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ORIGINAL ARTICLE

Comparison of the effect of isotretionin and alitretionin on endometriotic implants and serum vascular endothelial growth factor level: an experimental study

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ABSTRACT

Objective: To compare the effects of alitretionin and isotretionin on endometrial peritoneal implants and serum vascular endothelial growth factor (VEGF) levels.

Study design: Forty-eight female Sprague Dawley rats were used. Initially surgical rat endometriosis model was done. The endometrial implant volume was measured and rats were randomly divided into four groups. Group 1: Control group (rats did not get any drug but having endometriotic implants), group 2: rats receiving po isotretionin 10 mg/kg per day for 10 d, group 3: rats receiving po isotretionin 20 mg/kg per day for 10 d and group 4: rats receiving po alitretionin 80 mg/kg per day for 10 d. After 1-week medication, rats were sacrificed and size, histopathology of endometriotic implant and levels of VEGF were evaluated.

Results: Volumes of peritoneal endometrial implants were significantly decreased in Group 2 and Group 3 compared with initial values. However, there were no significant changes in histopathological scores and serum VEGF levels in all groups.

Conclusions: This study finding may suggest the possible medical treatment modality of isotretionin on endometriosis. However, alitretionin (potent retinoid) does not have potent regressive effect on endometriotic implants as in isotretionin.

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Alitretionin; endometriosis; isotretionin; retinoid; VEGF

Introduction

Angiogenesis plays an important role in the formation of endometriotic lesions in which there is an increased vascularization. VEGF (vascular endothelial growth factor) and soluble tyrosine kinase receptor have been suggested to have a role in the pathogenesis of endometriosis [1]. Women with endometriosis have high endometrial and peritoneal VEGF level, this may suggest the ethiopathogenic role of VEGF in endometriosis [2,3].

The therapeutic potential of retinoic acid on endometriosis has been recently reported [4,5]. However, the effect of alitretionin on endometriosis has not been known. Alitretionin has been widely used successfully in many refractory dermatologic conditions. It is a vitamin A analogue, inhibits VEGF production and has anti-proliferative, anti-inflammatory and immunomodulating properties [6]. Considering endometrial tissue implantation in the peritoneum and the role of angiogenesis in the development of endometriosis, and the four known property of alitretionin, we suggest alitretionin for the medical treatment of endometriosis. To our best of knowledge, there has been no reported studies noted the comparison of isotretionin and alitretionin therapeutic efficacy on endometriosis. In this experimental study, we aimed to compare the effects of alitretionin and isotretionin on endometrial peritoneal implants and serum VEGF levels.

Materials and methods

Animals

This study was conducted in the animal experiments skill development laboratory of Karadeniz Technical University Medical School. Approval of animal care and ethics committee from the same faculty was obtained (Date: 12 June 2013, registration number: 172235/402).

Forty-eight female Sprague Dawley rats (10–12 weeks old and 180–220 g of weight) were used. Rats were kept under controlled conditions with a 12-h of light and dark cycle and an environment with 22° C of temperature. Standard rat feed and water were given orally. All rats were housed for a week prior to the experiment in these environmental conditions.

Study protocol

Surgical procedures of endometriosis

First operation

Laparotomy (lower middle line incision of 3 cm) was performed following 50 mg/kg of intraperitoneal ketamine hydrochloride (Ketalar, Eczacibasi, Istanbul, Turkey) and 7 mg/kg of xylazine hydrochloride (Rompun, Bayer, Istanbul, Turkey) injections under aseptic conditions. One (DK) of the authors performed all

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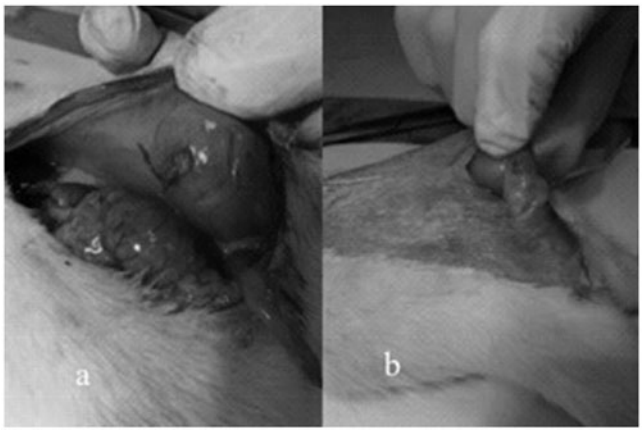

Figure 1 Rat model of endometriosis (a: abscess formation, b: endometriotic implant formation).

surgery procedures. Initially, uterine horn area (between tube and cervical region) was removed. Endometrium was dissected from the resected uterine horn and placed in saline solution. Then, the endometrial tissue was attached to the peritoneum of anterior abdominal wall where intense vascularization was observed with 4/0 absorbable suture (Polyglactin 910, Ethicon, Istanbul, Turkey) [7]. The abdomen was closed and rats were followed for 14 d without any additional drug treatment.

Second operation

Fourteen days after initial operation, second laparotomy was performed with the same technique to monitor the situation of endometrial implants and to make measurements of the implant site. The diameters of the focal implants in rats were measured *in vivo* and calculated by ellipsoid volume formula ($\pi/6 \times$ length $\times$ width $\times$ height). Following the second operation, 8 out of 48 rats were excluded (four of which were died and the other four due to the no development of endometriosis) from the study.

The remaining 40 rats were randomly divided into four groups each including 10 rats. Group 1: Control group (rats did not receive any drug but having endometriotic implants), group 2: rats receiving po isotretionin 10 mg/kg per day for 10 d, group 3: rats receiving po isotretionin 20 mg/kg per day for 10 d and group 4: rats receiving po alitretionin 80 mg/kg per day for 10 d. Selection of doses and days of administration were applied from previous studies [1,8]. Isotretionin and alitretionin were given for 10 d with orogastric tube.

Third operation

One week after completing 10 day of isotretionin/alitretionin treatment, third laparotomy procedure was carried out for all rats. Following the third operation, two animals from group 1, one from group 3 and three from group 4 were excluded from the study due to the development of abscess. The diameters of the endometriotic lesions in the remaining rats were individually measured and noted, the endometrial implants were excised and blood samples were taken from abdominal aorta for VEGF measurement. Following that, rats were euthanized by exsanguination (Figure 1(a,b)).

Histopathology and immunohistochemistry

Excised endometrial implants were stained with hematoxyline and eosin staining and examined under the light microscope. The presence of epithelial cells in endometrial implants were scored semi-quantitatively [9] (Figure 2).

Biochemical determination of VEGF

After centrifugation of blood samples at 300 *g* for 15 min, supernatants were collected and stored at − 80 °C and VEGF levels were measured quantitatively by commercially available ELISA kits (Rat VEGF Quantikine ELISA Kit Catalog number RRVoo, R&D Sytems Inc., USA).

Statistical analysis

The values of pre-treatment surface areas of the peritoneal endometrial implants were compared with the values following treatment. After the treatment, blood VEGF levels were compared among groups and histopathological scores obtained after staining with hematoxyline–eosin of endometriosis tissue were compared among groups.

Statistical analyses were performed using Statistical Package for the Social Sciences for windows, version 13.0. Kruskal–Wallis one-way variance analysis was used for the comparison. Wilcoxon sign test was performed for the comparison of volumes of endometrial implants before and after the treatment for each group. $p < .05$ was considered to be significant.

Results

Volumes of peritoneal endometrial implants were significantly decreased in Group 2 and Group 3 compared with initial values. Average volume changes of the endometrial implants before and after medical treatment (isotretionin or alitretionin) were found as from $39.3 \pm 2.3\,mm^3$ to $35.9 \pm 1.2\,mm^3$ in Group 2 ($p = .018$, Wilcoxon sign test); from $41.2 \pm 2.4\,mm^3$ to $29.6 \pm 1.2\,mm^3$ ($p = .012$, Wilcoxon sign test) in Group 3; from $39.3 \pm 3.1\,mm^3$ to $36.9 \pm 3.7\,mm^3$ ($p = .173$, Wilcoxon sign test) in Group 4; and from $39.9 \pm 3.2\,mm^3$ to $39.1 \pm 2.4\,mm^3$ ($p = .475$, Wilcoxon sign test) in Group 1.

Comparison of histopathological scores and serum VEGF levels are given in Table 1. VEGF levels were found to be higher in isotretinoin groups (groups 2 and 3) than those in alitretinoin and control groups ($p = .847$, Kruskal–Wallis variance analysis). There were also no statistically significant changes in histopathological scores among all groups ($p = .105$, Kruskal–Wallis variance analysis).

Discussion

The aim of conventional medical treatment for endometriosis is to relieve the symptoms associated with pain, by reducing the production of endogenous steroids. Danazol, medroxyprogesterone acetate, gestrinone, oral contraceptives and GnRH analogues are used to treat endometriosis; however, these treatments do not exhibit a positive development on infertility. Additionally, long-term usage of these agents is limited due to side effects [10].

Bevacizumab, an anti-angiogenic agent, was studied in rats for postoperative adhesions and observed to be beneficial to

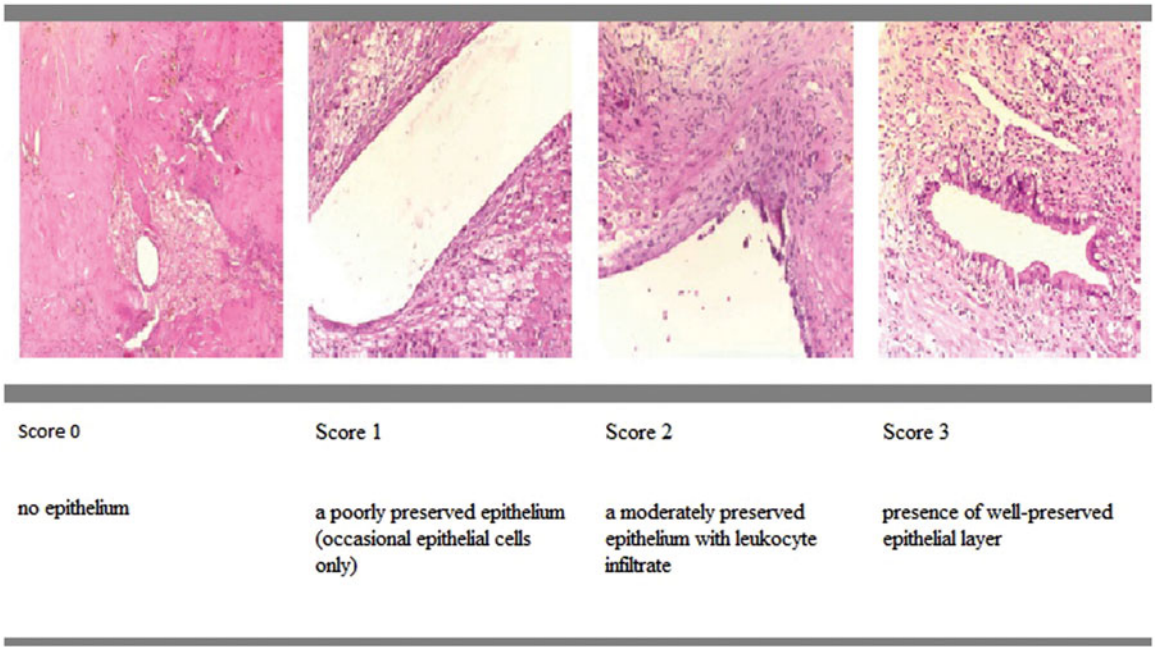

Figure 2 Histopathological scoring of endometriosis.

Table 1. Histopathological scores and serum VEGF levels in whole experimental groups (all values are given as mean ± SD).

Groups	Histopathological scoring[a]	Serum VEGF (pg/ml)[b]
1 (Control, $n = 10$)	1.44 ± 1.01	250.2 ± 14.9
2 (Isotretionin low dose, $n = 8$)	1.85 ± 1.21	252.4 ± 5.6
3 (Isotretionin high dose, $n = 9$)	1.50 ± 0.92	261.5 ± 50.5
4 (Alitretionin, $n = 7$)	1.66 ± 0.81	236.3 ± 5.6

[a] $p = .847$.
[b] $p = .105$, Kruskal–Wallis variance analysis.

reduce the adhesions and to increase the success of infertility treatment [11]. This effect is provided by VEGF inhibition. Therefore, it may be suggested that isotretionin (either in low or high dose) may have recovery effect on infertility by reducing adhesions by VEGF production inhibition.

Retinoic acid inhibited VEGF in HL-60 cells that were accepted as neutrophil-like cells in the endometrium. These neutrophils in the endometrium are rich sources for VEGF [12]. By considering this effect, we planned to suppress angiogenesis locally in the development of endometriosis. In our study, rats received isotretionin had been found to have smaller surface areas of endometriotic lesions, although a systemic VEGF reduction was not observed. This might be due to small sample size.

In endometriosis, the retinoic acid synthesis is impaired [5,13]. Retinoic acid inhibits development of endometriotic implants [14]. Retinoic acid treatment in endometriotic stromal cells induces autophagy and Beclin-1 expression [15]. Pavone et al. [16,17] stated that retinoid signaling affects decidualization in human endometriosis cell lines. Based on these scientific data, we believe that retinoic acid therapy (alitretionin, isotretionin) may inhibit the abnormal functions (inflammation, invasion, growth, differentiation) of endometriotic cells and treat all endometriosis-associated symptoms.

Our study is based on the principle of the regression of endometriotic lesions with isotretionin (in two different doses) and alitretionin, in accordance with the theory of retrograde menstruation. Unlike previous studies, we preferred to use blood VEGF levels instead of peritoneal fluid. There were no statistically significant differences between the groups in blood VEGF, although there was decreasing tendency in VEGF levels of alitretionin used group compared with control group. This can be resulted from the local effect of retinoic acid on angiogenesis in the cells of endometriotic lesion. In addition, low uptake of retinoic acid into these cells may also be the reason.

In the comparison of scores of endometriotic lesions among groups, no significant difference was found. This situation may be explained by the effect of retinoic acid causing the shrinkage of endometriotic lesion through angiogenesis but not creating a direct cytotoxic effect on endometrial cells. Resistance against isotretionin/alitretionin and other antiangiogenic factors may also be seen in the cells of endometriotic lesions [5].

Endometrial implant volumes before and after treatment were compared, a significant decrease occurred by isotretionin, but did not by alitretionin. Intracellular transport of retinoic acid derivatives and receptors may vary [4,5]. Therefore, they may act at different doses on different cells in the local domain. The unsuccessful effect of alitretionin on endometriosis may be related to this effect. New studies using different dose of alitretionin are needed.

Anti-angiogenic agents can be used in the treatment of endometriosis that is similar to the basic principles of cancer treatment. With the effects on VEGF, they may contribute to reduction of endometriotic lesions. Alitretionin, the new synthetic retinoid analog may not have effect on endometriosis-like isotretionin. It was concluded that isotretionin in low or high doses may give a new contribution to the treatment of endometriosis.

The limitations of the study are as follows:

a. The presented study was limited by its design as an experimental rat model, which may have limited applicability to humans.
b. The relatively small sample size.
c. We examined only serum VEGF levels, which was the important limitation. Further research regarding the effects of isotretionin on endometriotic implants would help us to establish a better understanding of how it acts and could provide more definitive evidence on the efficacy of isotretionin in the treatment of endometriosis.

Disclosure statement

No potential conflict of interests was reported by the author(s).

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