Journal of Traditional and Complementary Medicine xxx (2017) 1–4

Contents lists available at ScienceDirect

Journal of Traditional and Complementary Medicine

journal homepage: http://www.elsevier.com/locate/jtcme

Original Article

Genistein modulates the estrogen receptor and suppresses angiogenesis and inflammation in the murine model of peritoneal endometriosis

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ARTICLE INFO

Article history:
Received 13 August 2016
Received in revised form
13 March 2017
Accepted 28 March 2017
Available online xxx

Keywords:
Estrogen receptor
Growth factor
Inflammation
Angiogenesis
Peritoneum

ABSTRACT

The purpose of this study was to investigate the effect of genistein administration on the modulation of the estrogen receptor, inhibition of inflammation and angiogenesis in the murine model of peritoneal endometriosis. A total of thirty-six mice (*Mus musculus*) were divided into six groups (n = 6), including the control group, endometriosis group, endometriosis group treated with various doses of genistein (0.78; 1.04; 1.3 mg/day), and endometriosis group treated with leuprolide acetate (0.00975 mg/day every 5 days for 15 days). Analysis of estrogen receptor-α, estrogen receptor-β, TNF-α, IL-6, VEGF, and HIF-1α were performed immunohistochemically. Expression of estrogen receptor-α, estrogen receptor-β, TNF-α, IL-6, VEGF and HIF-1α increased significantly compared with the control group ($p < 0.05$). All doses of genistein decreased the expression of estrogen receptor-α, increased estrogen receptor-β, lowered VEGF and HIF-1α significantly compared with endometriosis group ($p > 0.05$). Genistein also decreased the expression of TNF-α and IL-6 (1.04 and 1.3 mg/day) compared with the endometriosis group, reaching level comparable to that of the control group ($p > 0.05$). It was concluded that genistein is able to modulate estrogen receptor-α and estrogen receptor-β and inhibit the development of inflammation and angiogenesis in the murine model of peritoneal endometriosis. Thus, genistein can be a candidate in the treatment of endometriosis.

1. Introduction

Endometriosis is the proliferation of endometrial tissue outside the uterine cavity. Until now, endometriosis is a gynecological disorder that often occurs among women at reproductive age (10%) or infertile women (20–50%).[1–5] Peritoneal environment seems to be a very important media in the development of endometriosis pathology. Estrogen receptor-β is one of the two nuclear receptors that mediates estrogen action. In the context of endometriosis, estrogen receptor-β is significantly higher (more than 100 times) in endometrial lesion than ectopic endometrium. It is thought to be caused by changes in gene promoter. This overexpression will lead

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Peer review under responsibility of The Center for Food and Biomolecules, National Taiwan University.

http://dx.doi.org/10.1016/j.jtcme.2017.03.002

to a decrease in the expression of estrogen receptor-α.[6–9] Furthermore, the ratio of estrogen receptor-β/estrogen receptor-α is very high in endometrial stromal cells associated with the increased inflammation.[10]

In addition to the estrogen receptor, a peritoneal fluid of endometriosis contains macrophages and other secreted products, including growth factors, cytokines, and angiogenic factors.[11,12] Vascular endothelial growth factor (VEGF) is an angiogenic factor, and it will trigger biological effects by binding to two receptors, namely VEGFR-1 or VEGFR-2.[13,14] VEGFR-2 binds to VEGF-A, VEGF-C and VEGF-D. VEGFR-2 signal is essential for vascular dilation, endothelial cell migration and cell proliferation.[15–18] Previous researches proved that VEGF was found to increase in peritoneal fluid of patients with endometriosis.[19–21]

Genistein has common name of 5,7-dihydroxy-3-(4-hydro xyphenyl)-4H-1-benzopyran-4-one. This compound is belonging to the group of isoflavones, heterocyclic polyphenols formed in plants.[22] Genistein is one of two main isoflavone aglycone which can be found in soybeans and it gets more attentions for two decades due to its roles in inhibiting hormones-related diseases.[23] Genistein is phytoestrogen which is frequently consumed and may influence cell functions through selective interaction with estrogen-β receptor in relation to co-activator and provokes downstream signal.[24] Genistein as one of the active substances of soy has various biological effects, such as an anti-neoplastic effect in various types of tumors through various mechanisms.[25,26] Previous researches also indicated that genistein has an anti-inflammatory and anti-angiogenic effects. Anti-inflammatory action of genistein is demonstrated in inhibiting the expression and release of pro-inflammatory cytokines induced by lipopolysaccharide.[27] Anti-angiogenic action of genistein is demonstrated *in vitro* and *in vivo* through the inhibitory ability of expression/excretion of VEGF.[28] As far as we know there is still controversy about genistein action against endometriosis. Therefore, this study aimed to determine whether genistein is able to modulate the estrogen receptor, inhibit inflammation and angiogenesis in the murine model of peritoneal endometriosis.

2. Materials and methods

2.1. Animals

This research used female, young adults mice (*Mus musculus*) (2–3 months of age), weighing 20–30 g, and never mating. Mice were purchased from experimental animal development unit at the Laboratory of Reproductive Physiology and Embryology, Faculty of Veterinary Medicine, University of Airlangga, Surabaya, East Java, Indonesia. A total of thirty-six female mice were divided into six groups, including the control group (no treatment), endometriosis model groups, endometriosis model group treated with genistein orally at doses of 0.78; 1.04; and 1.3 mg/day, and endometriosis model group treated with leuprolide acetate (0.00975 mg/5 days) as the standard drug. This research was conducted at the Laboratory of Molecular Physiology, Faculty of Medicine, University of Brawijaya, Malang, East Java, Indonesia.

2.2. Genistein administration

Genistein (Bioword, USA) in the form of powder was dissolved in sesame oil (1 ml volume of oil containing 1 g genistein). The genistein solution was administered to the mice orally with the sonde. The administration of this solution was started after 14 days of induction of endometriosis.[29,30] The genistein solution was administered once a day in the morning according to the dosage for 15 days.

2.3. Administration of leuprolide acetate

Leuprolide acetate is a synthetic analog drug known as GnRH (Tapros, Takeda Laboratories, Japan).[31] Leuprolide acetate was administered to mice intramuscularly every 5 days at a dose of 0.00975 mg/5 days to 15 days.

2.4. Development of endometriosis model

The myometrial and endometrial tissues were implanted in immunosuppressed mice to induce endometriosis. Immunodeficiency was induced by injection of cyclosporine A (0.2 ml/mouse as a single dose) intraperitoneally. Implant tissues were derived from patients with adenomyosis. Implant tissues in size of 1 cm^3 were washed twice at 300 rpm 4 °C. Supernatant of implant tissues was then sampled, and the residue of the tissue was supplemented with phosphate buffer saline. Products of implantation were implanted in the peritoneal cavity by injection (0.1 ml). After planting the implant, ethinyl estradiol (0.1 ml) was administered intramuscularly in the first and fifth day. The development of endometriosis in mice was observed for 14 days and categorized as a model of endometriosis.[32]

2.5. Peritoneal tissue sampling

After some treatments were done completely, the mice were sectioned for the removal of peritoneal tissues. Before being sectioned, the mice were administered with anesthetic diethyl ether. Peritoneal tissue samples were stored at −80 °C until they were analyzed.

2.6. Immunohistochemistry

The expressions of estrogen receptor-α, estrogen receptor-β, TNF-α, IL-6, VEGF and HIF-1α in peritoneal tissues were analyzed using immunohistochemical techniques in accordance with previous procedures.[33]

2.7. Ethics

This research has obtained an ethical approval from Faculty of Medicine of Brawijaya University, Malang, East Java, Indonesia. All methods in this research were conducted based on the relevant manual and regulations.

2.8. Statistical analysis

The expression was presented in mean ± SD. The differences between treatment groups were analyzed by a one-way analysis of variance (ANOVA) test. The analysis was performed using SPSS 15.0 statistical package for Windows. Probability value ($p < 0.05$) is considered as significantly different and this was continued with Least Significant Difference test.

3. Results

Table 1 shows the expression of estrogen receptor-α and estrogen receptor-β in several groups. Expression of estrogen receptor-α and estrogen receptor-β in the endometriosis group was significantly higher than the control group ($p < 0.05$). The administration of three doses of genistein decreased the expression of estrogen receptor-α, reaching a significant difference with endometriosis group ($p < 0.05$), reaching level comparable to the control group ($p > 0.05$). The expression of estrogen receptor-α among different doses of genistein was not significantly different

Please cite this article in press as: Sutrisno S, et al., Genistein modulates the estrogen receptor and suppresses angiogenesis and inflammation in the murine model of peritoneal endometriosis, Journal of Traditional and Complementary Medicine (2017), http://dx.doi.org/10.1016/j.jtcme.2017.03.002