Middle East Fertility Society Journal (2016) 21, 180–183

Middle East Fertility Society

Middle East Fertility Society Journal

www.mefsjournal.org
www.sciencedirect.com

ORIGINAL ARTICLE

The expression of vascular endothelial growth factor is affected by hypoxia inducible factor-1α in peritoneum of endometriosis mice treated with genistein

Sylvi Wafda Nur Amellia [a], **Yuyun Yueniwati** [b], **Sutrisno Sutrisno** [c,*]

[a] *Midwifery Master Study Programme, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia*
[b] *Department of Radiology, Saiful Anwar General Hospital, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia*
[c] *Division of Fertility, Endocrinology and Reproduction, Department of Obstetric and Ginaecology, Saiful Anwar General Hospital, Faculty of Medicine, Brawijaya University, Malang, East Java, Indonesia*

Received 30 May 2015; revised 9 December 2015; accepted 22 December 2015
Available online 10 February 2016

KEYWORDS

Hypoxia;
Growth factor;
Angiogenesis;
Peritoneal;
Endometriosis

Abstract This study aimed to investigate whether the genistein is able to decrease the expressions of vascular endothelial growth factor-A (VEGF-A) and hypoxia inducible factor-1α (HIF-1α) in mouse model of endometriosis. Forty female mice (*Mus musculus*) were divided into eight groups ($n = 5$ each), including the control (untreated) group, endometriosis group, and endometriosis groups treated with various doses of genistein (50; 100; 200; 300; 400; and 500 mg/day). VEGF-A and HIF-1α analyses were performed by immunohistochemistry. We found significant increases in the VEGF-A and HIF-α expressions in endometriosis group compared to the control group. The increased expressions of VEGF-A and HIF-1α were significantly ($p < 0.05$) attenuated by the administration of all doses of genistein. In conclusion, in mouse model of endometriosis, genistein potentially inhibits the increase in angiogenesis in peritoneal tissue. Therefore, this result may provide a novel anti-angiogenic treatment strategy for the therapy of endometriosis.

1. Introduction

Angiogenesis is a complex physiological process that is strictly controlled. Angiogenesis involves the proliferation and migration of endothelial cells. Angiogenesis plays a role in regeneration, tissue repair, and wound healing. Dysregulation of angiogenesis is a characteristic pathology of tumor metastasis, tumor neovascularization, vascular malformations, and various diseases. Angiogenesis involves the interaction between

* Corresponding author at: Jl. Jaksa Agung Suprapto, Malang, East Java, Indonesia.
E-mail addresses: habibah.vivie@gmail.com (S.W.N. Amellia), yyueniwati@yahoo.com (Y. Yueniwati), snospogk@gmail.com (S. Sutrisno).
Peer review under responsibility of Middle East Fertility Society.

ELSEVIER Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.mefs.2015.12.005

vascular endothelial growth factor (VEGF) and its receptors. A various physiological processes involving VEGF activity are the bone formation, hematopoiesis, wound healing, and also tissue growth and regeneration. The upregulation of VEGF is the presence in various tumors (1,2).

Endometriosis or growth of endometrial tissue outside the uterus is a disease in women of reproductive age (3). Pathogenesis of endometriosis has not been revealed clearly (4,5). However, the proposed mechanisms of endometriosis are vascular growth and development of endometriosis lesions (6,7). These indicate that treatment with angiogenesis inhibitor may be therapeutically beneficial (8). Several therapeutic approaches have been demonstrated in preclinical studies (9–12).

Genistein is a specific compound isolated from soy. Genistein has a broad spectrum of biological activity, such as anti-neoplastic, antiangiogenic, anticancer, and regeneration activities (2,13,14). Anti-angiogenic activity of genistein has been revealed in zebrafish larvae at a dose of 2 μM able to suppress the VEGF signaling by binding to VEGF receptor (15). The present study was performed to investigate the effect of genistein on angiogenesis signaling in mouse model of endometriosis.

2. Material and methods

2.1. Animal

Forty female mice (*Mus musculus*), aged 2–3 months, weighing 20–30 g were divided into eight groups, including the control (untreated) group, endometriosis group, and endometriosis groups administered with genistein (at doses of 50, 100, 200, 300, 400, and 500 mg/day). Mice were obtained from the Laboratory of Reproductive Physiology and Embryology, Faculty of Veterinary Medicine, Airlangga University, Surabaya. This study was conducted at the Laboratory of Reproductive Physiology and Embryology, Faculty of Veterinary Medicine, Airlangga University, Surabaya and Laboratory of Physiology, Faculty of Medicine, Brawijaya University, Malang.

2.2. Genistein treatment

Genistein (Tokyo Chemical Industries, Japan) was dissolved in sesame oil to achieve the desired dose (1 ml volume containing 1 g). Furthermore, genistein was administered orally for 14 days with an oral gavage. Genistein treatment was started after 14 days of endometriosis induction in mice (16,17).

2.3. Endometriosis induction

Mice model of endometriosis was made by myometrium and endometrium tissue implantation in immunodeficient mice according to our previous study (18).

2.4. Immunohistochemistry

The expressions of VEGF-A and HIF-1α in peritoneal tissue were examined according to previous protocol (19).

2.5. Statistical analysis

Data are presented as mean ± SD and the differences between groups were analyzed using one-way analysis of variance (ANOVA) with SPSS 15.0 statistical package for Windows. Only probability values of $P < 0.05$ were considered statistically significant and later subjected to Post hoc test.

3. Results

Table 1 presents the VEGF-A expression in the peritoneal tissue from each experimental group. The VEGF-A expression was significantly higher in the endometriosis group compared to the control group ($p < 0.05$). All doses of genistein significantly inhibited endometriosis-induced increase in VEGF-A expression. There were no significant differences between the genistein treated groups and the control group ($p > 0.05$).

Table 1 also presents the HIF-1α expression in the peritoneal tissue of all experimental groups. The HIF-1α expression was significantly greater in the endometriosis group compared to the control group. The increased expression of HIF-1α in endometriosis group was significantly reduced by the administration of all doses of genistein ($p < 0.05$), near to the expression in control group ($p > 0.05$). There were no significant differences between the genistein treated groups ($p > 0.05$).

4. Discussion

Endometriosis is a chronic and progressive disease, involving abnormal angiogenesis. Although the complete mechanism is not fully known, angiogenesis in this disorder involves the degradation of the whole organ, including blood vessels and the endothelial cells (20–22). In the present study, we found that the VEGF-A and HIF-1α expression in peritoneal tissue

Table 1 The expression of VEGF and HIF-1α in mice model of endometriosis treated with genistein.

Expression	Control	EM	EM + genistein treatment (mg/day)					
			50	100	200	300	400	500
VEGF (%)	0.90 ± 0.42	5.40 ± 1.70[a]	1.70 ± 1.24[b]	1.00 ± 0.99[b]	1.83 ± 0.75[b]	2.50 ± 0.66[b]	1.45 ± 0.96[b]	1.10 ± 0.53[b]
HIF-1α (%)	1.10 ± 1.40	5.40 ± 1.90[a]	1.98 ± 0.83[b]	1.90 ± 1.10[b]	0.95 ± 0.62[b]	0.75 ± 0.34[b]	1.00 ± 0.73[b]	0.65 ± 0.55[b]

Note: Data are presented as mean ± SD.

[a] $p < 0.05$; in comparison with control group.

[b] $p < 0.05$; in comparison with EM group; EM: endometriosis group; VEGF: vascular endothelial growth factor; HIF-1α: hypoxia inducible factor-1α; %: percentage; mg/day: milligram/day.