates LPS-induced endometritis by inhibiting the TLR4/NF-κB signaling pathway and that Ori might be an effective drug for the prevention and treatment of LPS-induced endometritis.

KEY WORDS: oridonin; endometritis; NF-κB; inflammatory cytokines.

INTRODUCTION

Endometritis is characterized by persistent inflammation of the endometrial lining and causes many health problems, such as spontaneous preterm labor, premature birth, and recurrent miscarriages [1–3]. *Escherichia coli* (*E. coli*) is the major cause of endometritis in the uterine cavity, as it produces the endotoxin lipopolysaccharide (LPS) [4, 5]. LPS is a major constituent of the outer membrane of Gram-negative bacteria and has been recognized as an immunostimulatory molecule; it has been used to establish many inflammatory disease animal models, such as those of endometritis, acute lung injury (ALI), and mastitis [6–8].

Toll-like receptors (TLRs) are a family of pattern recognition receptors (PRRs) involved in the regulation of both innate and adaptive immunity *via* the recognition of microbe-associated molecular patterns (MAMPs), including LPS [9]. TLR4 is a member of the TLR family and is well known to recognize exogenous ligands such as LPS [10]. When LPS stimulates the expression of TLR4, it induces the activation of intracellular signaling cascades, including nuclear factor (NF)-κB signaling [11]. NF-κB is important for inflammatory responses, and once activated, it can regulate the expression of inflammatory genes and the release of cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6),

[1] Department of Gynecology and Obstetrics, Renmin Hospital of Wuhan University, No.238 Jiefang Road, Wuchang District, Wuhan City, Hubei Province, People's Republic of China
[2] To whom correspondence should be addressed at Department of Gynecology and Obstetrics, Renmin Hospital of Wuhan University, No.238 Jiefang Road, Wuchang District, Wuhan City, Hubei Province, People's Republic of China. E-mail: drhongli7777@gmail.com

to aggravate inflammatory damage [12, 13]. Therefore, treatments aimed at inhibiting the activation of NF-κB may have potential therapeutic advantages for inflammatory diseases.

Oridonin (Ori, $C_{20}H_{28}O_6$) is a natural diterpenoid isolated from the traditional Chinese herb *Rabdosiarubescens* (*R.rubescens*) and has been widely used as a traditional Chinese medicine for centuries. Numerous studies have demonstrated that Ori has multiple health-promoting effects, including antioxidant, anti-inflammatory, and antitumor effects [14–16]. However, it is unknown whether Ori plays a role in endometritis, and the potential protective mechanism of Ori is not well characterized. In this study, we investigated the effects of Ori on LPS-induced endometritis *in vivo* and examined the effects of Ori on LPS-stimulated mouse endometrial epithelial cells (mEECs). Our examination of the antagonistic function of Ori *in vitro* and *in vivo* provided experimental evidence that Ori may be a potential therapeutic agent for the treatment of patients with endometritis or other inflammatory diseases.

MATERIALS AND METHODS

Reagents

Oridonin (purity > 98% (HPLC), cell culture grade, endotoxin free) was purchased from the National Institutes for Food and Drug Control (Beijing, China), solubilized in 100% dimethyl sulfoxide (DMSO, Sigma Chemical Co. St. Louis, MO, USA), and then diluted for a final concentration of 0.1% (*v*/*v*) DMSO. LPS (*E. coli* 055: B5) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). The antibodies used in this study were purchased from Cell Signaling Technologies (Beverly, MA,USA). qPCR was carried out using the SYBR Green Plus Reagent Kit (Roche Applied Science, Mannheim, Germany). All other chemicals and reagents used were of the highest commercial grade available.

Animal Treatments

Six- to eight-week-old BALB/c mice were obtained from the Animal Experiment Center of Wuhan University (Wuhan, China). The mice were housed at a constant temperature (23 °C) and relative humidity (60%) with a fixed 12 h light:12 h dark cycle and free access to food and water. All of the experimental procedures involving animals and their care conformed to the Guide for the Care and Use of Laboratory Animals of the National Veterinary Research Council. This study was approved by the ethics committee of the Institutional Animal Care and Use Committee of Renmin Hospital of Wuhan University. The collection work was performed under sodium pentobarbital anesthesia to minimize suffering.

The mice were randomly divided into five groups ($n = 6$): DMSO control group, Ori control group, LPS group, and LPS + Ori groups (10 and 40 mg/kg). LPS-induced endometritis was achieved as follows [8]: briefly, each side of the mouse uterus was perfused with 50 μL of LPS (1 mg/mL) under anesthesia. After 24 h, the LPS + Ori groups were intraperitoneally injected with 10and 40 mg/kg Ori three times every 8 h (for a total of three times, Fig. 1). The DMSO control group, Ori control group, and LPS group were intraperitoneally administered equal volumes of saline (containing 0.1% DMSO), Ori (40 mg/kg), and saline, respectively. The mice were euthanized with CO_2, and the uterine tissues were collected and stored at − 80 °C.

Cell Culture and Treatment

The mEECs were prepared as previously described [17]. Briefly, uterine tissues were minced into a paste and then digested with trypsin. The homogenized tissues were vortexed and then centrifuged and filtered through a 20-μm nylon mesh. The cells were cultured in Dulbecco's modified Eagle's medium/F-12 (DMEM/F-12, HyClone, USA) containing 10% (*v*/*v*) FBS (fetal bovine serum, Sigma-Aldrich, AUS), 10 ng/mL EGF (epidermal growth factor, PEPRO TECH, USA), 2 mM L-glutamine, 50 U/mL penicillin, and 50 μg/mL streptomycin and were incubated in a 37 °C humidified incubator containing5% CO2. The cells were treated with LPS (1 μg/mL) alone or in combination with Ori pretreatment (5, 20 μg/mL) or other treatments. After these treatments, the cells were prepared for further studies.

Cell Viability Assay

The mEECs were seeded in 96-well plates at a density of 4×10^5 cells/mL and then cultured with different concentrations of Ori (5, 10, 20, and 40, 100 μg/mL) for 24 h. Cell viability was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Optical density was measured at 570 nm on a Victor31420 Multilabel Counter (Perkin Elmer, USA).

RNA Extraction and qPCR

Total RNA was isolated from the uterine tissues and cells using TRIzol according to the manufacturer's

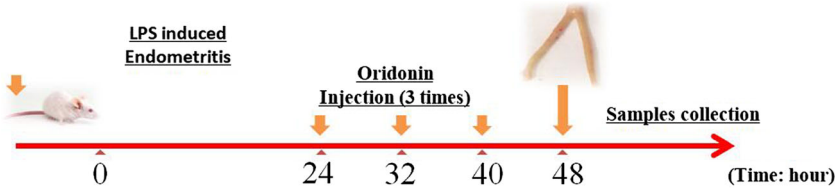

Fig. 1. Time line of experimental animal treatment.

instructions (Invitrogen, USA). Then, complementary DNA (cDNA) was synthesized using a reverse transcription kit (Takara, Japan). Total cDNA was used as the starting material for real-time PCR with FastStart Universal SYBR Green Master Mix (Roche Applied Science, Germany). The specific primers for TNF-α, IL-1β, IL-6, and GAPDH were designed based on known sequences (Table 1). The housekeeping gene GAPDH served as an internal standard. Relative quantification of the target gene expression levels was performed using the $2^{-\Delta\Delta Ct}$ method.

Cytokine Assays

The mEECs were seeded onto a six-well plate and treated as previously indicated. The uterine tissues were homogenized in prechilled PBS and centrifuged, and the supernatants were collected. Cytokine levels (IL-1β, TNF-α, and IL-6) in the tissues and supernatants were measured using ELISA kits according to the manufacturer's instructions (BioLegend, Thermo, USA). The absorbance value was read at 450 nm using a Victor31420 Multilabel Counter (Perkin Elmer, USA).

Histopathological Analysis

The uterine tissues were harvested and fixed in 10% formalin for subsequent histopathological experiments. Next, the tissues were embedded in paraffin, sectioned, and dehydrated. The 5-μm-thick sections were used for hematoxylin and eosin (H&E) staining. Finally, the sections were observed using an optical microscope (Olympus, Japan).

MPO Activity Assay

Uterine tissues (weighing approximately 100 mg) were homogenized with reaction buffer (*w*/*v* 1/19), and myeloperoxidase (MPO) activity was detected by the MPO Assay Kit (Jiancheng Biotechnology, China) according to the manufacturer's instructions.

Western Blot Analysis

Total protein was extracted from uterine tissues and cells, and the protein concentration was determined using a BCA kit (Vazyme, China). Samples with equal amounts of protein (50 μg) were separated on 10% SDS polyacrylamide gels, transferred to polyvinylidene difluoride membranes, and blocked in 5% skim milk for 1 h at room temperature (R.T.). Then, they were incubated with primary antibodies (1:1000 dilution) at 4 °C. Next, the membranes were treated with secondary antibody for 1 h at R.T., and the protein expression levels were determined using the Odyssey infrared imaging system (LI-COR, USA). β-actin served as an internal standard.

Table 1. Oligonucleotide Primers Used for qPCR

Name	Accession number	Primer sequence (5′-3′)	Product size (bp)
TLR-4	NM_021297.2	Forward: TTCAGAGCCGTTGGTGTATC Reverse: CTCCCATTCCAGGTAGGTGT	170
TNF-α	NM_013693.3	Forward: CTTCTCATTCCTGCTTGTG Reverse: ACTTGGTGGTTTGCTACG	198
IL-1β	NM_008361.4	Forward: CCTGGGCTGTCCTGATGAGAG Reverse: TCCACGGGAAAGACACAGGTA	131
IL-6	NM_031168.1	Forward: GGCGGATCGGATGTTGTGAT Reverse: GGACCCCAGACAATCGGTTG	199
GAPDH	NM_001289726.1	Forward: CAATGTGTCCGTCGTGGATCT Reverse: GTCCTCAGTGTAGCCCAAGATG	124

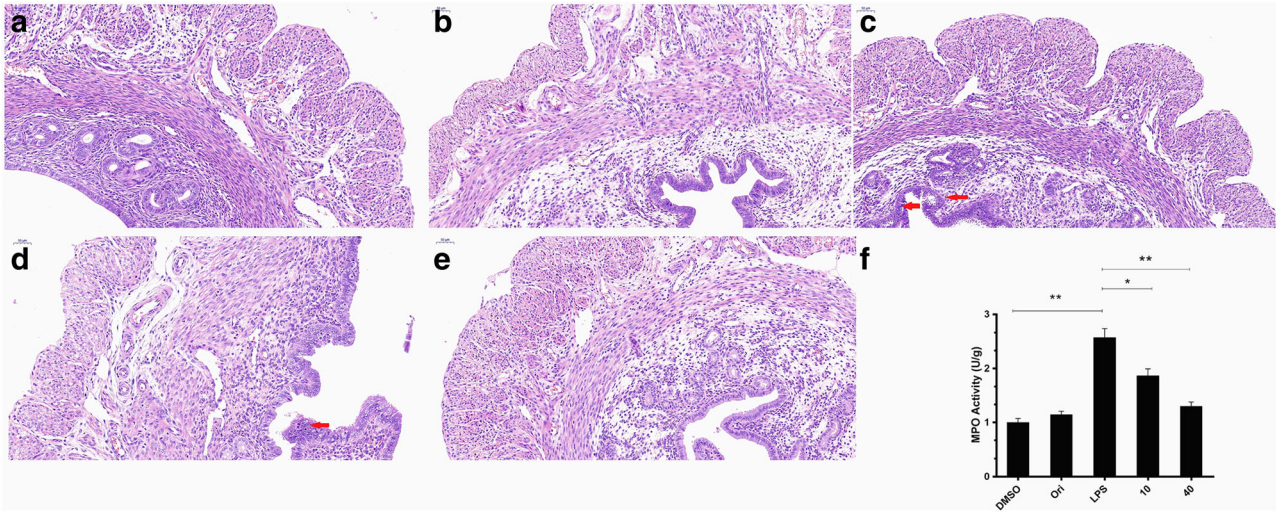

Fig. 2. Histopathological sections of uterine tissues. **a** DMSO control group. **b** Oridonin (40 mg/kg) control group. **c** LPS group. **d**, **e** LPS + oridonin (10 and 40 mg/kg, respectively) treatment groups. **f** MPO activity. The red arrow indicates the tissue lesion area. Data represent the mean ± S.E.M. of three independent experiments. #$p < 0.05$ and ##$p < 0.01$ *vs.* the DMSO control group. *$p < 0.05$ *vs.* the LPS group. **$p < 0.01$ *vs.* the LPS group.

Statistical Analysis

The results were analyzed using GraphPad Prism 5 (GraphPad InStat Software, USA). Comparisons among groups were performed with one-way ANOVA. Data are expressed as the mean ± standard error of the mean (S.E.M.). P values < 0.05 were considered statistically significant.

RESULTS

Oridonin Alleviates LPS-Induced Endometritis

H&E staining and MPO analysis were performed to evaluate the damage to the uterine tissue. Histological analysis displayed normal morphology and no histopathological changes in the DMSO control group (Fig. 2a) and Ori control group (Fig. 2b). Administration of LPS resulted in severe inflammation characterized by inflammatory cell infiltration, increased uterine cavity effusion, and uterine epithelial cell detachment and necrosis (Fig. 2c). However, treatment with Ori (10 and 40 mg/kg) attenuated the pathological conditions (Fig. 2d, e). An additional experiment was carried out to estimate the effectiveness of Ori on LPS-induced endometritis. The results showed that MPO activity was significantly increased in the LPS group ($p < 0.05$). However, MPO activity was markedly decreased in the Ori groups ($p < 0.05$, Fig. 2f).

Oridonin Has Little Effect on mEEC Viability

The potential cytotoxicity of Ori on mEECs was determined using the MTT assay. These results showed that cell viability was not affected by Ori administration (Fig. 3).

Oridonin Decreases the Expression of Proinflammatory Cytokines

TNF-α, IL-6, and IL-1β are major proinflammatory cytokines that play an important role in inflammatory

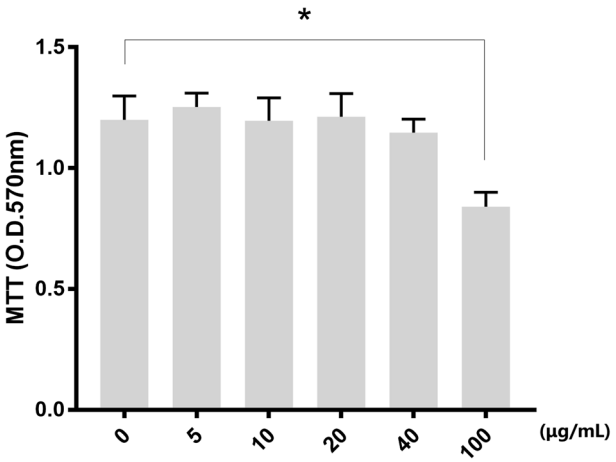

Fig. 3. Effects of oridonin on mEEC viability. The values presented are the mean ± S.E.M. ($n = 5$). *$p < 0.05$.

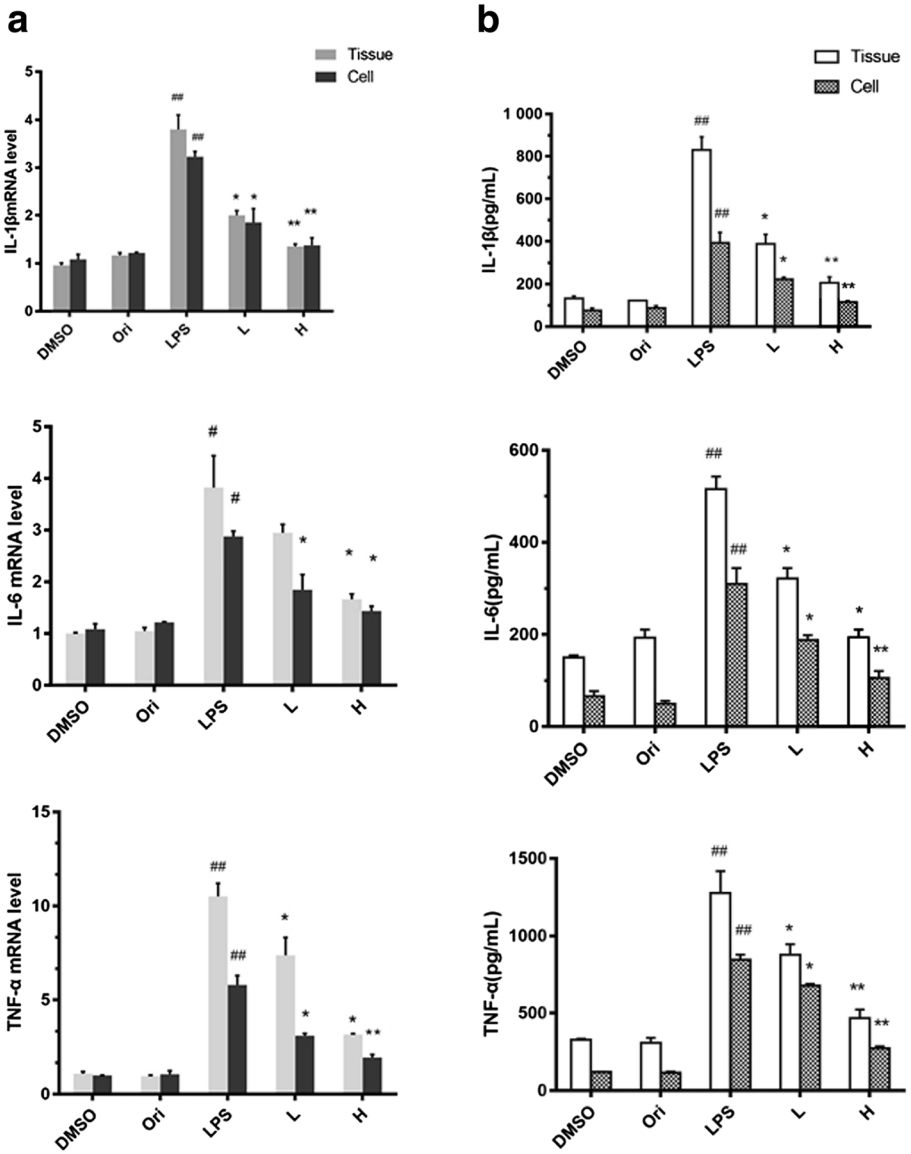

Fig. 4. Effects of oridonin on cytokine expression. **a** mRNA levels of IL-1β, IL-6 and TNF-α in LPS-stimulated uterine tissues and mEECs. *GAPDH* was used as a control. **b** The expression levels of the cytokines IL-1β, IL-6, and TNF-α were detected by ELISA. DMSO indicates the DMSO control group, Ori indicates the oridonin (20 μg/mL or 40 mg/kg) control group, and L and H indicate the oridonin groups treated with 10 and 40 mg/kg Ori per animal, respectively, and 5and 20 μg/mL Ori for cells, respectively. Data represent the mean ± S.E.M. of three independent experiments. #$p < 0.05$ and ##$p < 0.01$ *vs.* the DMSO control group. *$p < 0.05$ *vs.* the LPS group. **$p < 0.01$ *vs.* the LPS group.

diseases. Therefore, we detected the effects of Ori on the expression of IL-1β, IL-6, and TNF-αby qPCR (Fig. 4a) and ELISA (Fig. 4b). The results showed that LPS challenge caused a significant increase in TNF-α, IL-1β, and IL-6 expression in both tissues and cells. Compared with those in the LPS group, Ori significantly reduced the levels of TNF-α, IL-1β, and IL-6 in a dose-dependent manner. These results suggested that Ori decreased the production of proinflammatory cytokines.

Oridonin Inhibits the Expression of TLR4

TLR4 is the first identified mammalian TLR and well documented to recognize exogenous ligands such as LPS

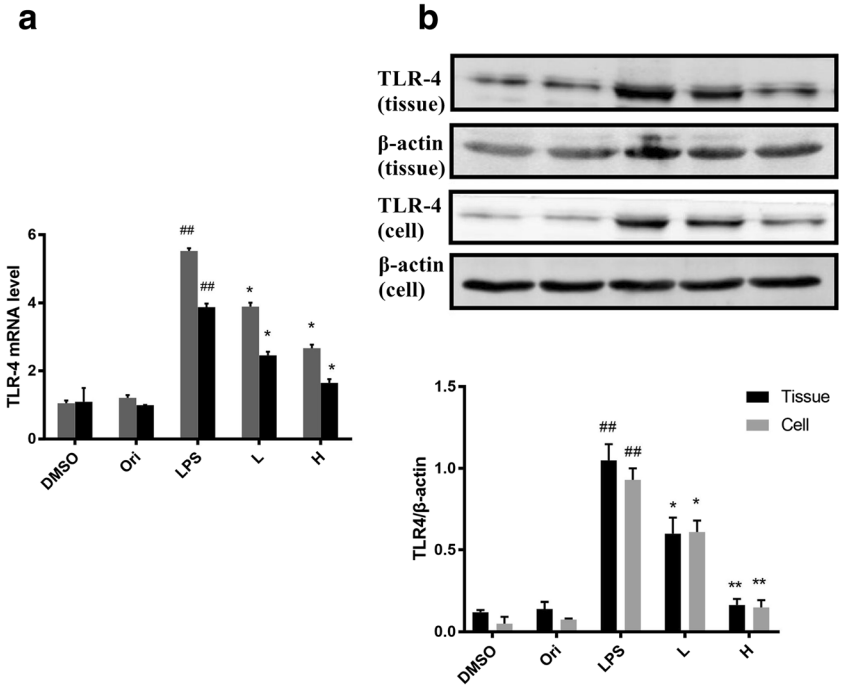

Fig. 5. Effects of oridonin on the expression of TLR4. **a** The expression level of TLR4 was detected by qPCR (**a**) and western blot (**b**) in uterine tissues and mEECs. *GAPDH* and β-actin were used as the control for mRNA and protein levels, respectively. DMSO indicates the DMSO control group, Ori indicates the oridonin (20 μg/mL or 40 mg/kg) control group, and L and H indicate the oridonin groups treated with 10 and 40 mg/kg Ori per animal, respectively, and 5 and 20 μg/mL Ori for cells, respectively. Data represent the mean ± S.E.M. of three independent experiments. #$p < 0.05$ and ##$p < 0.01$ *vs.* the DMSO control group. *$p < 0.05$ *vs.* the LPS group. **$p < 0.01$ *vs.* the LPS group.

[18]. Western blot and qPCR assays were performed to detect whether Ori reduced LPS-induced inflammation by inhibiting the expression level of TLR4. As shown in Fig. 5, LPS administration significantly increased TLR4 expression. However, the levels of TLR4 mRNA and protein were decreased by Ori treatment in the uterine tissues and LPS-induced mEECs ($p < 0.05$).

Oridonin Suppresses the Activation of TLR4 Signaling and the Downstream NF-κB Pathway

NF-κB is a key regulator of inflammatory processes with the ability to modulate the expression of many inflammatory mediators. Our results showed that Ori inhibited the LPS-induced activation of TLR4. However, activation of TLR4 directly affects the NF-κB pathway. Western blot assays were used to explore whether the anti-inflammatory mechanism of Ori in LPS-induced endometritis acts *via* the NF-κB pathway. The results showed that LPS induced the phosphorylation of p65 and IκBα protein; however, these values were decreased by Ori treatment both in tissues (Fig. 6a) and in cells (Fig. 6b).

DISCUSSION

Endometritis causes changes in the endometrium, which impedes fertilization, leads to implantation failure, may lead to early abortions, and is a huge economic burden [19, 20]. In recent years, the role of natural products in the treatment of clinical diseases has become increasingly prominent. A number of botanical and natural drugs, such as anti-infective berberine and antitumor colchicine, have been mass-produced and widely used in China. In the treatment of infectious diseases, natural products have unique advantages; for example, natural products do not lead to the development of drug-resistant bacteria, and indeed, they have been the source of inspiration for most FDA-approved drugs for many years [21]. The plant *R. rubescens*is used in traditional Chinese medicine to treat tumors and inflammatory diseases. Ori is an active

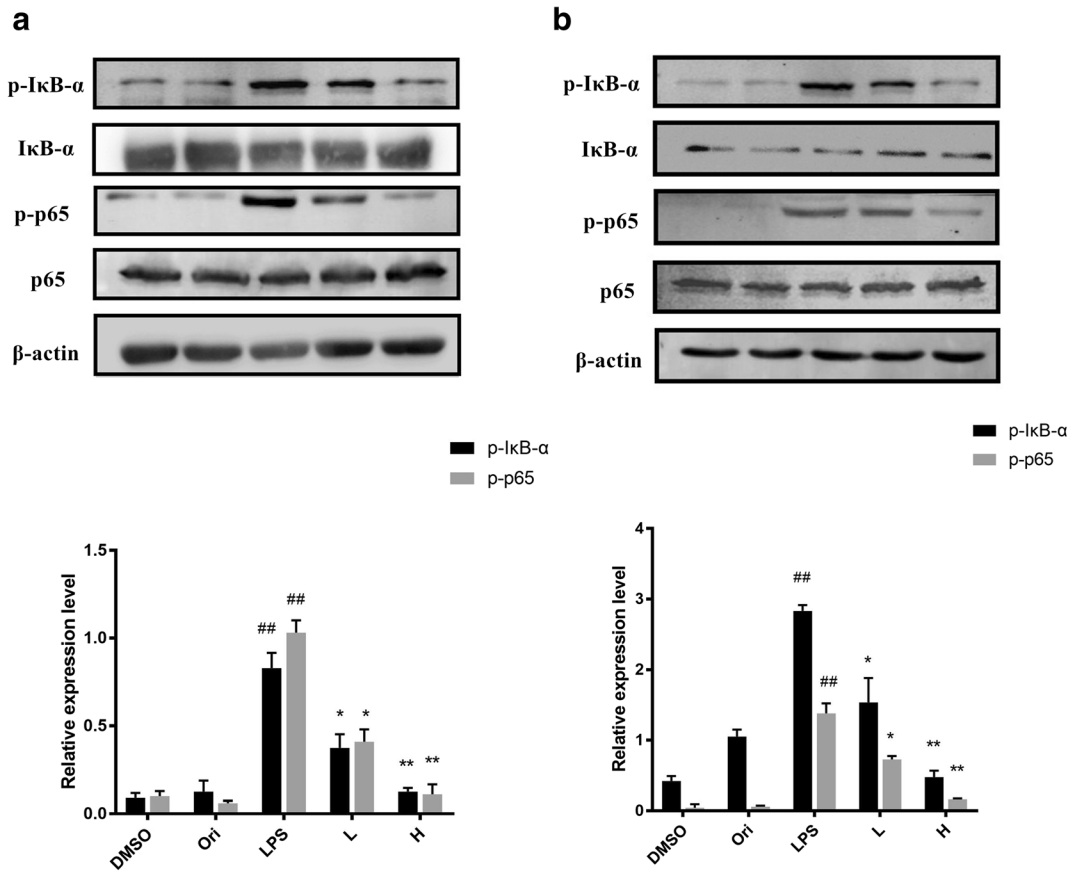

Fig. 6. Effects of oridonin on NF-κB pathway activation. **a** p65 and IκBα protein levels in uterine tissues. **b** p65 and IκBα protein levels in mEECs after LPS stimulation for 3 h. DMSO indicates the DMSO control group, Ori indicates the oridonin (20 μg/mL or 40 mg/kg) control group, and L and H indicate the oridonin groups treated with 10 and 40 mg/kg Ori per animal, respectively, and 5 and 20 μg/mL Ori for cells, respectively. Data represent the mean ± S.E.M. of three independent experiments. #$p < 0.05$ and ##$p < 0.01$ *vs.* the DMSO control group. *$p < 0.05$ *vs.* the LPS group. **$p < 0.01$ *vs.* the LPS group.

diterpenoid isolated from *R. rubescens* that possesses various pharmacological and physiological properties, including anti-inflammatory, antibacterial, and antitumor properties. Previous studies have demonstrated that Ori decreases the production of proinflammatory cytokines and thus attenuates LPS-induced ALI and liver injury [16, 22]. Our study obtained similar results; namely, Ori improved LPS-induced endometritis in a mouse model, confirming that Ori inhibits LPS-induced activation of NF-κB in primary mEECs, thereby attenuating the release of proinflammatory cytokines. We infer from these results that treatment with Ori may be beneficial for patients with endometritis or other uterine disorders.

In the present study, we observed the effects of Ori on an LPS-induced mouse endometritis model, which has been frequently used to explore the anti-inflammatory mechanisms of drugs [23]. Our results showed that administration of Ori significantly attenuated the pathological changes in the uterus. Ori treatment decreased inflammatory conditions, including inflammatory cell infiltration, uterine cavity effusion, uterine epithelial cell detachment, and necrosis (Fig. 2). MPO, a polymorph nuclear (PMN) marker enzyme, is used as a direct measurement of the content of active neutrophil granulocytes in tissues [24]. Our results showed that LPS-induced MPO activity was significantly decreased by Ori treatment; thus, Ori alleviated the LPS-induced inflammatory response *in vivo*, suggesting that Ori has a protective effect on LPS-stimulated endometritis.

LPS acts as a primary infectious stimulus that leads to severe inflammatory diseases, and treatment of endometrial epithelial cells (EECs) with LPS is commonly performed to generate an *in vitro* endometritis model [25]. Sheldon et al. showed that exposure to LPS caused a rapid

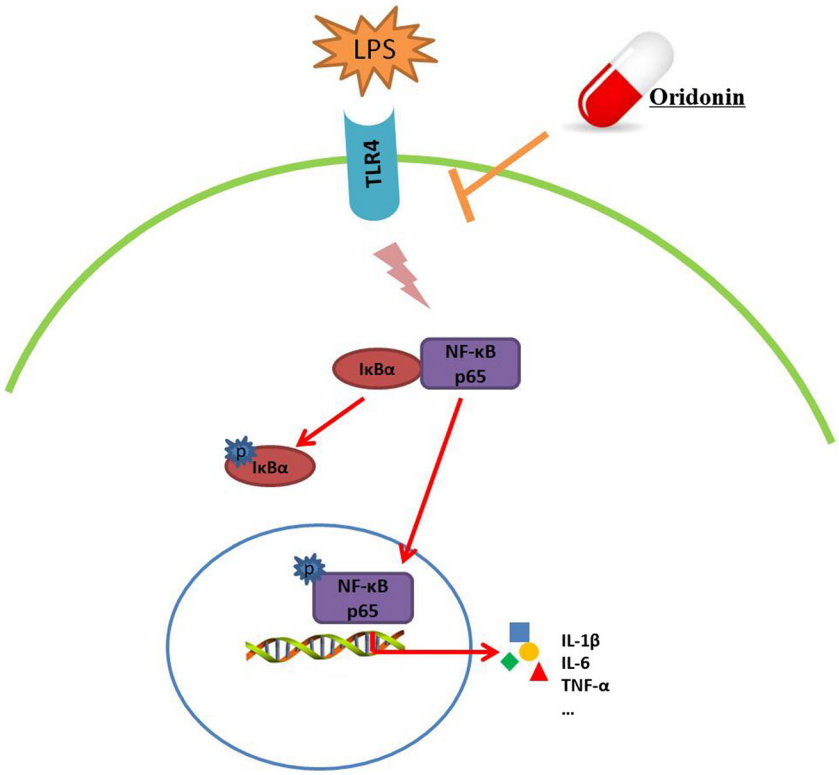

Fig. 7. Schematic diagram of signaling pathways related to the anti-inflammatory effects of oridonin on LPS-induced inflammation.

inflammatory response using primary bovine EECs [26]. In this study, LPS-treated primary mouse EECs were used to explore the anti-inflammatory mechanisms of Ori. In addition, due to the potential cytotoxicity of drug candidates, we evaluated the toxic effects of Ori on mEECs by MTT assay. Our results showed that treatment with Ori does not affect the normal growth activity of cells until the concentration of Ori reaches 100 μg/mL, over which cell viability drops significantly. This observation suggests that the concentration of the drug we selected for treatment does not cause significant cytotoxicity. After activation of the innate immune system by LPS, strong proinflammatory signals are produced; these signals are important both for maintaining a stable balance in the immune system and for protecting the host from harmful effects [27, 28]. Excessive proinflammatory cytokine release increases the level of the immune response, leading to an inflammatory cascade and tissue damage [29]. Therefore, inhibition of the release of inflammatory cytokines may serve as a target for anti-inflammatory drug development. IL-6 is rapidly produced in response to infection and tissue damage, promoting host defense by stimulating acute responses, hematopoiesis, and immune responses [30]. One of the best-characterized pro-tumorigenic cytokines, IL-6, is involved in tumor angiogenesis during inflammatory tumor progression [31]. IL-1β is a proinflammatory cytokine with a wide range of systemic and local effects and regulates the function of immune cells and non-immune cells. Stimulation with IL-1β promotes the activation and effector function of dendritic cells, macrophages, and neutrophils [32]. TNF-α is an acute-phase protein that initiates the cytokine cascade and increases vascular permeability, promoting the recruitment of macrophages and neutrophils to the site of infection and participating in inflammatory disorders [33]. These proinflammatory cytokines have been shown to play an important role in inflammatory diseases, including cancer-related inflammation [34], and control of their expression is beneficial to the local inflammatory microenvironment and recovery from the disease. We have demonstrated both *in vivo* and *in vitro* that Ori could significantly inhibit the production of IL-1β, TNF-α, and IL-6. In addition, Ori did not show cytotoxicity to the mEECs at a concentration of 20 μg/mL. These results show that Ori has important application potential in the development of novel

anti-inflammatory therapies against LPS-stimulated endometritis.

TLR4 is a member of the TLR family and acts as a receptor for LPS, which induces the immune response through activation of TLR4, culminating in NF-κB transcriptional activity [35]. The expression of proinflammatory mediators can be aggravated by the NF-κB pathway [36]. Thus, we hypothesized that the anti-inflammatory effect of Ori is achieved by suppressing TLR4-mediated NF-κB pathway activation. Our results showed that TLR4 expression was increased during LPS challenge but was decreased by Ori treatment. IκBα is a known inhibitor of NF-κB; however, upon NF-κB pathway activation, IκBα is phosphorylated and then degraded [37, 38]. The NF-κB subunit p65 then translocates from the cytoplasm to the nucleus, triggering the transcription of target genes, including TNF-α, IL-1β, and IL-6, and increasing the production of these proinflammatory cytokines, thereby aggravating inflammation [39]. Our study results demonstrated that Ori inhibited the phosphorylation of IκBα and NF-κB p65, which were increased in an LPS-induced mouse model and mEECs. These results indicated that Ori exerted its potential protective effects on LPS-stimulated endometritis by reducing the production of proinflammatory cytokines through theTLR4/NF-κB signaling pathway (Fig. 7).

Collectively, our data suggest that Ori exerted anti-inflammatory properties in LPS-induced endometritis and that the underlying mechanisms might act by suppressing the production of proinflammatory cytokines through the expression of TLR4 and the downstream NF-κB signaling pathway. Accordingly, Ori might be an effective drug in preventing and treating LPS-induced endometritis.

ACKNOWLEDGMENTS

The authors would like to thank all the teachers in the Department of Gynecology and Obstetrics and Central Laboratory at Renmin Hospital of Wuhan University for their technical assistance.

Funding Information

This study was supported by a grant from the National Natural Science Foundation of China (nos. 81471442).

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest. The authors declare that they have no conflicts of interest.

REFERENCES

1. Cicinelli, Ettore, Maria Matteo, Raffaele Tinelli, Vincenzo Pinto, Marco Marinaccio, Ugo Indraccolo, Dominique De Ziegler, and Leonardo Resta. 2013. Chronic Endometritis Due to Common Bacteria Is Prevalent in Women With Recurrent Miscarriage as Confirmed by Improved Pregnancy Outcome After Antibiotic Treatment. *Reproductive Sciences* 21 (5): 640–647.
2. Romero, R., and M. Mazor. 1988. Infection and preterm labor. *Clinical Obstetrics and Gynecology* 31 (3): 553–584.
3. Espinoza, J., O. Erez, and R. Romero. 2006. Preconceptional antibiotic treatment to prevent preterm birth in women with a previous preterm delivery. *American Journal of Obstetrics & Gynecology* 194 (3): 630–637.
4. Pino, A., G. Giunta, C.L. Randazzo, S. Caruso, C. Caggia, and A. Cianci. 2017. Bacterial biota of women with bacterial vaginosis treated with lactoferrin: an open prospective randomized trial. *Microbial Ecology in Health & Disease* 28 (1): 1357417.
5. Swangchanuthai, Theerawat, Chloe R.M. Lavender, Zhangrui Cheng, Ali A. Fouladinashta, and D. Claire Wathes. 2012. Time Course of Defense Mechanisms in Bovine Endometrium in Response to Lipopolysaccharide1. *Biology of Reproduction* 87 (6): 135.
6. Wu, H., G. Zhao, K. Jiang, X. Chen, Z. Zhu, C. Qiu, C. Li, and G. Deng. 2016. Plantamajoside ameliorates lipopolysaccharide-induced acute lung injury via suppressing NF-kappaB and MAPK activation. *International Immunopharmacology* 35: 315–322. https://doi.org/10.1016/j.intimp.2016.04.013.
7. Jiang, K., X. Ma, S. Guo, T. Zhang, G. Zhao, H. Wu, X. Wang, and G. Deng. 2017. Anti-inflammatory Effects of Rosmarinic Acid in Lipopolysaccharide-Induced Mastitis in Mice. Inflammation:1–12.
8. Wang, J., W. Wang, and Y. Pang. 2018. Saikosaponin A Inhibits LPS-Induced Endometritis in Mice Through Activating Nrf2 Signaling Pathway. *Inflammation* 41: 1508–1514. https://doi.org/10.1007/s10753-018-0796-5.
9. Barton, G.M., and R. Medzhitov. 2003. Toll-like receptor signaling pathways. *Science* 300 (5625): 1524–1525.
10. Vartanian, Keri B., Susan L. Stevens, Brenda J. Marsh, Rebecca Williamskarnesky, Nikola S. Lessov, and Mary P. Stenzelpoore. 2011. LPS preconditioning redirects TLR signaling following stroke: TRIF-IRF3 plays a seminal role in mediating tolerance to ischemic injury. *Journal of Neuroinflammation* 8 (1): 140.
11. Wu, Haichong, Zhao Gan, Kangfeng Jiang, Chengye Li, Changwei-Qiu, and Ganzhen Deng. 2016. Engeletin Alleviates Lipopolysaccharide-Induced Endometritis in Mice by Inhibiting TLR4-mediated NF-κB Activation. *Journal of Agricultural and Food Chemistry* 64 (31): 6171–6178.
12. Kagan, Jonathan C., and Ruslan Medzhitov. 2006. Phosphoinositide-Mediated Adaptor Recruitment Controls Toll-like Receptor Signaling. *Cell* 125 (5): 943–955.
13. Jung, H.W., R. Mahesh, J.H. Park, Y.C. Boo, K.M. Park, and Y.K. Park. 2010. Bisabolangelone isolated from Ostericumkoreanum inhibits the production of inflammatory mediators by down-regulation of NF-kappaB and ERK MAP kinase activity in LPS-stimulated RAW264.7 cells. *International Immunopharmacology* 10 (2): 155–162.
14. Zheng, X.C., Q.J. Wu, Z.H. Song, H. Zhang, J.F. Zhang, L.L. Zhang, T.Y. Zhang, C. Wang, and T. Wang. 2016. Effects of Oridonin on growth performance and oxidative stress in broilers challenged with lipopolysaccharide. *Poultry Science* 95 (10): 2281–2289.

15. Shi, Min, Xiao Jie Lu, Juan Zhang, Hua Diao, Guangming Li, Ling Xu, Ting Wang, Jue Wei, Wenying Meng, and Jia Li Ma. 2016. Oridonin, a novel lysine acetyltransferases inhibitor, inhibits proliferation and induces apoptosis in gastric cancer cells through p53- and caspase-3-mediated mechanisms. *Oncotarget* 7 (16): 22623–22631.
16. Zhao, G., T. Zhang, X. Ma, K. Jiang, H. Wu, C. Qiu, M. Guo, and G. Deng. 2017. Oridonin attenuates the release of pro-inflammatory cytokines in lipopolysaccharide-induced RAW264.7 cells and acute lung injury. *Oncotarget* 8 (40).
17. Jiang, K., X. Chen, G. Zhao, H. Wu, J. Mi, C. Qiu, X. Peng, and G. Deng. 2016. IFN-τ Plays an Anti-Inflammatory Role in Staphylococcus aureus-Induced Endometritis in Mice Through the Suppression of NF-κB Pathway and MMP9 Expression. *Journal of Interferon & Cytokine Research the Official Journal of the International Society for Interferon & Cytokine Research* 37 (2).
18. Jiang, K., S. Guo, T. Zhang, Y. Yang, G. Zhao, A. Shaukat, H. Wu, and G. Deng. 2018. Downregulation of TLR4 by miR-181a Provides Negative Feedback Regulation to Lipopolysaccharide-Induced Inflammation. *Frontiers in Pharmacology* 9.
19. Brodzki, P., K. Kostro, A. Brodzki, and U. Lisiecka. 2014. Determination of selected parameters for non-specific and specific immunity in cows with subclinical endometritis. *Animal Reproduction Science* 148 (3–4): 109–114.
20. Di Pietro, C., S. Caruso, R. Battaglia, M. IraciSareri, A. La Ferlita, F. Strino, G. Bonaventura, et al. 2018. MiR-27a-3p and miR-124-3p, upregulated in endometrium and serum from women affected by chronic Endometritis, are new potential molecular markers of endometrial receptivity. *American Journal of Reproductive Immunology*: e12858. https://doi.org/10.1111/aji.12858.
21. Mishra, Bhuwan B., and Vinod K. Tiwari. 2011. Natural products: An evolving role in future drug discovery. *European Journal of Medicinal Chemistry* 46 (10): 4769–4807.
22. Deng, Y., C. Chen, H. Yu, H. Diao, C. Shi, Y. Wang, G. Li, and M. Shi. 2017. Oridonin ameliorates lipopolysaccharide/D-galactosamine-induced acute liver injury in mice via inhibition of apoptosis. *American Journal of Translational Research* 9 (9): 4271–4279.
23. Wu, Y., J. Zhang, and Y. Qin. 2018. S100A4 promotes the development of lipopolysaccharide-induced mouse endometritis. *Biology of Reproduction*. https://doi.org/10.1093/biolre/ioy124.
24. Borregaard, N., O.E. Sorensen, and K. Theilgaard-Monch. 2007. Neutrophil granules: a library of innate immunity proteins. *Trends in Immunology* 28 (8): 340–345. https://doi.org/10.1016/j.it.2007.06.002.
25. Cao, S.Y., W. Wang, F.F. Nan, Y.N. Liu, S.Y. Wei, F.F. Li, and L. Chen. 2018. Asiatic acid inhibits LPS-induced inflammatory response in endometrial epithelial cells. *Microbial Pathogenesis* 116: 195–199.
26. Herath, S., D.P. Fischer, D. Werling, E.J. Williams, S.T. Lilly, H. Dobson, C.E. Bryant, and I.M. Sheldon. 2006. Expression and function of Toll-like receptor 4 in the endometrial cells of the uterus. *Endocrinology* 147: 562–570.
27. Cohen, J. 2002. The immunopathogenesis of sepsis. *Nature* 420 (6917): 885–891.
28. Netea, M.G., and Van Der Meer JwkullbergBj. 2004. Toll-like receptors as an escape mechanism from the host defense. *Trends in Microbiology* 12 (11): 484–488.
29. Zhao, G., K. Jiang, H. Wu, C. Qiu, G. Deng, and X. Peng. 2017. Polydatin reduces Staphylococcus aureus lipoteichoic acid-induced injury by attenuating reactive oxygen species generation and TLR2-NFkappaB signalling. *Journal of Cellular and Molecular Medicine* 21 (11): 2796–2808. https://doi.org/10.1111/jcmm.13194.
30. Tanaka, T., M. Narazaki, and T. Kishimoto. 2014. IL-6 in inflammation, immunity, and disease. *Cold Spring HarbPerspect Biol* 6: a016295.
31. Yang, X., B. Shi, L. Li, Z. Xu, Y. Ge, J. Shi, Y. Liu, and D. Zheng. 2016. Death receptor 6 (DR6) is required for mouse B16 tumor angiogenesis via the NF-κB, P38 MAPK and STAT3 pathways. *Oncogenesis* 5: e206.
32. Dinarello, C.A. 1996. Biologic basis for interleukin-1 in disease. *Blood* 87: 2095–2147.
33. Esposito, E., and S. Cuzzocrea. 2009. TNF-alpha as a therapeutic target in inflammatory diseases, ischemia-reperfusion injury and trauma. *Current Medicinal Chemistry* 16: 3152–3167.
34. Colotta, F., P. Allavena, A. Sica, C. Garlanda, and A. Mantovani. 2009. Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. *Carcinogenesis* 30: 1073–1081.
35. Mateu, A., L. Ramudo, M.A. Manso, and I. De Dios. 2015. Cross-talk between TLR4 and PPARγ pathways in the arachidonic acid-induced inflammatory response in pancreatic acini. *International Journal of Biochemistry & Cell Biology* 69: 132–141.
36. Jiang, Q., M. Yi, Q. Guo, C. Wang, H. Wang, S. Meng, C. Liu, Y. Fu, H. Ji, and T. Chen. 2015. Protective effects of polydatin on lipopolysaccharide-induced acute lung injury through TLR4-MyD88-NF-κB pathway. *International Immunopharmacology* 29 (2): 370–376.
37. Zhao, G., H. Wu, K. Jiang, X. Chen, X. Wang, C. Qiu, M. Guo, and G. Deng. 2016. The Anti-Inflammatory Effects of Interferon Tau by Suppressing NF-κB/MMP9 in Macrophages Stimulated with Staphylococcus aureus. *Journal of Interferon & Cytokine Research the Official Journal of the International Society for Interferon & Cytokine Research* 36 (8): 516–524.
38. Baeuerle, P.A. 1988. IκB: a specific inhibitor of the NF-κB transcription factor. *Science* 242 (4878): 540–546.
39. Nicholas, Courtney, Sanjay Batra, Melissa A. Vargo, Oliver H. Voss, Mikhail A. Gavrilin, Mark D. Wewers, Denis C. Guttridge, Erich Grotewold, and Andrea I. Doseff. 2007. Apigenin Blocks Lipopolysaccharide-Induced Lethality In Vivo and Proinflammatory Cytokines Expression by Inactivating NF-κB through the Suppression of p65 Phosphorylation. *Journal of Immunology* 179 (10): 7121–7127.