International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.10, pp 612-617, 2017

Effect of Genistein, Leuprolide Acetate and Dienogest towards Progesterone Receptor, IL-8, MMP-2 Expression of Lesions in a Peritoneal Mice Model of Endometriosis

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Abstract : Endometriosis isestrogen-dependent disease that related to the inflammatory process in the peritoneal cavity. To being activated by pro-inflammatory cytokines, endometriosis is also influenced by the response of progesterone receptors. Effective treatments are required to reduce the endometriosis symptoms. The treatments should affordable, have minimal side effects, and can reduce the recurrence rate. Previous studies have stated that genistein, leuprolideacetate, and dienogest are able to induce the regression of endometriosis cells. The aims of the study is to identify the effect of genistein, leuprolide acetate and dienogest on the expression of the progesterone receptor, IL-8, and MMP-2 in a mice model of endometriosis. This study used a laboratory experimental research design with a post-test only control group design. Female mice (*Musmusculus*) were divided into 7 groups; 1 negative control group, 1 positive control group and 5 experimental groups: endometriosis miceadministered by various dose of genistein (0.78 mg/hr, 1.04mg/hr, and 1.30 mg/hr), leuprolide acetate, and dienogest. On day 30, mice were dislocated and the expression of PR, IL-8, and MMP-2 was assessed with IHK staining.The effect of genistein, leuprolide acetate, and dienogesttoward the expression of progesterone receptor, IL-8, and MMP-2 was assessed using one-way ANOVA. The significant effect was seen in the positive control group compared to the treatment group. The delivery of genistein, leuprolide acetate, and dienogest showed an increasing trend on progesterone receptor levels and decreased levels of receptor IL-8 and MMP-2 in the mice model of peritoneal endometriosis lesions.
Keywords : Progesterone receptor, interleukin 8, matrix metalloprotein 2, genistein, leuprolide acetate, dienogest, endometriosis.

Introduction

Endometriosis is defined as endometrial tissue dysfunction (glandula and stroma) located outside from the uterus[1,2]. Endometriosis is affecting 5-10% in women of reproductive age[1]. Endometriosis has been recognized as an estrogen-dependent disease, and is associated with genetic, endocrine, immunological, and

environmental factors in the formation and development of the disease. Exposure to uterine diethylstilbestrol, prolonged exposure to endogenous estrogen (due to early menarche, late menopause, or obesity), short menstrual cycles, low birth weight, and exposure to chemicals that interfere with endocrine function can be a risk factor for endometriosis. On microscopic examination, characteristics of endometriosis can be observed in the form of glands and endometrial stroma, with or without the former, and new bleeding in the form of erythrocytes, hemosiderin pigments, and macrophage cells containing hemosiderin. Symptoms that are often found in this disease include progressive lower abdominal pain that occurs during menstruation (dysmenorrhea), dyspareunia, painful defecation, menometrorrhagia (Dysfunctional Uterine Bleeding), and infertility.

The diagnosis of endometriosis is usually made on the basis of anamnesis and physical examination, ascertained by laparoscopy examination. The standard method of diagnosis for endometriosis is direct visualization through laparoscopy and histological examination. The latest trend in the treatment of endometriosis is to use non-hormonal drugs such as cytokine modulators, progesterone receptor modulators, anti-inflammatory drugs, and drugs which inhibit metalloproteinase matrices[3].

Under conditions of endometriosis, NK cell activity is decreasedthen followed by increased activated peritoneal macrophages indicates a disruption of phagocytic function[1]. This macrophage activation status will increase TNF secretion, facilitating attachment of the endometrium to the peritoneum. Activated lesions and macrophages will also secrete proinflammatory cytokines such as IL-8[1]. The progesterone receptor consists of two shapes (isoforms): RP-α and RP-β[4].

Genistein is one of the isoflavones that act as selective estrogen receptor modulators (SERMs), which have estrogenic and anti-estrogenic effects and can bind to estrogen receptors. Leuprolide acetate is a synthetic analognonapeptide of the agonist gonadotropin releasing hormone (GnRH) which is currently the gold standard for the treatment of endometriosis[5]. Dienogest is an oral progestin that has been systematically studied for the treatment of endometriosis[6]. Dienogest works as an anti-ovulatory, antiproliferative drug in endometrial cells, and has the effect of inhibiting the secretion of cytokines[7].

Experimental

The research design used experimental laboratories (true experimental design) with the research design *post-test only control group design*. The experimental animals used in this study were 2-3 months old female mice (*Musmusculus*) weighed 20-30 mg.Mice were randomly divided into 7 experimental group, including of 1 negative control group (healthy mice), 1 positive control group (mice model of endometriosis) and 5 treatment groups, which are groups of endometriosis mice administered genistein at various doses (0.78 mg, 1.04mg and 1.30 mg) referring to Schindler *et al.*[6,8], leuprolide acetate (0.00975mg), and dienogest (0.0052mg). The variables measured in this study were RP, IL-8, and MMP-2 expression in peripheral endometriosis lesions of an endometriosis model.

Mice were dislocated on 30th day of experiment. This endometriosis model refers to a study performed by Aoki[9]. The visual examination was performed to determine whether there were hypervascularization and peritoneal lesions. Immunohistochemical tests of ER-α and ER-β expression in peritoneal lesions were also performed.

The tests for the effects of genistein, leuprolide acetate and dienogest on progesterone receptor expression, IL-8 expression and MMP-2 expression using 1 control group (K), 3 genistein dose levels, 1 leuprolide acetate level, and 1 dienogest level were performed using ANOVA.

Result and Discussion

This study showed that there was a significant difference between the expression of progesterone receptors on the peritoneum of treated mice from the endometriosis model, and untreated mice (positive control group) (Figure 1.). This suggests that the administration of genistein, leuprolideacetate, and dienogest may increase the expression of the progesterone receptor on the peritoneum of the mice in endometriosis models. The group with the highest increase in progesterone receptor expression was the leuprolide acetate (P4) group. Leuprolide acetate therapy can inhibit the formation of endometriosis cells by binding to the pituitary GnRH

receptor[10,11]. In a study conducted by Mitchell *et al*.[12] patients administered with leuprolide acetate had increased levels of progesterone receptors, although this was statistically insignificant. In women with endometriosis, lower stromal expression was reported in ectopic endometrium compared with eutopic endometrium[13]. Progesterone receptor expression increases in ectopic endometrium only at the end of the secretion phase[5,14].

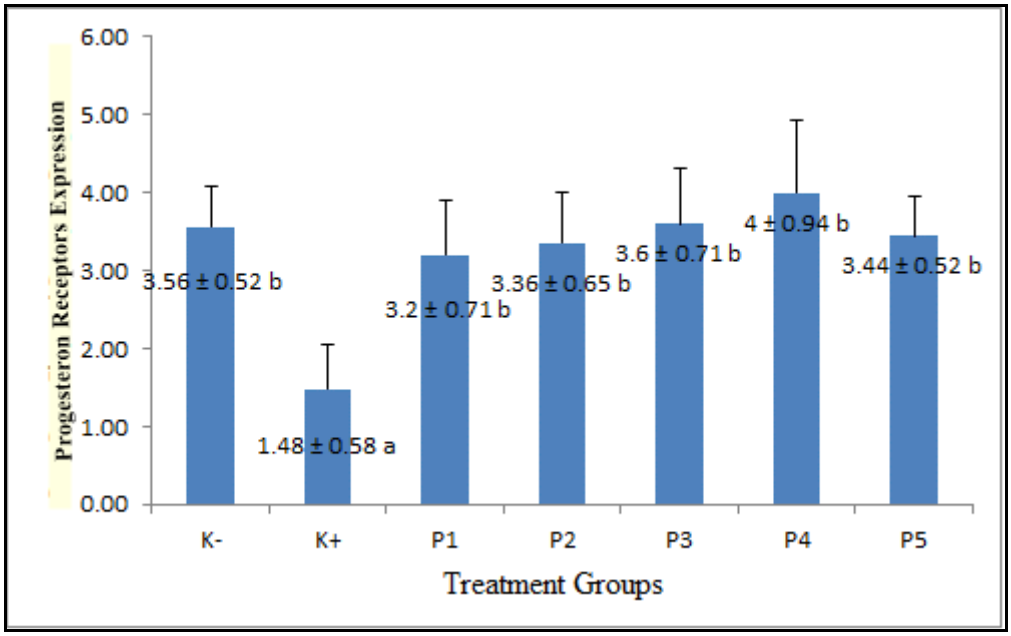

Figure 1.Mean Histogram of Progesterone Receptor Expression.

Dienogest is a selective progestin that combines 19-norprogestin and progesterone derivatives so that it only provides localized effects on endometrial tissue. The progestin in dienogest is able to inhibit the proliferation of endometrial stromal cells by increasing the expression of progesterone receptors and decreasing the production of estrogen (hypoestrogenic) hormones in the body[15]. The study by Hayashi *et al.* proves that dienogest is able to significantly increase progesterone receptor expression compared with the group that did not receive therapy[15]. The P3 group was treated with a genistein dose of 1.3 mg/day. Genistein therapy can inhibit the formation of endometriosis cells by binding to estrogen receptors[10,11]. Genistein is able to bind to estrogen receptors and active competitors with endogenous estrogens because they are structurally and functionally similar to 17β-estradiol[16]. This causes methylation of the progesterone receptor, preventing the inhibition of proteins[17].

The results of this study showed a significant difference between IL-8 expression in the peritoneum of treated mice and those without endometriosis treatment (positive control group) (Figure 2.). This suggests that the administration of genistein, leuprolide acetate, and dienogest may decrease IL-8 expression in the mice model of endometriosis. Endometrial cells were able to induce immune cells to emerge in the peritoneal cavity due to inflammation, especially macrophages. Increasing the number of endometriosis lesions contributes to the increase in estrogen-mediated macrophages. Estrogen will induce macrophage production and activate pro-inflammatory cytokines, such as IL-8, which will promote endometrial adhesion on the peritoneum and contribute to the development of endometriosis[18,19].In the cells of patients receiving GnRH therapy, the expression of IL-8 in endometriosis stromal cells was decreased. In the treatment group (P), it was found that the expression of IL-8 in each group decreased. The greatest decrease in IL-8 expression was in the group with the administration of a dose of 1.3 mg/day of genistein (P3). In hyperestrogenic conditions, such as endometriosis, genistein will act as an anti-estrogenic. Genistein may bind to RE because the genistein structure is similar to that of 17β-estradiol. These receptors function in assisting the transmission of hormones to the transcription of genes in the nuclei[13]. Dienogest is a selective progestin that combines 19-norprogestin and progesterone derivatives so that it only provides localized effects on endometrial tissue. The progestin in dienogest is able to inhibit the proliferation of endometrial stromal cells by increasing the expression of progesterone receptors and decreasing the production of the hormone estrogen (hypoestrogenic) in the body; thus, the activation of pro-inflammatory cytokines is reduced[15, 20].

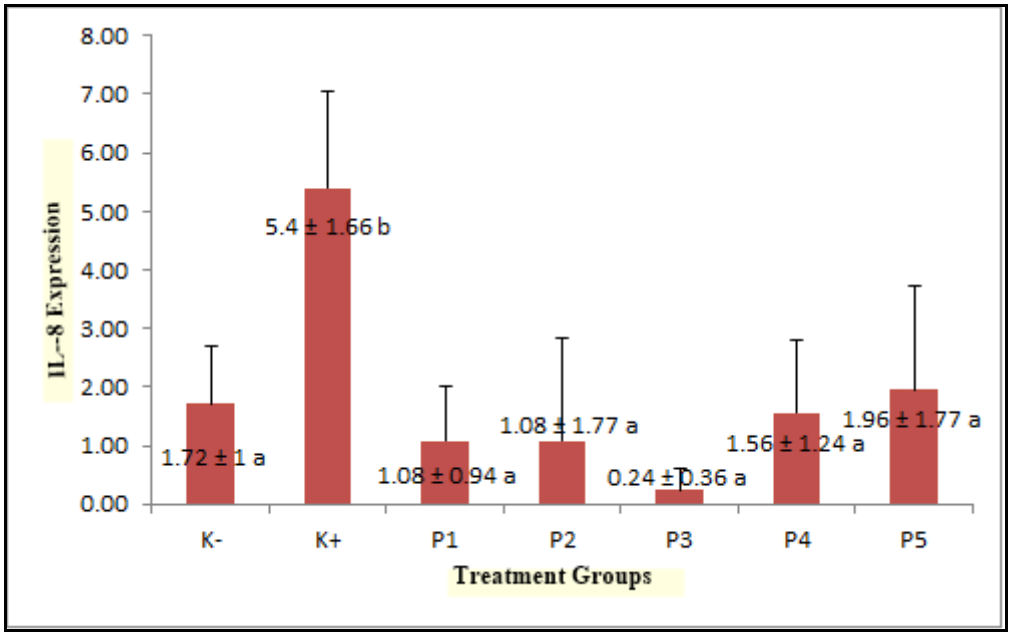

Figure2.Mean Histogram of IL-8 Expression.

Upon examination of peritoneal lesions in the mice model of endometriosis, there was a significant difference in MMP-2 expression between the treatment group (P) and the positive control group, with a decrease in MMP-2 expression in the treatment group (Figure 3.). This showed that administration of genistein, leuprolide acetate, and dienogest is able to decrease MMP-2 expression in the peritoneal lesion of the mice model of endometriosis. This is supported by research conducted by Harada*et al.*, who stated that progestin is able to suppress angiogenesis factor processes including MMP, VEGF-A, FGF in endometrial tissue grafted in mice[7]. Genistein was also used to suppress the progression of breast cancer by lowering MMP levels[21]. GnRH analogs were able to decrease MMP-9 expression in breast cancer cells. The lowest decrease in MMP-2 expression was found in the P4 group, which was the group treated with leuprolide acetate. GnRH (Gonadotropin releasing hormone) is synthesized in the hypothalamus and secreted to the pituitary via the hypothalamus-pituitary portal circulation pathway; this binds to the gonadotropin cell surface receptor in the anterior pituitary[13]. Meanwhile, the decrease in MMP-2 expression was strongest in the P3 group, which was the treatment group with the administration of genistein at a dose of 1.3 mg/day. Genistein binds to RE-β, which inhibits transcriptional activation in endometriosis cells to form MMP-2. Other studies have shown that genistein inhibits the expression of MMP-2 and MMP-9 in the mice model of endometriosis[22]. Similarly, the administration of Dienogest binding to progesterone receptors results in a hypoestrogenic condition that could reduce endometriosis cells from forming MMP-2[23].

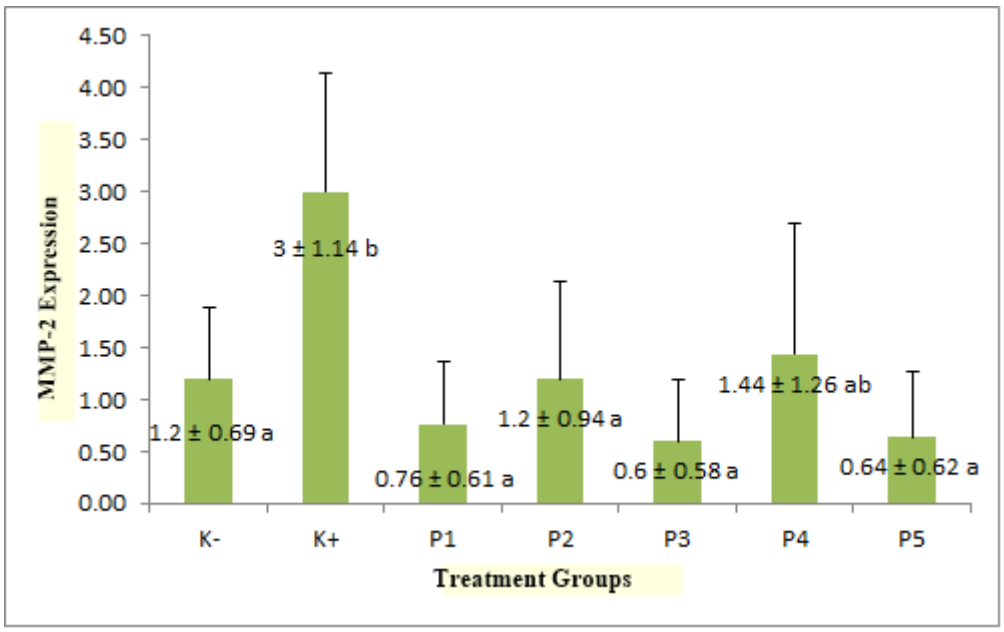

Figure 3. Mean Histogram of MMP-2 Expression.

The results of a correlation test between genistein dose and progesterone receptor expression were obtained, with a p-value less than 0.05 ($p < 0.05$), showing that there was a significant relationship between genistein dosage and progesterone receptor expression. The results of the correlation test between genistein dose and IL-8 expression has a p-value less than 0.05 ($p < 0.05$) showed that there was a significant relationship between genistein dose and IL-8 and MMP-2 expression. Comparison of each treatment group with various doses obtained a p-value of more than 0.05 ($p > 0.05$). This suggests that the administration of genistein, leuprolide acetate, and dienogest produce relatively equal progesterone receptor expression.

Based on the results of the study, the increased expression of the progesterone receptor and the decreased expression of IL-8 and MMP-2 fluctuated with increasing doses of Genistein.In this study, Genistein was administered at various doses in a mice model of endometriosis, but various doses of leuprolide acetate and dienogest were not administered.

Conclusion

1. Administering genistein, leuprolide acetate and dienogest may increase the expression of progesterone receptor in lesions in a peritoneal mice model of endometriosis
2. Administering genistein, leuprolide acetate and dienogest can decrease the expression of IL-8 in lesions a peritoneal mice model of endometriosis
3. Administering genistein, leuprolide acetate and dienogest decrease MMP-2 expression in lesions of the peritoneal mice model of endometriosis
4. Administering genistein, leuprolide acetate, or dienogest showed no significant difference in the expression of progesterone receptor, IL-8, and MMP-2 in lesions of the peritoneal mice model of endometriosis

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