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Original Research Article

Effect of bazedoxifene on expressions of VEGF, VEGFR2, COX-2 and inflammatory factors in a rat endometriosis model

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Abstract

Purpose: *To study the effect of bazedoxifene on the expressions of vascular endothelial growth factor (VEGF), soluble vascular endothelial growth factor receptor 2 (sVEGFR2), cyclooxygenase synthase 2 (COX-2) and inflammatory factors in a rat endometriosis (EMS) model.*
Methods: *Thirty rats (EMS) were divided into untreated control group, bazedoxifene group and celecoxib group (10 rats per group). Bazedoxifene and celecoxib were administered at doses of 3 and 25 mg/kg, respectively. Negative control rats served as control, and were given 0.9 % sodium chloride at a dose of 10 mL/kg. All treatments were given intragastrically for 21 days. Levels of VEGF, sVEGFR2 and COX-2 in uterine ectopic endometrium were assayed.*
Results: *The levels of VEGF and sVEGFR2 in the serum and peritoneal fluid of the untreated control group were significantly higher than the corresponding control values, while VEGF and sVEGFR2 levels in the serum and peritoneal fluid of the bazedoxifene group were significantly lower than those in the untreated control group ($p < 0.05$). Bazedoxifene group had lower VEGFR2 and COX-2 levels than untreated control group ($p < 0.05$). Expression of COX-2 protein in the ectopic endometrium of celecoxib group was significantly lower than that in the untreated control group ($p < 0.05$).*
Conclusion: *Bazedoxifene alleviates angiogenesis and inflammatory factors in serum and peritoneal fluid of EMS rats, inhibits the expression of sVEGFR2 and COX-2 in ectopic endometrium, and also inhibits the growth of ectopic endometrium. This finding may help to discover additional new drugs.*

Keywords: *Bazedoxifene, Endometriosis, VEGF, VEGFR2, COX-2, Inflammatory factors*

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INTRODUCTION

Endometriosis (EMS) refers to the appearance, growth and formation of nodules and masses of endometrial tissue with growth function outside the endometrium [1]. The main clinical manifestations are chronic lower abdominal pain, dysmenorrhea and infertility. The associated histopathological changes are benign, but they have strong infiltration, invasion and metastasis [2]. In spite of several theories, the pathogenesis of EMS is still unknown. However, inflammation and vascular formation are considered as very important factors in the pathogenesis of EMS.

Being an estrogen-dependent disease, the endometrium of postmenopausal ectopic lesions may gradually undergo atrophic absorption, and pregnancy and sex hormone drugs are used to inhibit ovarian function and prevent disease progression [3,4]. Clinically, EMS is treated with drugs that can reduce the production of endogenous estrogen, but long-term drug usage is needed before low estrogen symptoms appear. Therefore, there is clinical need for a drug that can inhibit the growth of ectopic lesions, improve pregnancy efficiency, and reduce EMS recurrence, with acceptable tolerance by patients [5].

Bazedoxifene is a third-generation estrogen receptor (ER) regulator with tissue selectivity which is antagonized by ER binding in the breast and uterus. Studies have shown that bazedoxifene significantly reduces ectopic lesions in the rat EMS model, and also reduces the expression of ER; these findings have raised its potential for development into a new drug for the treatment of EMS [6]. In the present study, an EMS rat model was established by autologous transplantation, and the mechanism of bazedoxifene action on EMS was investigated in two aspects: regulation of angiogenesis and inhibition of inflammatory factors.

EXPERIMENTAL

Materials

Thirty non-mating female SD rats, weighing 180 - 200 g at 6 - 8 weeks old, were purchased from Shanghai Slack Laboratory Animals Co., Ltd. Bazedoxifene was product of Pfizer, USA; Celecoxib (> 99%) was purchased from Shanghai Qiyi Biotechnology Co., Ltd. (Lot: 169590-42-5), while VEGF, VEGFR2, IL-1, IL-2, IL-6 and TNF-α ELISA kits were products of TSZ. Rabbit anti-VEGFR2 antibody and rabbit anti-COX-2 antibody were obtained from CST; DAB chromogenic reagent was product of Vector Labs (Lot: SK4800); ECL Chemiluminescence detection kit was produced by Shanghai Tianneng Technology Co. Ltd (Lot: 180-501).

This research was approved by the Animal Ethical Committee of Department of Traditional Chinese Medicine, Affiliated Hangzhou First People's Hospital, Zhejiang University School of Medicine, Hanzghou City China (approval no. 20188379), and carried out according to "Principles of Laboratory Animal Care" (NIH publication no. 85-23, revised 1985) [7].

Paraffin slicer (RM2235) and DM750 optical microscope were purchased from Leica, Germany; Cryogenic centrifuge (5430) was product of Eppendorf; Real-Time PCR instrument was produced by Applied Biosystems (ABI7500, AME), while Tanon4100 digital gel image processing system was product of Shanghai Tianneng Technology Co. Ltd, China.

Establishment of rat EMS model

The EMS rat model was established by autologous transplantation. Thirty-two (32) rats were anesthetized with 40 % chloral hydrate (0.4g/100g), following the steps of skin preparation, laparotomy, dye stripping, suture, and abdominal closure. Part of the endometrium was implanted between the abdominal muscles and the subcutaneous fascia, with the intima surface clinging to the abdominal muscles. After 3 weeks, secondary laparotomy indicated that the graft showed a cystic enlargement which was vesicular or nodular. The surface of the cyst wall had blood vessels and a small amount of connective tissue, indicating that the model was successfully established. Two rats did not have ectopic endometrial tissue on the inside of the abdominal wall.

Rat grouping and drug administration

Thirty model rats were randomly divided into untreated control group, bazedoxifene group and celecoxib group (10 rats per group). Bazedoxifene and celecoxib were administered at doses of 3 mg/kg and 25 mg/kg, respectively. Un-operated rats served as control, and were given 0.9 % sodium chloride at a dose of 10 mL/kg. All treatments were given intragastrically for 21 days. Twelve hours after the last administration, the rats were anesthetized, and intraperitoneal injection of PBS buffer was given. The peritoneal fluid was collected by gently pressing the abdomen. The abdominal cavity was opened, blood was collected from the abdominal aorta, and the rats were sacrificed. The ectopic endometrial lesions were obtained. The blood and peritoneal fluid were centrifuged at 3000 rpm for 15 min at 4 °C, and the supernatant portions were fixed in 4 % paraformaldehyde, and partially stored in liquid nitrogen.

Treatment indices

The contents of VEGF, sVEGFR2 and inflammatory factors (IL-1, IL-2, IL-6 and TNF-α) in serum and peritoneal fluid were determined using enzyme-linked immunosorbent assay (ELISA). All operations were performed in line with kit instructions. The expressions of VEGFR2

and COX-2 were assayed using immune-histochemistry and Western blot.

Statistical analysis

Data were statistically analyzed using SPSS 17.0 software and are expressed as mean ± standard deviation (mean ± SD). Multiple comparison was performed with one-way analysis of variance (ANOVA), and LSD-t (Fisher's Least Significant Difference-t) test was used for comparison between two groups. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Levels of VEGF and sVEGFR2

The levels of VEGF and sVEGFR2 in serum and peritoneal fluid of untreated control group were significantly higher than those of the control group, and their levels in the bazedoxifene group were significantly lower than those in the untreated control group ($p < 0.05$). The content of VEGF in celecoxib group was significantly lower than that in untreated control group ($p < 0.05$), but VEGF and sVEGFR2 levels in serum and peritoneal fluid were not significantly different from those in untreated control group ($p > 0.05$). These results are shown in Table 1.

Expressions of inflammatory factors in peritoneal fluid of rats

Table 2 shows that the contents of IL-1, IL-2, IL-6 and TNF-α in the peritoneal fluid of the untreated control group were significantly higher than those in the control group, while IL-1 IL-2, IL-6 and TNF-α levels in the peritoneal fluid of rats in the bazedoxifene group were significantly lower than those in the untreated control group ($p < 0.05$). The levels of IL-1, IL-2 and IL-6 in the peritoneal fluid of rats in the celecoxib group were significantly lower than those in untreated control group. However, the TNF-α contents of the celecoxib and untreated control groups were comparable ($p > 0.05$).

Expressions of VEGFR2 and COX-2 in ectopic endometrium of rats

Results from immunohistochemistry showed that VEGFR2 was expressed mainly in cytoplasm and membrane of vascular endothelial cells and glandular epithelial cells, and also slightly expressed in interstitial cells. In contrast, COX-2 was expressed mainly in glandular epithelial cells and interstitial cells in ectopic endometrium of rats. The positive expression levels of VEGFR2 and COX-2 in the ectopic endometrium of the bazedoxifene group were significantly decreased. In the celecoxib group, only the positive expression of COX-2 was significantly decreased (Figure 1 and Figure 2). Western blot analysis showed that the levels of VEGFR2 and COX-2 in the bazedoxifene group were significantly lower than those in the untreated control group ($p < 0.05$). COX-2 expression in the ectopic endometrium of the celecoxib group was significantly lower than in the untreated control group ($p < 0.05$). These results are shown in Figure 3.

Table 1: Levels of VEGF and sVEGFR2 in serum and peritoneal fluid of rats after treatment (mean ± SD)

Group	n	VEGF (pg/mL)		sVEGFR2 (pg/mL)	
		Serum	***Peritoneal fluid***	***Serum***	***Peritoneal fluid***
Control	5	78.80 ± 13.01	83.05 ± 5.74	1286.27 ± 285.47	124.88 ± 407.69
Untreated control group	10	173.67 ± 28.56*	185.35 ± 12.74*	3273.68 ± 594.25*	3714.25 ± 152.98*
Bazedoxifene	10	120.96 ± 30.14$^{\#}$	116.32 ± 18.01$^{\#}$	2115.32 ± 276.14$^{\#}$	2232.42 ± 554.10$^{\#}$
Celecoxib	10	134.56 ± 16.74$^{*\#}$	122.99 ± 22.91$^{\#}$	2758.47 ± 723.28	2812.76 ± 331.65

*$P < 0.05$, *compared with the control group; #*$p < 0.05$, *compared with untreated control group*

Table 2: Levels of VEGF and sVEGFR2 in serum and peritoneal fluid of rats after treatment ($\bar{x} \pm s$)

Group	n	IL-1(ng/L)	IL-2(ng/L)	IL-6(pg/mL)	TNF-α(ng/L)
Control	5	126.95 ± 15.35	993.97 ± 101.25	52.34 ± 12.45	221.54 ± 24.26
Untreated control group	10	226.54 ± 16.82*	2135.54 ± 99.32*	143.25 ± 7.63*	511.64 ± 19.46*
Bazedoxifene	10	161.25 ± 30.35$^{\#}$	1426.35 ± 300.12$^{\#}$	100.64 ± 23.54$^{\#}$	354.33 ± 115.64$^{\#}$
Celecoxib	10	141.42 ± 36.05$^{\#}$	1308.14 ± 312.45$^{\#}$	74.63 ± 16.45$^{\#}$	338.45 ± 40.98$^{*\#}$

*$P < 0.05$, *compared with the control group; #*$p < 0.05$, *compared with untreated control group*

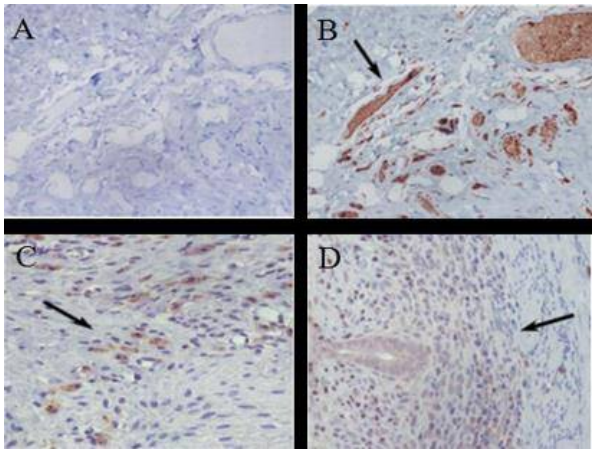

Figure 1: VEGFR2 expression in ectopic endometrium after treatment (DAB staining; ×400). A: Control, B: Model, C: Bazedoxifene, D: Celecoxib

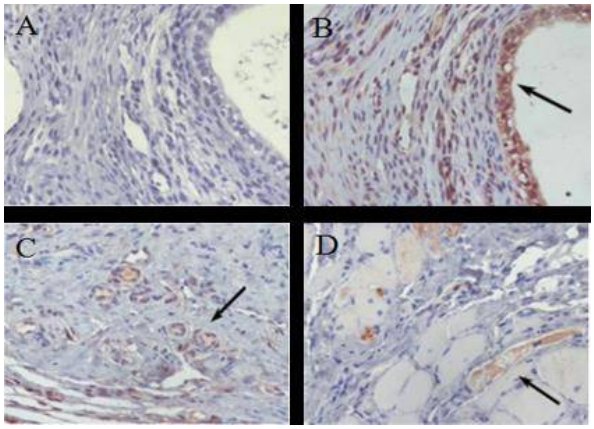

Figure 2: COX-2 expression in ectopic endometrium after treatment (DAB staining; ×400). A Control; B Model; C Bazedoxifene; D Celecoxib

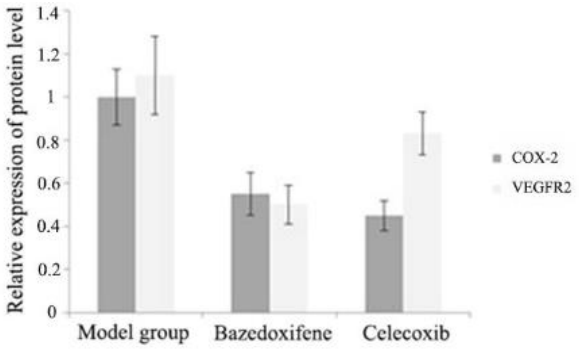

Figure 3: Expressions of VEGFR2 and COX-2 in ectopic endometrium of rats

DISCUSSION

Endometriosis (EMS) is a benign estrogen-dependent disease with strong malignant biological characteristics such as invasion, adhesion, recurrence, and malignant transformation [8]. The symptoms are caused by ectopic endometrial tissue implanted outside the uterus. Cyclooxygenase-2 (COX-2) is an important inducible enzyme in the inflammatory process. Studies have confirmed that COX-2 is involved in the formation of EMS, and is closely related to the occurrence and development of EMS [9].

Currently, clinical treatment of EMS is based mainly on surgery and drugs. Due to certain drawbacks of radical surgery, and poor receptivity, drug treatment is relatively more convenient. However, the clinically-used anti-estrogen drugs cause adverse reactions such as high androgen symptoms, impairment of liver function, and dyslipidemia. Thus, patients' tolerance of these drugs is poor. Bazedoxifene is a representative drug of the third-generation SERM. It is used for postmenopausal osteoporosis, and it has no adverse effects on the endometrium. Moreover, it has a stronger antagonism against endometrial ER than raloxifene, and it is expected to become a new drug for the treatment of EMS [10,11].

Vascular endothelial growth factor (VEGF) is a highly specific vascular endothelial cell mitogen which is currently recognized as the most important pro-angiogenic factor. It enhances the formation of new blood vessels by increasing vascular permeability and altering the gene expression of vascular endothelial cells [12]. On the other hand, VEGF also promotes mitosis of vascular endothelial cells by activating phospholipase C. It participates in extracellular proteolysis and degradation of basement membrane, and facilitates migration and proliferation of vascular endothelial cells. Studies have found that VEGF and its free receptor sVEGFR2 are upregulated in the peritoneal fluid of patients with EMS [13].

In the present study, the levels of VEGF and sVEGFR2 in the serum and peritoneal fluid of the untreated control group were significantly higher than those in the control group. The VEGF levels in the serum and peritoneal fluid of the two treatment groups were significantly decreased, and sVEGFR2 in the serum and peritoneal fluid of the celecoxib group decreased slowly. These results suggest that bazedoxifene acts on VEGF and its receptors, blocks VEGF from binding to its receptor, and affects angiogenesis. These findings are consistent with those reported by other scholars [14].

Cyclooxygenase-2 (COX-2) is a rate-limiting enzyme that catalyzes the conversion of phospholipid arachidonic acid to prostaglandins (PGs). It is upregulated by inflammatory mediators and cytokines, and it is involved

mainly in the inflammatory process, transmission of pain signals and the occurrence of tumors. Studies have shown that COX-2 expression is significantly increased in the endometrium of patients with endometriosis [15]. This results in increased adhesion and invasiveness of endometrial cells. In recent years, studies have shown that immune regulation plays an important role in the occurrence and development of EMS. Impairment of the immune system is an intrinsic factor in the occurrence of EMS.

Some scholars have suggested that the number of macrophages in the peritoneal fluid of EMS model mice increase significantly, secreting a variety of inflammatory factors which promote the adhesion and invasion of the intima [16]. The results of the present study showed that the levels of IL-1, IL-2, IL-6 and TNF-α in the peritoneal fluid of the untreated control group were significantly higher than those in the control group. The COX-2 expression was strong in the untreated control group, and in the bazedoxifene group, the contents of IL-1, IL-2, IL-6 and TNF-α in the peritoneal fluid of rats were significantly lower than those in the untreated control group. The reduction in COX-2 content in the ectopic endometrium of the two treatment groups was statistically significant, suggesting that bazedoxifene can significantly reduce the degree of inflammation in EMS rats. The underlying mechanism may be that bazedoxifene directly antagonizes the ER, but does not affect the expression of PR during the treatment.

CONCLUSION

Bazedoxifene significantly reduces the levels of angiogenesis and inflammatory factors in serum and peritoneal fluid of EMS rats; it inhibits the expression of VEGFR2 and COX-2 in ectopic endometrium, and affects the formation of ectopic intimal neovascularization as well as alters the inflammatory environment of the abdominal cavity, thereby inhibiting the growth of ectopic endometrium. Thus, bazedoxifene may be suitable for the clinical management of EMS.

DECLARATIONS

Acknowledgement

We acknowledge the support received from the management of the institutional hospital.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors, all authors read and approved the manuscript for publication. Hao Xu conceived and designed the study, Hao Xu collected and analysed the data, Hao Xu wrote the manuscript.

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