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Review Article

Angiogenesis and Antiangiogenic Treatment in Endometriosis

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Abstract

Endometriosis, defined as the presence of endometrial-like tissue outside the uterus, is associated with a chronic inflammatory reaction. Cellular proliferation, invasion, and neoangiogenesis are key to the establishment, progression, and recurrence of the disease.

Understanding the pathogenesis and the endocrinology of endometriosis allows for the improvement of the currently existing treatment options and the introduction of new treatments.

The ideal pharmacologic agent must fulfill the following criteria: 1. High effectiveness in blocking disease progression and symptoms 2. Ability to inhibit and suppress established lesions 3. High specificity, not interfering with physiologic processes in which the same molecules that are targeted in the treatment are involved 4. Demonstrated safety for long-term use in humans 5. High lingering effect in the inhibition of endometriotic lesions

Almost all currently available treatments of endometriosis are suppressive, not curative. They are associated with the temporary relief of symptoms during treatment. On treatment discontinuation, recurrence of the symptoms is the rule.

Keywords: *Endometriosis; Angiogenesis; Antiangiogenic Agents; Proinflammatory/Proangiogenic Cytokines; Anti-VEGF Antibodies; VEGFR Inhibitors; VEGF/VEGFR Pathway*

Abbreviations

VEGF: Vascular Endothelial Growth Factor; uPA: Urokinase-Type Plasminogen Activator; MMP-2 and -9: Matrix Metalloproteinase-2 and -9; KDR: Kinase Domain Receptor; VCAM-1: Vascular Cell Adhesion Molecule-1

Introduction

Endometriosis, one of the most common gynecological diseases, is defined as the presence of endometrial glands and stroma tissue outside the uterus. This disease affects 5% to 10% of women of reproductive age. However, in sub fertile women the prevalence seems to be considerably higher, ranging from 20% to 50%, but with significant variation over time periods and the age of patients and the risk of infertility. It is increased two-fold in women < 35 years with endometriosis compared with women without endometriosis. The presence of this ectopic tissue evokes an estrogen-dependent chronic inflammatory process. The causes of infertility in women with endometriosis

may range from anatomical distortions due to adhesions and fibrosis to endocrine abnormalities and immunological disturbances. The diagnosis currently requires direct visualization of the lesions under surgery. Traditionally both surgical and medical therapy have focused on alleviation of symptoms, prevention of disease progression, and promotion of fertility. In spite of significant developments in medical and surgical approaches, the optimal therapy for treating endometriosis-associated infertility has yet to be established [1].

It is widely known that angiogenesis plays a key role in endometriotic lesion formation and development [2].

This study explores the role of angiogenesis and the potential, limitations and challenges of antiangiogenic therapy for the treatment of endometriosis in terms of efficacy and risk of side effects. Antiangiogenic agents have recently emerged as promising drugs for targeting vessels in tumors, and their potential use in the context of endometriosis is discussed [3].

Several theories for its pathogenesis were proposed in recent years**,** such as implantation theory and angiogenesis. The presence of endometrial cells in the peritoneal cavity is explained by retrograde menstruation (Sampson, 1927), whereby menstrual tissue refluxes through the fallopian tubes and implants on pelvic structures. This mechanism has been consistently observed in humans and is supported by the anatomic distribution of implants of endometriotic tissue. Epithelial progenitor cells derived from the shedding of endometrial tissue can implant on the peritoneum, ovaries, or in the rectovaginal pouch. Once established, these hormone responsive and cyclically active endometriotic lesions drive acute then chronic inflammatory reactions, and lead to pelvic adhesions, pain, and infertility [4]. The phenomenon of retrograde menstruation commonly occurs in 90% of women undergoing laparoscopy. This theory does not explain the observation that reflux menstruation occurs in most women but the disease in only 5% to 10% of the female population.

Angiogenesis is a complex process involving a number of different but coordinated functions that must be undertaken before this process can be fulfilled. These include the proliferation, migration and extension of endothelial cells, adherence of these cells to extracellular matrix, remodeling of the extracellular matrix and ultimate formation of a new lumen [5]. Angiogenesis is important in the pathophysiology of endometriosis, a condition characterized by implantation of ectopic endometrium in the peritoneal cavity.

Angiogenic factors are increased in the peritoneal fluid of patients with endometriosis, in peritoneal implants, and in ovarian endometriomas. Although many angiogenic factors associated with endometriosis have been identified, the mechanisms underlying revascularization of endometriotic lesions remain largely unknown. It appears that angiogenic processes in endometriosis share common markers with tumor angiogenesis, since many factors overexpressed in endothelial cells from eutopic and ectopic endometrium of endometriosis patients, such as vascular endothelial growth factor (VEGF) receptor-2, endoglin, αvβ3 integrin, urokinase-type plasminogen activator (uPA), interleukin interleukin-8, matrix metalloproteinase-2 and -9 (MMP-2 and -9) and fibronectin, are also found in activated endothelial cells in tumors. Angiogenesis involves the interaction of a number of tightly regulated molecules including vascular endothelial growth factor (VEGF), which is recognized as a pivotal angiogenic factor [6].

An early event in angiogenesis consists of the extravasation of plasma and proteins through leaky blood vessels leading to the deposition of a fibrin-rich gel, which then promotes angiogenesis. This process is probably induced by hypoxia in the transplanted lesions, which leads to the expression of vascular endothelial growth factor (VEGF), one of the most potent and well-studied pro-angiogenic cytokines [7].

It has been shown that the subjects with a specific genotype in the VEGF gene polymorphism had a significantly increased risk of endometriosis than those without the genotype [8]. VEGF is a 23 - 45 kDa heparin binding glycoprotein with potent endothelial cell specific mitogenic and vascular permeability activities [9].

The VEGF family consists of five members and placental growth factor. Two principle VEGF receptor kinases have been identified. The VEGFR-1 and VEGFR-2, both bind VEGF family with high affinity. Binding of VEGF to one of several tyrosine kinase receptors triggers their autophosphorylation, resulting in the activation of mitogen activated protein kinases. VEGF receptors flt and KDR (kinase domain

receptor) were also detected, suggesting autocrine regulation. During the menstrual cycle, expression of flt was constant but that of KDR was increased in the luteal phase, at which time the cells migrated in response to VEGF. KDR expression and the migratory response were significantly higher in patients with endometriosis. VEGF-A has been identified in the luminal and glandular epithelium in the proliferative phase as well as the stroma, with the expression remaining in the epithelial cells but not in the stroma during the secretory phase [10]. VEGF-A increases the expression of vascular cell adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1) in endothelial cells and the promotion of monocyte chemotaxis and is capable of inducing vascular permeability [11].

This fact suggest that retrograde menstruation expresses VEGF-A, indeed menstrual effluent does contain high values of VEGF-A expressing glandular cells. The expression of VEGF-A in the menstrual effluent would suggest this tissue has the potential to undergo angiogenic blood vessel development and the abnormalities in this may contribute to the development of endometriosis [12].

VEGF mRNA and protein expression have also been detected in the eutopic endometrium and are known to play an important role in blood vessel formation during the menstrual cycle. VEGF mRNA and protein expression have been detected in endometriotic lesions and were reported to be greater in red lesions (active) than in black lesions, a finding associated with higher vascularization in active red lesions.

These results also suggest that before retrograde menstruation occurs, eutopic endometrial tissue already has the capacity to promote its implantation at ectopic sites, through the secretion of VEGF. The significantly higher microvessel density in the secretory phase eutopic endometrium from women with endometriosis, compared with women without endometriosis, was reflected by a significantly higher expression of VEGF-A in glandular epithelium and of VEGFR-2 in blood vessels as well as a higher level of VEGF-A in peritoneal fluid. The higher microvessel density in the secretory phase eutopic endometrium from women with endometriosis might be due to a higher production of VEGF-A within the eutopic endometrium or an extensive endometrial exposure of VEGF-A that is produced and contained in the peritoneal fluid.

Neovascularization of newly formed endometriotic lesions is likely to play a key role in their development and persistence, by providing them with nutrients and growth factors and promoting the recruitment of inflammatory cells. Early endometriotic lesions, which are considered to be the most active, are characterized by a high vascular density that gives them the typical pink-red appearance [12].

These lesions contain angiogenic vessels, as indicated by the high mitotic index and absence of α-smooth muscle actin (αSMA)-positive pericytes, as well as mature pericyte-covered vessels. There is a greater percentage of mature vessels in black endometriotic lesions, which are thought to be a later stage of lesion development. Ovarian endometriotic lesions are also strongly vascularized with a high proportion (84%) of immature pericyte-free vessels [13].

The ovarian vascular surface density index is higher in endometriotic than in follicular and luteal ovarian samples [14].

There are two possible ways by which peritoneal fluid VEGFA can reach the eutopic endometrium and stimulate endometrial angiogenesis: (1) by transport of peritoneal fluid via the uterine tubes to the uterine cavity and (2) through the local pelvic network of lymph and blood vessels. Ectopic endometrium showed a higher microvessel density in the proliferative phase, in contrast to the finding in eutopic endometrium. Since we found no differences in the expression of VEGF-A or its receptors (data not shown), or in the content of VEGF-A in peritoneal fluid or serum, between the proliferative and secretory menstrual phases, other reasons for this may need to be sought.

Possible explanatory mechanisms include the ectopic site of growth, the presence of endogenous aromatase activity in the endometriotic lesions and the continuous exposure to peritoneal fluid with its content of pro-inflammatory and pro-angiogenic substances, factors that might override or bias the control of proliferation and function that is normally exerted by the ovarian sex steroid hormones. It is believed, rather, that angiogenesis in eutopic endometrium occurs mainly through vessel elongation and to some extent also via intussusceptive microvascular growth and incorporation of circulating endothelial progenitor cells into the growing blood vessels. However, it is not known whether the same mechanisms are responsible for angiogenesis in ectopic endometrium. It has previously been shown that higher the concentration of VEGF in the peritoneal fluid is, the more advanced is the endometriosis and that the amount of vascularization in and around the endometriotic lesion correlates with the mitotic activity within the lesion.

VEGF are found in peritoneal fluid of patients with endometriosis. VEGF immunoreactivity was found in activated peritoneal fluid macrophages. Macrophage activation was highest in women with endometriosis, and media conditioned by peritoneal fluid macrophages from these women caused a VEGF-dependent increase in endothelial cell proliferation above that seen from normal women. Peritoneal fluid macrophages secreted VEGF in response to ovarian steroids, and this secretion was enhanced after activation with lipopolysaccharide. Peritoneal fluid macrophages expressed receptors for steroid hormones. Peritoneal macrophages and activated lymphocytes seem to play an integral role in the secretion of proinflammatory/proangiogenic cytokines. For example, in patients with endometriosis, interleukin-1β (IL-1β) is produced by activated macrophages and results in the increased expression of VEGF.

There is an imbalance between pro- and antiangiogenic growth factors in peritoneal fluid from endometriosis patients, and inflammatory cells such as activated macrophages produce proangiogenic factors, contributing to this proangiogenic milieu [15].

To date, endometriosis is hormonally treated, aimed at achieving a hypoestrogenic state. Hormonal therapy only suppresses symptoms but will not eradicate the ectopic implant. Moreover, there are significant side-effects. Long-term hormonal therapy, therefore, is not an attractive option. Alternatively, endometriosis can be treated surgically. Conservative surgery consists of ablation of endometriosis lesions, resulting in pain relief, but symptoms recur in time in majority of women. Radical surgery includes removal of uterus and/or ovaries, giving more permanent symptom relief, but resulting in the end of reproductive life. An effective therapeutic agent for endometriosis would be a compound that not only prevents the development of endometriosis lesions but would also be effective against the growth of established lesions.

Angiogenesis is now widely accepted to play a pivotal role in endometriosis. Antiangiogenic therapy has therefore been suggested as a novel therapeutic approach. Considering the characteristic of endometriosis, an ideal reagent should have followed these features: reducing or removing the ectopic lesion, reducing side effects, especially to the reproductive system; preventing recurrence.

Development of appropriate bioassays made possible the discovery and assessment of the agents that target endometriosis-associated angiogenesis. These compounds can be classified as either exclusive antiangiogenic agents, which only one known function is the suppression of angiogenesis, or inclusive agents that have anti-angiogenic activity associated with other functions [16].

Thanks to recent advances in the understanding of the mechanisms underlying the development of this multifactorial disease, new therapeutic strategies have been proposed and are currently being tested in experimental models**.** Antiangiogenic approaches, designed to prevent new vessel formation, have been the subject of growing interest during the last ten years**.** The aim of this review is to explore the potential, limitations and challenges of antiangiogenic therapy for the treatment of endometriosis in terms of efficacy and risk of side effects. Vascular-disrupting agents (VDAs) have recently emerged as promising drugs for targeting vessels in tumors, and their potential use in the context of endometriosis is discussed.

These include growth factor inhibitors, endogenous angiogenesis inhibitors, fumagillin analogues, statins, cyclo-oxygenase-2 inhibitors, phytochemical compounds, immunomodulators, dopamine agonists, peroxisome proliferator-activated receptor agonists, progestins, danazol and gonadotropin-releasing hormone (GnRH) agonists. However, clinical evidence for their efficacy in anti-angiogenic endometriosis therapy is still lacking.

Bevacizumab (Avastin) - a humanized monoclonal IgG1 antibody that specifically inhibits all major isoforms of human VEGF-A - received US FDA approval in February 2004 for the use in 5-fluorouracilbased regimens for treatment of metastatic colorectal cancer. Few years later, Ricci and colleagues tested bevacizumab against surgically induced endometriotic lesions of BALB/c mice and demonstrated that the experimental therapy significantly decreases VEGF-A peritoneal fluid level, cell proliferation within the lesions and their vascular density [17].

Endostatin, endogenous angiogenesis inhibitor, is derived from extracellular matrix molecules, which is formed in the body and have been shown to inhibit the development of new blood vessels, suppresses matrix metalloproteinase (MMP)-2, -9, -13 activity and blocks the binding of VEGF to its receptor [18].

In endometriosis models and/or patients, wide range of compounds, from hormones to anti-inflammatory and lipid lowering drugs have been found to interfere with both lesion growth and their neovascularization, as inclusive antiangiogenic agents. Suppressing the ovarian function, progestins, synthetic testosterone derivative danazol and gonadotropin releasing hormone (GnRH) agonists have been used in endometriosis patients for decades. There is evidence that these drugs possess anti-angiogenic activity as well. While estrogen increases VEGF-A expression, progesterone, dydrogesterone and dihydrodydrogesterone, such as danazol, reduce VEGF-A levels. Importantly, dienogest, an oral progestin, inhibited development and maturation of neovessels in a rat endometriosis model. Also, that leuprolide acetate, a GnRH agonist, reduces macrophage infiltration and microvessel density in lesions collected from endometriosis patients. Thus, beneficial mechanisms of progestins, danazol and GnRH agonists encompass their angiostatic activity [19].

Dopamine agonists, such as ergot-derived cabergoline may cause the regression of endometriotic lesions by inhibiting mitosis and VEGF-A-mediated angiogenesis. Dopamine agonists inhibit angiogenesis in an autocrine fashion by binding to the dopamine receptor D2 (DRD2) on endothelial cells that inactivates VEGFR -2. The mechanisms by which dopamine agonists (DAs) inhibit VEGF action and angiogenesis are not fully established. Two possibilities have been more extensively considered: [1] binding to the dopamine receptor-2 (Dp-r2), leading to the inhibition of the VEGFR-2 phosphorylation and downstream intracellular signaling(the presence of Dp-r2 in eutopic and ectopic endometrium is confirmed); [2] inhibited VEGF and VEGFR-2 expression [20].

The principal enzyme in the conversion of arachidonic acid to prostaglandins, cyclo-oxygenase 2 (COX-2), is over-expressed in endometriotic lesions and eutopic endometrium of affected women, seeming to play multiple roles in the pathogenesis of endometriosis. The third-generation aromatase inhibitors (AIs) anastrozole, letrozole, exemestane, and vorozole are more potent than the original aminoglutethimide and are more specific for the aromatase enzyme. In addition, the associated side effects (headache, nausea, and diarrhea) are fewer. By inhibiting aromatase activity, these AIs impair the conversion of androgens to estrogens, and, like danazol, suppress ovarian and local estrogen formation in endometriotic tissue.

Some COX-2 inhibitors suppress the endometriotic lesion growth, partially by the inhibition of angiogenesis. Since endometriosis-associated inflammation and angiogenesis are closely related processes, numerous studies have been focused on anti-inflammatory drugs and immunomodulators.

Pentoxifylline is another antiangiogenic agent. It is a methylxanthine with anti-inflammatory and antioxidant properties. It acts as a competitive nonselective phosphodiesterase inhibitor and decreases platelet aggregation. It is reported that pentoxifylline exerts an antiangiogenic effect on developing endometriotic lesions in rats [21].

Immunomodulatory agents including the cytokines IL-12 and IFN-γ-2b and two synthetic immunomodulators, the guanosine analogue loxoribine and the acetylcholine nicotinic receptor analogue levamisole have been suggested for the treatment of the disease. It has been shown to inhibit VEGF-stimulated endothelial proliferation and angiogenesis [22].

Histone Deacetylase Inhibitors-Accumulating evidence indicates that survival of endometriotic cells is mediated by down-regulation of genes involved in apoptosis. Aberrant methylation seems to take part in this process of gene silencing. Because demethylation agents and histone deacetylase inhibitors (HDACI) can reactivate the genes silenced by promoter hypermethylation, it has been proposed that HDACI might be used for treating endometriosis [23].

Progesterone receptor-binding molecules: Progesterone receptor modulators interact with the progesterone receptor to block or modify downstream effects. In premenopausal women, they induce secondary amenorrhea, accompanied by decreased systemic levels of

progesterone and estradiol. Modulators with potent progesterone antagonist activity, comprising the progesterone antagonist mifepristone (RU-486) and the selective progesterone receptor modulators (SPMRs) asoprisnil and CDB-4124 (a 21-substituted-19-norprogestin), have been proposed as therapeutic agents for endometriosis [23].

Danazol: The synthetic androgen 2,3-isoxazol (danazol), a derivative of 17a-ethynyl testosterone, has mild androgenic but strong antiestrogenic activity. It induces the inhibition of gonadotropin release, determines the competitive inhibition of steroidogenic enzymes, modulates immunologic function, and suppresses cell proliferation [23].

Conclusion

Anti-angiogenic compounds hold great promise for the future treatment of endometriosis because they may inhibit the establishment of new endometriotic lesions in early stages of the disease or after surgical treatment. In conclusion, although current medical treatments are helpful for many women with endometriosis, these treatments have limitations that include side effects in some women and contraceptive action for women desiring to conceive.

The present study reviewed relevant studies of endometriosis therapies that applied either anti-VEGF antibodies or VEGFR inhibitors using animal models of the disease.

Angiogenesis is mainly mediated by vascular endothelial growth factor (VEGF) and its receptor (VEGFR). Efforts to suppress angiogenesis have targeted the VEGF/VEGFR pathway through anti-VEGF antibodies and VEGFR inhibitors.

Endometriosis has a highly variable phenotype, and thus a wide variety of medical treatments targeting different pathways is likely to be important to move toward precision health (personalized medicine) in endometriosis.

Conflict of Interest

No financial interest and no conflict of interest.

Bibliography

1. Elsheikh A., *et al*. "Endometriosis and reproductive disorders". *Annals of the New York Academy of Sciences* 997 (2003): 247-254.

2. Abbas S., *et al*. "Prevalence and incidence of diagnosed endometriosis and risk of endometriosis in patients with endometriosis related symptoms: findings from a statutory health insurance based cohort in Germany". *uropean Journal of Obstetrics and Gynecology and Reproductive Biology* 160.1 (2012): 79-83.

3. Anne Van Langendonckt., *et al*. "Antiangiogenic and vascular-disrupting agents in endometriosis: Pitfalls and promise". *Molecular Human Reproduction* 14.5 (2008): 259-268.

4. Taylor RN and Lebovic DI. "Endometriosis". In: Strauss JF, Barbieri RL (eds). Yen and Jaffe's Reproductive Endocrinology", 7th edition. Philadelphia, PA: Elsevier Saunders (2014): 565-585.

5. Folkman J and Shing Y. "Angiogenesis". *Journal of Biological Chemistry* 267.19 (1992): 10931-10934.

6. Smith SK. "Regulation of angiogenesis in the endometrium". *Trends in Endocrinology and Metabolism* 12.4 (2001): 147-151.

7. Feng D., *et al*. "Different pathways of macromolecule extravasation from hyperpermeable tumor vessels". *Microvascular Research* 59.1 (2000): 24-37.

8. Sung HK., *et al*. "Vascular endothelial growth factor gene + 405 C/G polymorphism is associated with susceptibility to advanced stage endometriosis". *Human Reproduction* 20.10 (2005): 2904-2908.

9. Taylor RN., *et al*. "Angiogenic factors in endometriosis". *Annals of the New York Academy of Sciences* 955 (2002): 89-100.

10. Torry DS and Torry RJ. "Angiogenesis and the expression of vascular endothelial growth factor in endometrium and placenta". *American Journal of Reproductive Immunology* 37.1 (1997): 21-29.

11. Melder RJ., *et al*. "During angiogenesis vascular endothelial growth factor and basic growth factor regulate natural killer adhesion to tumor endothelium". *Nature Medicine* 2.9 (1996): 992-997.

12. McLaren J. "Vascular endothelial growth factor and endometriotic anguiogenesis". *Human Reproduction* 6.1 (2000): 45-55.

13. Hull ML., *et al*. "Antiangiogenic agents are effective inhibitors of endometriosis". *Journal of Clinical Endocrinology and Metabolism* 88.6 (2003): 2889-2899.

14. Inan S., *et al*. "Increased vascular surface density in ovarian endometriosis". *Gynecological Endocrinology* 17.2 (2003): 143-150.

15. Laschke MW., *et al*. "In vivo analysis of angiogenesis in endometriosis-like lesions by intravital fluorescence microscopy". *Fertility and Sterility* 84.2 (2005): 1199-1209.

16. Figg WD and Folkman MJ. "Angiogenesis: an integrative approach from science to medicine". New York: Springer (2008).

17. Ricci AG., *et al*. "Effect of vascular endothelial growth factor inhibition on endometrial implant development in a murine model of endometriosis". *Reproductive Sciences* 18.7 (2011): 614-622.

18. Kim YM., *et al*. "Endostatin blocks vascular endothelial growth factor-mediated signaling via direct interaction with KDR/ Flk-1". *Journal of Biological Chemistry* 277.31 (2002): 27872-27879.

19. Dusan Djokovic and Carlos Calhaz-Jorge. "Angiogenesis as a Therapeutic Target in Endometriosis". *Acta Médica Portuguesa* 27.4 (2014): 489-497.

20. Novella-Maestre E., *et al*. "Dopamine agonist administration causes a reduction in endometrial implants through modulation of angiogenesis in experimentally induced endometriosis". *Human Reproduction* 24.5 (2009): 1025-1035.

21. Lu D., *et al*. "Pentoxifylline for endometriosis". *Cochrane Database of Systematic Reviews* 1 (2012): CD007677.

22. Xu Z., *et al*. "Lipoxin A4 inhibits the development of endometriosis in mice: the role of antinflammation and anti-angiogenesis". *American Journal of Reproductive Immunology* 67.6 (2012): 491-497.

23. Kawano Y., *et al*. "Application of the histone deacetylase inhibitors for the treatment of endometriosis: histone modifications as pathogenesis and novel therapeutic target". *Human Reproduction* 26.9 (2011): 2486-2498.

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