INTRAUTERINE PERFUSION OF PRP IMPROVES THE INFLAMMATORY RESPONSE OF ENDOMETRITIS IN RATS THROUGH MIR-19A AND NF-KB PATHWAY

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ABSTRACT

Objective*: To investigate the potential mechanism of PRP intrauterine infusion in treating inflammatory response of endometritis.*

Method: *SD female rats were selected and randomly divided into control group (CG), model group (MG), PRP group, PRP+NC inhibitor group and PRP+miR-19a inhibitor group. In the MG, PRP group, PRP+NC inhibitor group and PRP+miR-19a inhibitor group, rats were all inoculated with Escherichia coli.*

Methods: *In the PRP group, PRP+NC inhibitor group and PRP+miR-19a inhibitor group, rats received PRP intrauterine infusion. In the PRP+NC inhibitor group, rats were injected with NC inhibitor via tail vein after PRP treatment. In the PRP+miR-19a inhibitor group, rats were injected with miR-19a inhibitor via tail vein after PRP treatment. The levels of serum inflammatory factors, MDA and SDO were recorded in each group. The pathological changes of endometritis were detected by HE staining. The NF-kB protein was detected by Western blot. The miR-19a was detected by qPCR.*

Results: *Endometritis caused an increase in serum phosphorylation levels of TNF-, IL-1, MCP, IL-10 and NF-kB protein, and a decrease in MDA, SOD and miR-19a. Endometritis resulted in degeneration and necrosis of endometrial epithelial cells, infiltration of scattered or lamellar inflammatory cells, and congestion and edema of endometrium. Intrauterine perfusion of PRP inhibited the phosphorylation levels of serum TNF-α, IL-1β, MCP, IL-10 and NF-kB protein, and increased MDA, SOD and miR-19a. Down-regulation of miR-19a could counteract the therapeutic effect of PRP on endometritis.*

Conclusion: *Intrauterine perfusion of PRP could improve the inflammatory response of endometritis in rats through miR-19a and NF-kB pathway.*

Keywords: *PRP intrauterine perfusion, endometritis, inflammatory reaction, NF-kB pathway, miR-19a.*

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Introduction

Endometritis is a common postpartum infection, and bacterial infection is the cause of endometritis. Most of these endometrial bacterial infections are caused by anaerobic and aerobic bacteria[(1)]. Endometritis can be divided into acute endometritis and chronic endometritis[(2)]. If endometritis cannot be treated in time, it may lead to death (the mortality rate is about 17%)[(3)]. In addition, endometritis is accompanied by complications such as septicemia, which may cause uterine necrosis[(4)]. At present, antibiotic therapy is a common treatment for endometritis, but its therapeutic effect on patients with chronic endometritis is still poor[(5, 6)]. Therefore, it is urgent to find an alternative treatment for endometritis. Platelet-rich plasma (PRP) is widely used in the treatment of inflammatory diseases such as osteoarthritis because of its anti-inflammatory and antibacterial properties[(7-9)]. Platelet concentration

of PRP is significantly higher than that of normal plasma, and highly enriched platelets exert its therapeutic effect by releasing growth factors and cytokines[10]. Previous studies[11-13] have proved that PRP can promote endometrial regeneration, improve pregnancy outcome and inhibit the expression of endometrial inflammatory genes. In the research of Reghini et al. and Segabinazzi[14, 15], PRP intrauterine perfusion effectively inhibited the inflammatory reaction of endometritis in mares, thus increasing the possibility of pregnancy. Sfakianoudis et al.[6] have believed that conventional antibiotics have no obvious therapeutic effect on endometritis, while PRP intrauterine infusion may help to improve chronic endometritis. Therefore, the above research shows that PRP intrauterine perfusion has a considerable application prospect in endometritis.

At present, there are few evidences about PRP intrauterine perfusion in the treatment of endometritis, and its treatment mechanism is not clear. Therefore, rats were induced by Escherichia coli to construct the endometritis model in this study, and PRP intrauterine perfusion was used to observe the pathological changes of inflammatory factors and endometrial tissue in rats, so as to provide reliable experimental data for PRP intrauterine perfusion in vivo.

Methods

Endometritis model

SD female rats were selected and randomly divided into CG, MG, PRP group, PRP+NC inhibitor group and PRP+miR-19a inhibitor group, with 6 rats in each group. In the MG, PRP group, PRP+NC inhibitor group and PRP+miR-19a inhibitor group, rats were all inoculated with Escherichia coli.

Inoculation process

Rats were anesthetized by intraperitoneal injection of 10% chloral hydrate (350 mg/kg). The abdomen was shorn and disinfected routinely, and the abdominal cavity was opened. A total of 0.1ml escherichia coli fluid was injected into both sides of the uterus at the bifurcation point, and the abdomen was closed with layer by layer suture. In the PRP group, PRP+NC inhibitor group and PRP+miR-19a inhibitor group, rats received PRP intrauterine infusion. In the PRP+NC inhibitor group, rats were injected with NC inhibitor via tail vein after PRP treatment. In the PRP+miR-19a inhibitor group, rats were injected with miR-19a inhibitor via tail vein after PRP treatment.

After ELISA detection, the rats in each group were euthanized according to humanitarianism, and the endometrial tissues were taken for follow-up experiments.

ELISA

After modeling successfully, the tail venous blood was collected, the supernatant was collected after centrifugation, and the levels of serum TNF-α, IL-1β, MCP, IL-10 were detected by using TNF-α, IL-1β, MCP, IL-10 kits. The levels of superoxide dismutase (SOD) and malondialdehyde (MDA) in rat serum were determined by colorimetry.

Pathological evaluation of endometritis

In each group, the left uterus of rats was collected and fixed in 10% neutral formalin solution.

After that, the uterus was embedded in paraffin and sectioned, and the sections were stained with hematoxylin-eosin (HE) to observe the degeneration and necrosis of endometrial epithelial cells, interstitial inflammation infiltration and muscular hyperplasia or not.

The evaluation criteria of endometrial epithelial cell lesions from mild to severe were as follows:

- 0 for those without lesions;
- 1 point for patients with extremely mild lesions, 2 points for mild cases with lesion range less than 1/3;
- 3 points for moderate lesions and lesions ranging from 1/3 to 2/3;
- 4 points for severe cases with lesion range greater than 2/3.

The pathological comprehensive score was recorded in each group.

qPCR

The cells were prepared into cell suspension, and the total RNA of cells was extracted by Trizol to determine the purity of total RNA.

Among them, miR-19a was reversely transcribed and amplified. The primer sequence was designed and synthesized by Sangon Biotech (Shanghai) Co.,Ltd. The reverse transcription and qPCR amplification kits were purchased from Solarbio Co., Ltd. For the composition of the reaction system and the setting of the reaction procedure, we referred to the product manual. The expression level was standardized by $2^{-\triangle\triangle t}$ after obtaining the Ct value of the sample. More details are shown in Table 1 for primers sequence.

	Sequence information (5'-3')
miR-19a Forward	TGT GCA AAT CTA TGC AAA
miR-19a Reserve	CAG TGC GTG TCG TGG AGT
U6 Forward	CTC GCT TCG GCA GCA CA
U6 R	AAC GCT TCA CGA ATT TGC GT

Table 1: Primers sequence.

Western blot

RIPA lysis buffer was used to lyse cells, and the lysate was centrifuged for 20 min. The sediment was discarded, and the supernatant was obtained. The protein concentration in the supernatant was quantified by BCA method (Thermo Fisher Company). SDS-PAGE (Solarbio company) electrophoresis was used to separate the total protein. The protein was transferred to the polyvinylidene fluoride membrane (Solarbio), and the protein to be tested and the internal reference protein primary antibody were added and placed overnight at 4°C.

The goat anti-rabbit secondary antibody was added and placed at room temperature for 1 h. ECL luminescent liquid was used for visualization processing. The internal reference protein was GAPDH. Protein antibodies were all purchased from Abcam Company.

Statistics and analysis

One-way anova was used to compare the differences among multiple groups. Afterwards, the pairwise comparison was Dunnett's multiple comparison. Statistical software was SPSS 22.0, and drawing software was GraphPad 8.0. The experiment was repeated three times.

Results

PRP improved the inflammatory response of endometritis in rats

Table 2 showed the levels of inflammatory cytokines TNF-α, IL-1β, MCP and IL-10 in serum of rats in each group. Table 3 showed the serum MDA and SOD levels of rats in each group.

There were significant differences in TNF-α, IL-1β, MCP, IL-10, MDA and SOD among the five groups (P<0.05). Compared with normal saline group, the serum TNF-α, IL-1β, MCP and IL-10 in MG increased statistically, while MDA and SOD decreased. Compared with the MG, the serum TNF-α, IL-1β, MCP and IL-10 in PRP group decreased statistically, while MDA and SOD increased. Compared with PRP+NC inhibitor group, the serum TNF-α, IL-1β, MCP and IL-10 in PRP+miR-19a inhibitor group increased statistically, while MDA and SOD decreased.

	TNF-a (ng/ml)	IL-1b (ng/ml)	MCP (pg/ml)	IL-10 (ng/ml)
Normal saline group	35.21±6.92	12.52±4.12	57.21±11.85	23.14±5.63
MG	52.82±7.63	28.42±5.17	78.28±13.52	32.25±5.28
PRP group	37.49±7.34	14.26±4.67	58.17±11.24	24.22±5.12
PRP+NC inhibitor group	38.01±7.29	14.95±4.35	59.11±12.17	24.68±5.36
PRP+miR-19a ihibitor group	48.22±7.32	26.88±4.22	74.05±13.96	29.62±5.33
P	0.0008	<0.0001	0.0154	0.0277

Table 2: Serum inflammatory response factors of rats in each group.

	SOD (U/ml)	MDA (μmol/L)
Normal saline group	192.36±23.87	11.08±2.39
MG	143.74±21.07	16.22±2.53
PRP group	184.88±19.84	11.92±2.12
PRP+NC inhibitor group	183.52±18.62	12.01±1.98
PRP+miR-19a ihibitor group	146.85±19.26	16.13±2.47
P	0.0004	0.0006

Table 3: Serum MDA and SOD of rats in each group.

Table 4 showed the pathological comprehensive scores of rats in each group, and the total pathological scores among the five groups were statistically different (P<0.05). Compared with the MG, the pathological comprehensive score decreased in PRP group. The scores of PRP+ miR-19a inhibitor group were higher than those of PRP+NC inhibitor group.

	Pathological comprehensive score
Normal saline group	1.12±0.52
MG	8.22±2.21
PRP group	4.36±1.08
PRP+NC inhibitor group	4.99±1.23
PRP+miR-19a ihibitor group	7.26±2.14
P	<0.0001

Table 4: Pathological comprehensive scores of rats in each group.

Figure 1 was a HE staining image of endometrial tissue. In the normal saline group, the structure of uterine tissue was normal, and there was no degeneration or necrosis of endometrial epithelial cells and no hyperplasia of muscular layer. In the MG, there was degeneration and necrosis of

endometrial epithelial cells, scattered or lamellar inflammatory cell infiltration and endometrial congestion and edema in rats. In the PRP group, the surface epithelium of endometrium was slightly hyperplastic and thickened, and the infiltration of inflammatory cells was obviously reduced.

The pathological staining of PRP+NC inhibitor group was similar to that of PRP group. The PRP+miR-19a inhibitor group was more serious than the PRP group.

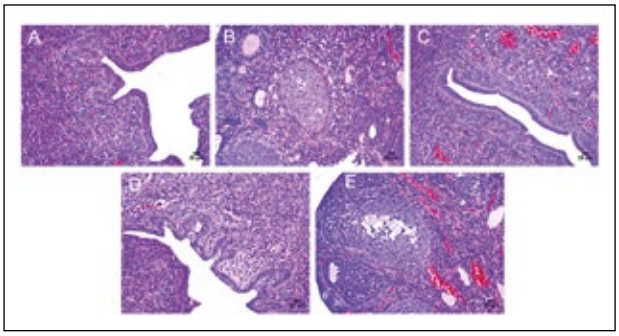

Figure 1: HE staining images of endometrial tissue in each group. All images were 20 μm in scale. A, Normal saline group. B, MG. C, PRP group. D, PRP+NC inhibitor group. E, PRP+miR-19a inhibitor group. The results of HE staining showed that the structure of uterine tissue was normal in the normal saline group, and there was no degeneration or necrosis of endometrial epithelial cells and no hyperplasia of muscular layer. In the MG, there was degeneration and necrosis of endometrial epithelial cells, scattered or lamellar inflammatory cell infiltration and endometrial congestion and edema in rats. In the PRP group, the surface epithelium of endometrium was slightly hyperplastic and thickened, and the infiltration of inflammatory cells was obviously reduced. The pathological staining of PRP+NC inhibitor group was similar to that of PRP group. The PRP+miR-19a inhibitor group was more serious than the PRP group.

PRP perfusion caused the increase of miR-19a and the decrease of NF-kB pathway in rats with endometritis

Figure 2 showed the expression of miR-19a in endometrial tissue of each group.

Figure 3 showed the activity of NF-kB protein (p65 and its phosphate delayed p-p65) in each group. Compared with normal saline group, miR-19a was down-regulated and NF-kB phosphorylation level increased in MG.

Compared with the MG, miR-19a was up-regulated and NF-kB phosphorylation level was down-regulated in PRP group. Compared with PRP+NC inhibitor group, miR-19a was down-regulated and NF-kB phosphorylation level was increased in PRP+miR-19a inhibitor group.

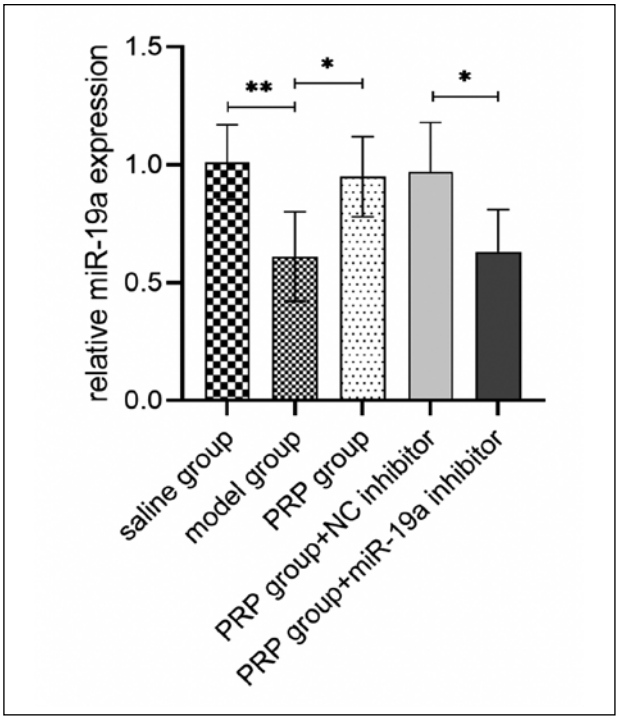

Figure 2: Expression of miR-19a in endometrial tissues of each group. Compared with normal saline group, miR-19a was down-regulated in MG. Compared with MG, miR-19a was up-regulated in PRP group. Compared with PRP+NC inhibitor group, miR-19a was down-regulated in PRP+miR-19a inhibitor group. **denotes P<0.01; ***denotes P<0.001. There were six rats in each group. According to humanitarianism, rats were euthanized, the endometrial tissues were isolated, and the expression of miR-19a was detected by qPCR.

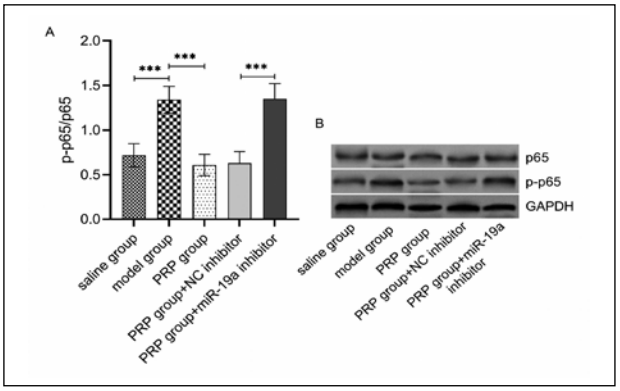

Figure 3: Expressions of p65 and its phosphate in endometrial tissues of each group. A, Changes of p-p65/p65 value in each group. Compared with normal saline group, the phosphorylation level of p65 was up-regulated in MG. Compared with MG, the phosphorylation level of p65 was down-regulated in PRP group. Compared with PRP+NC inhibitor group, the phosphorylation level was up-regulated in PRP+miR-19a inhibitor group. B, Western blot figure. ***denotes P<0.001. There were six rats in each group. According to humanitarianism, rats were euthanized, the endometrial tissues were isolated, and the p65 and its phosphorylation levels were detected by Western blot.

Discussion

Endometritis is a serious infectious disease, so it is necessary to carry out timely antibacterial and anti-inflammatory treatment according to the symptoms. PRP is widely used in clinic because of its remarkable anti-inflammatory and antibacterial effects. At present, it is still needed to do more research to discuss the application of PRP in endometritis. Therefore, rats were induced by Escherichia coli to construct the endometritis model in this study, and PRP intrauterine perfusion was used to observe the changes of serum inflammatory factors and endometrial tissue in rats. Endometritis resulted in elevated serum levels of TNF-α, IL-1β, MCP and IL-10, as well as degeneration and necrosis of endometrial epithelial cells in rats, inflammatory cell infiltration and endometrial congestion and edema. PRP intrauterine perfusion could significantly reduce the serum TNF-α, IL-1β, MCP and IL-10 levels of endometritis, and relieve the proliferation of endometrial epithelial cells and the infiltration inflammatory cells. These results indicated that PRP intrauterine perfusion could effectively improve the inflammatory response of endometritis.

While improving inflammatory response, we observed that miR-19a decreased and NF-kB pathway level increased in rats with endometritis. NF-kB pathway is a common inflammatory signaling pathway, which mediates inflammatory response, cell proliferation or apoptosis in sepsis and pulmonary fibrosis[16-20]. miR-19a is a miRNA located on human chromosome 14, which is involved in regulating the cellular biological functions of gastric cancer, myocardial infarction and ovarian cancer[21-24]. Yin et al.[25-27] have believed that miR-19a inhibited the expression of TNF-α and other inflammatory factors by negatively regulating the activity of NF-kB pathway in the model of endometrial inflammation induced by lipopolysaccharide. Similarly, the results of this study showed that the expression of miR-19a increased and the level of NF-kB pathway decreased in rats with endometritis induced by Escherichia coli after receiving PRP cervical perfusion therapy. Down-regulation of miR-19a could offset the regulatory effect of PRP intrauterine perfusion on the miR-19a and NF-KB pathways of endometritis, and aggravate endometrial hyperplasia and inflammatory cell infiltration. Therefore, it is speculated that PRP intrauterine perfusion may down-regulate the activity of NF-kB pathway through miR-19a, thus alleviating the inflammatory reaction and cell proliferation mediated by NF-kB pathway. Although the mechanism of PRP intrauterine perfusion has been studied by endometritis rat model, there are still some deficiencies in this study. First, this paper showed that the level imbalance of miR-19a and NF-KB pathways was involved in endometritis, but it failed to investigate whether there was a regulatory relationship between miR-19a and NF-KB pathways, and the downstream target genes of miR-19a in endometritis were not discussed in this paper. Secondly, the effect of PRP intrauterine perfusion on endometrial hyperplasia was qualitatively determined by HE staining in this paper, so the effect of PRP on endometrial cell proliferation will be further studied in future studies. Thirdly, only one PRP dose has been studied in this paper, so we will focus on optimizing the PRP dose to obtain the best therapeutic effect in the next study.

To sum up, this study showed that PRP intrauterine perfusion reduced the level of serum inflammatory factors in endometritis uterus through miR-19a and NF-kB pathway, and inhibited the infiltration of inflammatory cells and intimal hyperplasia and thickening. This study revealed that PRP intrauterine perfusion has potential therapeutic value for endometritis, but its specific dosage plan and treatment time still need to be further studied.

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