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Original Research Article

Polygonum cuspidatum glycoside mitigated LPS-induced human endometrial stromal cell inflammation by regulating NF-κB/Nrf2 signaling pathway-related proteins

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Abstract

Purpose: *To study the influence of polydatin on LPS-provoked human endometrial stromal cell inflammation, and its mechanism of action.*
Methods: *Fifty ICR female mice were selected and assigned to control and three-dose polydatin groups. Before establishment of the model, mice in low-dose group, middle-dose group and high-dose group were given polydatin at doses of 5, 10 and 20 mg/kg, respectively, by oral gavage for 5 days. Protein expression levels of interleukin 1β (IL-1 β), tumor necrosis factor- α (TNF-α) level, nuclear factor E2-related factor 2 (NRF-2) and nuclear transcription factor kappa-B (NF-2 κB) were determined with Western blot assay.*
Results: *Model group mice protein levels of NF- κB and nrf-2 were significantly reduced, relative to the corresponding control values ($p < 0.05$). The NF-κB and NRF-2 proteins in model group were markedly up-regulated, relative to control group, but they were and dose-dependently lower in the 3 polydatin groups than in control ($p < 0.05$).*
Conclusion: *Polydatin reduces LPS-induced inflammatory response in mouse endometrial stromal cells, and promotes the repair of endometrium and regeneration of glands via a mechanism related to regulation of NF-κ B/Nrf2 signaling pathway-related proteins.*

Keywords: *Polydatin, NF- κ B, Nrf2 signaling pathway, lipopolysaccharide, Human endometrial stromal cells, Inflammation*

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INTRODUCTION

Endometritis is a bacterial infectious disease of the reproductive tract which often occurs during abortion, curettage, placement of birth control rings, and puerperal infections. The disease is seen regularly in the Gynecology Department. Endometritis is caused by vaginal infection due to *Escherichia coli, Staphylococcus aureus* and *Streptococcus* which ultimately lead to inflammatory changes in the endometrium and related structures [1]. Endometritis often occurs in married women and women of childbearing age, and it affects 10 - 15 % of women of childbearing age, 20 % of whom eventually become infertile, thereby seriously affecting their lives and health [2]. Due to changes in lifestyle in recent years, endometritis has been on the

increase even among the younger population groups, thereby posing serious challenges to gynecologists [3].

Polydatin is a monomeric compound extracted from the dried root and stem of *Polygonum cuspidatum.* It is effective against dampness and *yellopathy*, blood circulation and *stasis* removal, as well as *heat clearing*, detoxification, cough, jaundice and rheumatism [4]. Recent studies have found that polydatin has anti-inflammatory, antioxidant and anti-shock effects [5].

In this study, 50 female ICR mice served as animal model for determination of the influence of polydatin on LPS-provoked human endometrial stromal cell inflammation, and the mechanisms involved.

EXPERIMENTAL

Animals

Fifty (50) female ICR mice obtained from Animal Medical Center of Yangzhou University were fed in animal house at a temperature of 24 ℃ under equal durations of day and night. The study received approved from Animal Ethics Division of Qinghai Provincial People's Hospital, and the study was conducted in line with the guidelines of "Principles of Laboratory Animal Care" (NIH guideline, 1985) [6].

Study design and treatments

Four mice groups were used: inflammation model, as well as low-dose polydatin, middle-dose polydatin and high-dose polydatin groups which were given (via gavage) polydatin at levels of 5, 10 and 20 mg/kg, in that order, for 5 days. Each mouse in the model group was given an equivalent volume of 0.5 % normal saline in place of polydatin. The control (5^{th} group) was not treated. Animals in model, low-dose, middle-dose and high-dose groups were LPS-induced endometritis model mice. One hour after the last polydatin administration, the mice were anesthetized via intraperitoneal injection of 4 % chloral hydrate. The abdominal cavity was opened, and 25 μL LPS saline solution (2.5 mg/mL) was injected into the uterus with a 1-mL insulin syringe. After the injection, the incision sites were sutured layer-by-layer. Following 24 h, the animals were sacrificed *via* decapitation, and the uterine tissues were excised. One part was fixed in 10 % formaldehyde for H & E staining, while the other part was stored at -80 ℃ for subsequent studies.

Assessment of parameters and histology

Histological examination

Uterine tissue was fixed in 10 % formaldehyde solution, followed by dehydration, embedding, and sectioning. The slices were heated at 60 ℃ for 1 h, dewaxed with xylene (Ⅰ and Ⅱ) and gradient concentrations alcohol, stained with hematoxylin for 7 - 8 min, and rinsed in water. Then, the slices were counterstained with eosin for 25 - 30 sec, washed with water for 10 sec, dehydrated in alcohol gradient, dried in a fume cabinet, sealed, dried, examined under a light microscope and photographed.

Levels of myeloperoxidase (MPO) and nitric oxide

A 10 % homogenate of uterine tissue of each mouse in each group was prepared. After centrifugation, the resultant supernatant was assayed for level of NO using the Griess reagent method, while the MPO content was measured using ELISA.

Levels of interleukin-1 β (IL-1β) and tumor necrosis factor-α (TNF-α)

Uterine tissue was taken up in a 2-mL EP tube, ground evenly on ice, and following centrifugation for 15 min at 3000 rpm, supernatant levels of these cytokines were assayed with ELISA.

Western blot assay

Uterine total protein extractions were done with radioimmunoprecipitation assay (RIPA) buffer containing protease inhibitor. The uterine tissues were cut into bits and homogenized with ultrasonic homogenizer. The tissue homogenates were placed on ice for several minutes, transferred to centrifuge tubes and centrifuged at 12000 g at 4 ℃ for 10 min, and supernatant protein contents were measured using BCA procedure.

Thereafter, the proteins were resolved with SDS-polyacrylamide gel electrophoresis, followed by membrane transfer, sealing, incubation with primary antibodies for NF-κB and NRF-2 overnight at 4 ℃, and incubation with secondary antibody linked to horse radish peroxidase. Finally, the bands were subjected to ECL chromogenic agent analysis for determination of the relative protein expressions of NF-κB and NRF-2.

Statistics

The SPSS 20.0 package was applied for statistical analysis of data. All measurement results consistent with normal distribution are presented as mean ± SD. Comparison amongst multiple groups was done with one-way analysis of variance (ANOVA), while pairs were compared with SNK-Q test. Statistical results are presented as percentage, and χ^2 test was employed for group comparison. Statistical significance was assumed at values of $p < 0.05$.

RESULTS

Histological features of mouse uterus

Model mouse endometrium lamina was infiltrated by inflammatory cells (mainly neutrophils), relative to control, and there were degeneration and necrosis of the epithelial cells of the uterus and glands. Uterine tissue damage was significantly and dose-dependently reduced in mice in the 3 polydatin groups. These results are shown in Figure 1.

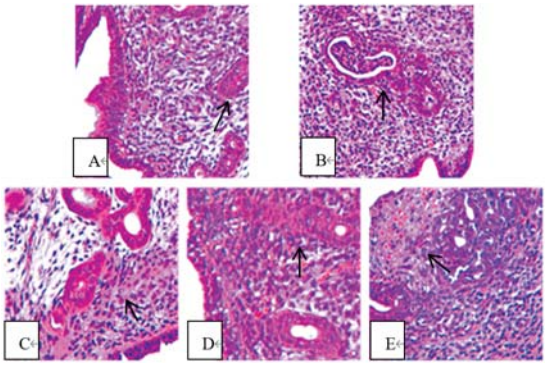

Figure 1: H & E-stained images of uterus of mice. A: Control; B, C & D: low-, medium- and high-dose polydatin, respectively

Number of uterine glands

The number of glands was markedly lower in endometriosis mice than in normal group. In contrast, the numbers of glands in low-, medium- and high-dose groups were significantly and dose-dependently increased, relative to the model group value ($p < 0.05$; Table 1).

MPO activity and NO level

As shown in Table 2, the activity of MPO and level of NO in model mice were markedly higher than control values, but they were markedly and dose-dependently reduced in middle- and high-dose mice, relative to model nice.

Table 1: Comparison of population of glands in the two groups

Group	Number of glands
Control	55.78±5.78
Model	26.65±5.28[a]
Low dose	32.65±4.15[ab]
Medium dose	38.62±4.89[abc]
High dose	49.81±5.16[abcd]
F	111.770
P-value	＜0.001

[a,b,c,d]$P < 0.05$, [a]vs normal mice; [b]vs model; [c]vs low-dose, [d]vs medium-dose

Table 2: MPO activity and NO levels in each group

Group	MPO (ng/mL)	NO (μmol/L)
Control	3.85±0.15	3.71±0.19
Model	7.46±0.51[a]	8.16±0.48[a]
Low dose	6.16±0.38[ab]	6.58±0.41[ab]
Medium dose	5.29±0.25[abc]	5.41±0.39[abc]
High dose	4.71±0.31[abcd]	4.71±0.31[abcd]
F	327.340	433.850

[a,b,c,d]$P < 0.05$, [a]vs control; [b]vs model; [c]vs low-dose, [d]vs medium-dose group

TNF-α and IL-1β

There were higher concentrations of TNF-α and IL-1β in model mice than in control mice, but they were markedly reduced in the 3 polydatin groups, relative to model group (Table 3).

Table 3: Levels of IL-1β and TNF-α in each group

Group	IL-1β (pg/mL)	TNF-α (pg/mL)
Control	71.26±5.64	63.25±4.69
Model	256.38±41.36[a]	463.52±40.31[a]
Low dose	136.52±20.14[ab]	352.68±30.12[ab]
Medium dose	118.95±15.34[abc]	300.05±20.15[abc]
High dose	102.64±13.26[abcd]	256.64±15.48[abcd]
F	196.150	676.840
P-value	＜0.001	＜0.001

[a,b,c,d]$P < 0.05$, [a]vs normal mice; [b]vs model; [c]vs low-dose, [d]vs middle-dose group

Expression levels of NF-κB/Nrf2 signaling pathway-related proteins

As shown in Figure 2, there were markedly higher NF-κB and NRF-2 protein concentrations in model group than in control group, but they were markedly and dose-dependently lower in the 3 polydatin groups than in model mice.

DISCUSSION

There are no obvious clinical symptoms and signs at the early stages of endometritis, but in the advanced stage of the disease, symptoms such as irregular or regular vaginal bleeding, lower abdominal pain and abundant leucorrhea may occur [7,8].

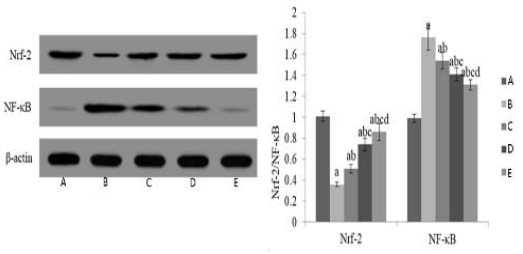

Figure 2: Expression levels of NF-κB/Nrf2 signaling pathway-related proteins in each group. $^{a,b,c,d}P < 0.05$, ^{a}vs control; ^{b}vs model; ^{c}vs low-dose; ^{d}vs medium-dose. A: Control, B: Model, C: Low dose, D: Medium dose, E: High dose

If patients do not receive adequate anti-inflammatory treatment during the attack stage of inflammatory diseases, repeated inflammatory reactions will eventually lead to chronic endometritis. This may result in prolonged menstrual period or amenorrhea in mild cases, and infertility, spontaneous abortion and myositis in severe cases, thereby impacting adversely on standard of life [9]. Polydatin, also known as resveratrol glycoside, is an astragalus compound which possesses anti-inflammatory and anti-oxidative stress properties, and it is beneficial in endometriosis, polycystic ovary syndrome, metabolic system diseases and cardiovascular system diseases [10]. It has been reported that polydatin inhibited the enhancing effect of IL-6 on the invasion and migration of breast cancer MDA-MB-231 cells through down-regulation of Akt signaling pathway and other pathways [11]. Moreover, a study has shown that polydatin improved the reproductive function of the ovary and the development of oocytes and embryos *in vitro* [12].

In the present study, relative to control, endometrial lamina in model mice was infiltrated by inflammatory cells dominated by neutrophils, and there was evidence of necrotic and degenerative lesions in the uterus and glands. The extent of uterine tissue damage in mice in the 3 polydatin groups was markedly and dose-dependently reduced. Moreover, the population of glands in model mice was markedly lower than the number of glands in control mice. However, polydatin treatment significantly and dose-dependently increased the number of viable glands, relative to the model group. These results indicate that polydatin markedly decreased the infiltration of inflammatory cells and inhibited the movement of neutrophils in mice with endometritis.

Excessive inflammatory response can lead to pathological damage in endometritis [13]. Myeloperoxidase, a marker of neutrophil function and activation, is a reflection of the degree of inflammatory cell infiltration [14]. Nitric oxide (NO) is generated in the body by several cells. Moreover, LPS stimulates macrophages to produce a large amount of NO and H_2O_2 which neutralize invading bacteria, fungi and other microorganisms, as well as inflammatory injury [15]. Neutrophils are cells that elaborate pro-inflammatory factors e.g. IL-1β and TNF-α which cause epithelial injury in LPS-induced endometritis [16]. In this study, there were markedly higher amounts of these cytokines and MPO and NO in model mice than in control mice, but their levels were significantly and dose-dependently reduced by polydatin exposure. These results suggest that polydatin mitigated inflammatory response and suppressed the development of endometritis in mice.

Nuclear factor kappa B (NF-κB) is a typical eukaryotic transcription factor involved in inflammation, immune response and anti-apoptotic processes. Activation of NF-κB in LPS-induced endometritis leads to production of inflammation-enhancing cytokines such as IL-1β and TNF-α [17]. A member of the cap and collar transcription factor family, NrF-2 has antioxidant stress effect and it is crucial in endometrioma and other cancers, as well as in cardiovascular diseases [18]. In this study, the protein levels of NF-κB and NRF-2 were markedly up-regulated in model mice, relative to control mice, but the expressions of these factors were significantly and dose-dependently reduced by polydatin treatment. These results indicate that polydatin reduced inflammatory response in mice with endometritis via a mechanism involving the regulation of expressions of NF-κB/Nrf2 signaling route-linked proteins.

CONCLUSION

Polydatin reduced LPS-induced inflammation in mouse endometrial stromal cell inflammation and also promoted endometrial repair and gland regeneration through a mechanism involving regulation of NF-κB/Nrf2 signaling pathway-related proteins.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was performed by the authors named in this article, and all liabilities

pertaining to claims relating to the content of this article will be borne by the authors. Wenjuan Wang designed the study, supervised the data collection, and analyzed the data. Wenjuan Wang interpreted the data and prepared the manuscript for publication. Wenjuan Wang, Zhengfang Xiong and Xianghui Zeng supervised the data collection, analyzed the data and reviewed the draft of the manuscript.

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