Newer treatment modalities in endometriosis: systematic review

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Abstract

Endometriosis is a chronic, benign gynecological disorder seen in women of reproductive age. It is characterized by the presence of endometrial gland and stromal tissue outside the uterine cavity and intraperitoneal inflammation. In this article, we review the mechanistic basis of traditional established treatment modalities, focusing on efficacy and adverse effects and adequacy of response and compare them with the newer treatment modalities currently undergoing investigation in human and animal models, including aromatase inhibitors, antiangiogenic agents, tumor necrosis factor-α (TNF-α) blockers, matrix metalloproteinase (MMP) inhibitors, selective progesterone receptor modulators (SPRMs) and selective estrogen receptor modulators (SERMs). Several open-label studies show that aromatase inhibitors are effective in reducing lesion size and alleviating pelvic pain in patients with endometriosis refractory to other treatment modalities. Literature reviewed shows that the antiangiogenic agents seem to be more beneficial in treating early-stage disease, and some research has been done on vascular disrupting agents (VDAs) to potentially treat advanced endometriosis. Vascular disrupting agents, currently in phase I and II clinical trials for cancer treatments, operate by inducing apoptosis of endothelial cells in existing blood vessels. However, the literature reports that the VDA's may serve as an adjunct therapy following laparoscopic surgery. More research needs to be conducted to further evaluate the selective estrogen and progesterone receptor modulators, ligands, vascular growth factor inhibitors, and immunomodulators in the management of endometriosis.

Key words: aromatose inhibitors, antiangiogenic agents, matrix metalloproteinase inhibitors, tumor necrosis factor-α.

Introduction

Endometriosis is a chronic, benign gynecological disorder seen in women of reproductive age. It is characterized by the presence of endometrial gland and stromal tissue outside the uterine cavity and intraperitoneal inflammation. Endometriosis is estimated to affect 10 to 20% of women of reproductive age. It commonly presents with symptoms of chronic pelvic pain, severe dysmenorrhea, dyspareunia, and infertility [1-3]. These associated symptoms have a negative impact on the quality of life of women affected with the disorder. Endometriosis contributes to more than 100,000 hysterectomies worldwide each year [4]. Over the past few decades, the incidence of endometriosis in patients who present with either pelvic pain or infertility has been increasing and has been reported to be as high as 80% [5].

Although endometriosis is a major contributor to female infertility, studies are inconclusive in determining the exact pathophysiology of the disease, and it remains an open field of research. Strong evidence suggests retrograde menstruation is the keystone in the development of endometriosis. The classic theory of metaplasia of the coelomic epithelium continues to contribute to the pathogenesis of endometriosis. Recent studies postulate immunological factors and inflammatory mediators as having critical roles in the progression of the disease [6].

As various studies have improved our understanding of the pathophysiology behind endometriosis, advances in medical intervention have been made possible. Current treatment regimens are limited to hormonal drugs that suppress the menstrual cycle and activity of endometriotic lesions, with the aim of relieving pain and bleeding; however, adverse effects of these drugs decrease their compliance. Medical therapy is rarely encouraged in women wishing to conceive since treatment further enhances infertility [7]. Furthermore, recurrence of symptoms is very common during treatment-free intervals. As a result, focus remains on newer treatment approaches seeking to minimize the systemic adverse effects typical of traditional treatment modalities.

In this article, we review the mechanistic basis of traditional established treatment modalities, focusing on efficacy and adverse effects, and adequately comparing them to the newer treatment modalities currently undergoing studies, including aromatase inhibitors, antiangiogenic agents, tumor necrosis factor-α (TNF-α) blockers, matrix metalloproteinase (MMP) inhibitors, selective progesterone receptor modulators (SPRMs), and selective estrogen receptor modulators (SERMs).

Classical theories of endometriosis

Various theories have been set forth to explain the mechanisms behind the nebulous pathology of endometriosis. No single mechanism can encompass the in-depth pathogenesis of endometriosis. The existing circulating theories include Sampson's theory of retrograde menstruation, Meyer's coelomic metaplasia theory with the offshoot induction theory, the embryonic rest theory, and the lymphatic and vascular metastasis theories.

Sampson's theory remains the most widely accepted theory for the development of endometriosis. Sampson proposed that endometriosis is caused by retrograde menstruation of endometrial tissue that implants in the peritoneal cavity by traveling through patent fallopian tubes. The theory is based on three assumptions: (1) retrograde menstruation does occur through the fallopian tubes, (2) refluxed endometrial cells are viable in the pelvic cavity, and (3) refluxed endometrial cells are able to adhere to peritoneum with subsequent invasion, implantation and proliferation.

Numerous studies have confirmed the high incidence of retrograde menstruation; it is a common occurrence seen in 76 to 90% of women with patent fallopian tubes [8-10]. Retrograde menstruation plays a critical role in endometriosis development, as women with endometriosis have been found to have higher volumes of refluxed blood and endometrial tissue than women without the disease [8]. A number of studies also have demonstrated the viability of refluxed endometrial cells and their ability to implant at ectopic sites [11-13]. Implantation and invasion of endometrial tissue in the peritoneal cavity is further supported by a number of potential serum markers, including vascular endothelial growth factor (VEGF) for neoangiogenesis and matrix metalloproteinase (MMP) enzymes for invasion.

The coelomic metaplasia theory proposes that endometriosis is caused by metaplasia of the cells lining the pelvic endometrium [14-16]. Meyer developed this theory and proposed that endometriosis develops as a result of metaplasia induced by various stimuli, such as hormones and infectious agents [17, 18]. The theory is based on clinical evidence of endometriosis demonstrated in subjects that deviate from the usual demographic distribution; these include men, prepubertal and adolescent girls, and women who never menstruated. Further evidence to the coelomic metaplasia theory is the presence of endometriosis in unconventional anatomical locations such as the pleural cavity [19]. The evidence supporting this theory is highly disputed and remains inconclusive.

The induction theory is an offshoot of the coelomic metaplasia theory. It suggests that endogenous physiological conditions, whether biochemical or immune-related, can induce undifferentiated cells to differentiate into endometrial tissue [19]. Levander and Normann performed an animal study conferring this theory. Uterine wall sections from pregnant rabbits were inserted into subcutaneous tissue of rabbits stimulated with gonadotropins. Results demonstrated cells with endometrium characteristics and cyst formation in surrounding tissue [20]. Matsuura et al. provided further evidence consistent with the induction theory. In their study, ovarian surface epithelium was co-cultured with endometrial stromal cells and treated with a concentration of 17 β-estradiol 10 times higher that that seen in peritoneal fluid; *in vitro* coelomic metaplasia was demonstrated. The findings were consistent with induction of coelomic metaplasia being responsible for some cases of endometriosis [21].

The embryonic rest theory proposes that mullerian cell rests can differentiate into endometrial cells when activated by a specific stimulus. In rare cases, endometriosis has been reported in men. Proponents of this theory suggest that estrogen acts as a stimulus to induce transformation of embryonic rests [19].

The lymphatic and vascular metastasis theory propose endometriosis is a result of lymphatic and hematogenous dissemination of endometrial cells. This is a plausible explanation, as the lymphatic system anatomically communicates with distant structures such as the pleura, umbilicus, retroperitoneal space, lower extremity, vagina, and cervix [15, 22-24].

Substantial evidence is consistent with the theory of endometrial cells metastasizing *via* lymphatic and hematogenous pathways. Sampson's study provided additional evidence to this theory by demonstrating women with adenomyosis presented with endometrial tissue in uterine veins [25]. Further substantiating the hematogenous dissemination theory, Hobbs and Borthnick successfully induced pulmonary endometriosis by intravenously injecting endometrial tissue in rabbits [10].

Traditional treatment modalities

The aim of medical therapy for endometriosis is to relieve pain and bleeding. Traditionally, endometriosis has been managed medically with the following therapeutic agents: gonadotropin-releasing hormone (GnRH) agonists, progestins, and danazol. As the disease is chronic and often relapses, long-term or repeated courses of treatment is often required. Overall, the efficacy and adverse profiles of the different treatment modalities vary enormously.

Gonadotropin-releasing hormone agonists

Gonadotropin-releasing hormone agonists are highly effective in relieving pain associated with endometriosis. Upon administration, there is an initial increase in gonadotropins; subsequently, a downregulation. The result is the adverse hypoestrogenic side effect leading to a pronounced loss of bone mineral density and suppression of ovulation, thereby further enhancing infertility. Other symptoms produced include vasomotor symptoms, atrophic vagina, insomnia, mood disorders, and cognitive dysfunction [5].

Efficacy of GnRH agonists in relieving pain symptoms remains questionable. Patients are initially started on a 6-month course of these drugs. Clinical trials have indicated that the majority of patients respond with pain relief; however, recurrence of pain is common as well. A study conducted by Dlugi et al. [26] demonstrated 54% of patients with moderate to severe pain before treatment with GnRH agonists reported recurrence of pain within 6 months. Moreover, only 37% of patients experienced a symptom-free interval after one year of treatment. Various other studies have established similar results. Miller [27] demonstrated recurrence of pain in endometriosis patients being treated with GnRH agonists is experienced, on average, in 5.2 months. Of the patients experiencing recurrence, 60% respond to another 6-month course of GnRH agonists.

The primary concern in long-term administration of GnRH agonists is the hypoestrogenic side effect and subsequent progressive loss in bone mineral density (BMD) and other associated adverse symptoms. To reduce the adverse effects, add-back therapy has been introduced in conjunction with GnRH agonist administration or after several months of initiating treatment. The most commonly used add-back regimens include norethindrone and low-dose estrogen. Higher doses of estrogen supplementation results in diminished efficacy; patients continue to experience pain symptoms as well as breakthrough bleeding and dysmenorrhea. Progestin causes unwanted side effects of mood disorders and weight gain. Lower dosages of both estrogens and progestins have been attempted as add-back regimens; however, bone mineral loss is not prevented [5].

Progestins

Progestin provides an alternate therapeutic approach to managing endometriosis-associated pain. Subcutaneous medroxyprogesterone acetate (Depo-SubQ-Provera 104, Pfizer, New York, N.Y.) is administered every 12 to 14 weeks to relieve pain symptoms. This agent is similar to the Depo-Provera (Pfizer) contraceptive agent, yet contains fewer hormones. Ovulation is thereby suppressed, and cessation of treatment results in delayed ovulation resumption. Although efficacious in managing chronic pain seen in endometriosis patients, progestin further compromises fertility. However, as with GnRH agonists, the primary concern with progestin use is the potential loss of BMD. Although the efficacy of both treatment regimens is comparable in relieving pain symptoms associated with endometriosis, GnRH agonists have a higher degree of BMD loss. Studies demonstrate after 6 months of therapy with both agents, patients using Depo-SubQ-Provera 104 experienced recovery in bone loss at 12 months, whereas patients treated with GnRH agonists experienced persistent loss in BMD. Prolonged use of Depo-SubQ-Provera 104 is not recommended and further studies evaluating the effects of long-term Depo-SubQ-Provera 104 need to be conducted [5].

Additional progestin agents have been approved for managing endometriosis pain and have shown promising efficacy, i.e. medroxyprogesterone acetate. Side effects, however, include weight gain, mood changes and irregular bleeding. These adverse effects are not well tolerated and result in lower patient compliance.

Danazol

An additional efficacious agent in relieving pain associated with endometriosis is danazol, a 17-ethinyl-testosterone derivative. Danazol has demonstrated similar efficacy to GnRH agonists in relieving chronic pain symptoms associated with endometriosis. The adverse effects differ, however, and are associated with the androgen properties of the agent. Patients experience weight gain, edema, acne, hirsuitism, and myalgia. The lipid profile is negatively altered and liver enzymes are raised. Danazol further suppresses lutenizing hormone (LH) and follicle stimulating hormone (FSH), thus, inducing amenorrhea and enhancing infertility. During the 6-month danazol treatment regimen, recurrence of pain symptoms occurs with the same frequency as with GnRH agonists. The combination of adverse side effects and recurrence of pain makes this agent less tolerated amongst patients, and compliance is low [5].

Pathophysiology: basic components of endometriosis

Sampson's theory of retrograde menstruation continues to prevail as the most widely accepted theory of endometriosis development. The basic components of the disease process include the accepted notion of an intrinsically altered endometrium, invasion of the peritoneum, angiogenesis, and an immune response and inflammation.

Under normal physiologic conditions, apoptosis functions to eliminate senescent cells from the functional layer of the late secretory and menstrual endometrium. Bcl-2 and Fas/FasL systems function to regulate apoptosis activity; Bcl-2 diminishes apoptosis, whereas the Fas/FasL system promotes apoptosis. In patients with endometriosis, these regulatory mechanisms are disturbed and, consequently, apoptosis is decreased. The result is an environment abundant in viable regurgitated endometrial cells, incapable of being cleared.

The endometrium of patients with endometriosis has also demonstrated a decreased sensitivity to progesterone. Of concern is the expression of progesterone-responsive genes, particularly those involved with tissue remodeling. Matrix metalloproteinases (MMPs) are enzymes critical to tissue remodeling by mediating normal tissue turnover. In an environment of decreased progesterone sensitivity, MMPs are inappropriately expressed and alterations in the endometrium incur, namely tumor cell invasion of tissue [17, 18].

In the peritoneal environment of endometriosis patients, after endometrial cells are regurgitated and invade the peritoneal surface, the new implants must establish a blood supply for survival. This is accomplished by angiogenic factors. Angiogenic factors are present in the peritoneal fluid of 58% of patients with endometriosis [28]. Numerous components are responsible for VEGF production: activated peritoneal macrophages, endometrial cells and endometriotic lesions [29]. Vascular endothelial growth factor creates the blood supply essential to newly implanted endometrial cell survival. Increased levels of VEGF in peritoneal fluid have been documented.

Patients with endometriosis are documented not only to have intrinsic abnormalities in their endometrium, they also demonstrate dysfunctional immune systems. Normally, natural killer cells have cytotoxic properties and anti-tumor effects. In patients with endometriosis, intraperitoneal cytoxicity is diminished. On the contrary, T lymphocytes are elevated in peritoneal fluid of these patients. Furthermore, levels of macrophages are increased in the peritoneal environment. However, the phagocytic activity normally associated with macrophages is impaired [29]; instead the increased macrophages function to enhance cytokine secretion. The impaired clearance observed in macrophages of endometriosis patients is consistent with Sampson's proposed theory of implantation.

As a result of the increased level of macrophages seen in patients with endometriosis, cytokines and growth factors are found in higher concentrations. In endometriosis, cytokines promote implantation, proliferation, and survival of the endometriotic tissue; they mediate the clinical manifestation of the disease. Interleukin 1 (IL-1) stimulates VEGF and IL-6. Interleukin 6, although not consistently elevated in peritoneal fluid, contributes to endometriosis development by promoting endometrial proliferation and macrophage activator. Elevated IL-8 also has been demonstrated in peritoneal fluid and promotes implantation and growth of ectopic endometrium in the peritoneal cavity. It promotes attachment of endometrial cells to the peritoneal surface and subsequent invasion. Furthermore, high levels of RANTES (regulated on activation, normal T-cell expressed and secreted) have been observed in the peritoneal fluid of endometriosis patients. Studies demonstrate RANTES not only act as cytokine chemoattractants, but higher levels directly correlate with the stage of disease [30]. Finally, higher concentrations of TNF are seen in the peritoneal fluid as well and have

been documented as the most commonly elevated cytokine observed in endometriosis patients [31]. Tumor necrosis factor acts to enhance infertility by having deleterious effects on the embryo and sperm motility.

Newer treatment modalities

As our understanding of the pathophysiology of endometriosis continues to expand, advances in the medical management of endometriosis have been made possible. Newer treatment modalities are currently being evaluated with the objective of minimizing the adverse systemic effects seen with traditional treatment modalities. Furthermore, investigations continue to establish therapies targeted at preserving fertility. With the traditional treatment options available, medical therapy is rarely offered to women potentially wanting to conceive as the therapeutic agents enhance infertility. Endometriosis is a disease in women of reproductive age; therefore, therapy should be individualized according to the severity of the disease and each woman's wishes. Potential new therapies currently being evaluated include: aromatase inhibitors, antiangiogenic agents, TNF-α blockers, matrix metalloproteinase (MMP) inhibitors, selective progesterone receptor modulators (SPRMs), and selective estrogen receptor modulators (SERMs).

Aromatase inhibitors

The physiologic basis for the treatment of endometriosis with aromatase inhibitors is based on the estrogen dependency of the disease. Aromatase P450 converts androstenedione and testosterone to estrone and estradiol, respectively. Because the severity of the disease regresses with menopause and worsens with menstrual cycles, this enzyme represents a good pharmacological target not only in the sense that it reduces estrogen production, but also because it is the final step in estrogen biosynthesis so enzymatic steps upstream of the inhibition are not affected [32]. Anastrozole and letrozole, both third-generation agents, are the most commonly used aromatase inhibitors in clinical trials with these drugs. They competitively bind to the heme group of the cytochrome P450 subunit, resulting in inhibition of aromatase [33].

In light of recent studies that elucidate a positive feedback loop involving estradiol (E2) and prostaglandin (PGE2), reduction of estradiol levels is a particularly attractive potential treatment. Endometriotic implants express abnormally high levels of estradiol and prostaglandin, and we now know that PGE2 is one of the strongest promoters of aromatase activity. Upregulation of aromatase results in elevated levels of E2, which in turn increases activity of cyclooxygenase (COX-2) enzyme, the producer of PGE2 [34].

In addition to ovarian estradiol synthesis, there are several extraovarian sites of production, including the brain, skin and adipose tissue. While aromatase inhibitors can block extraovarian conversion of adrenal androgens to estrogens, they cannot completely inhibit ovarian production. Furthermore, as we have seen in the setting of reproductive medicine [35], aromatase inhibitors may actually stimulate the hypothalamic-pituitary-ovarian axis to output higher levels of FSH in the absence of estrogen's negative feedback on the hypothalamus and pituitary. This could result in increased follicular recruitment, ovarian hyperstimulation, and ovarian cysts. To avoid these side effects, some investigators reasoned that addition of a GnRH analogue to an aromatase inhibitor regimen would both help completely block estrogen production and also prevent reflex increases in FSH levels from the hypothalamic-pituitary-ovarian (HPO) axis due to aromatase inhibitor treatment.

Soysal et al. [36] performed a well-designed randomized trial comparing goserelin alone to a combination treatment of anastrozole and goserelin. The study demonstrated good power, as they recruited 80 premenopausal patients to participate in the study and randomized 40 into each group. Both groups reported 100% pain relief 6 months after the treatment period, but the anastrozole- plus-goserelin group significantly prolonged the median time to symptom recurrence to greater than 24 months, compared with 17 months for goserelin alone (P=0.0089). Immediately following treatment, the most notable side effect was a loss in bone mineral density (BMD) due to the induction of a doubly hypoestrogenic state with the combination treatment vs. goserelin alone (P=0.003), but 24 months after treatment, BMD levels were similar (P=0.46).

In a recent open-label study with 12 patients, Remorgida et al. [37] used a combination of letrozole and desogestrel for women with stage IV disease refractory to conventional treatments. None of the patients completed the study due to development of ovarian cysts, but immediately following treatment cessation, they reported reductions in presence of dymenorrhea (P<0.001) and deep dyspareunia (P=0.005).

Several open-label studies show that aromatase inhibitors are effective in reducing lesion size and alleviating pelvic pain. Ailawadi et al. [38] treated 10 premenopausal patients with letrozole and norethindrone acetate daily for 6 months, which resulted in a reduction of laparoscopically measured lesion size (P=0.0013) and of pain scores as measured by visual analog scale (VAS) (P<0.005). Amsterdam et al. [39] conducted a study on

15 premenopausal women using anastrozole and ethinyl estradiol/levonorgestrel daily for 6 months. Visual analog scale pain scores showed a statistically significant improvement after just one month of treatment (P=0.001) and 93% pain relief after the 6-month treatment period.

Although no long-term studies have been done on the effects of aromatase inhibitors in reproductive-aged women, the clinical trials reported thus far in the literature show that aromatase inhibitors are effective in treating patients with endometriosis refractory to conventional medical and surgical options.

Antiangiogenic agents

Ectopic endometrial tissue must establish blood supply in order to thrive outside the uterine cavity. Because antiangiogenic agents, such as Avastin (Genentech, San Francisco, Calif.) have been used clinically to inhibit the growth of cancerous tissue [40], research has been conducted to evaluate the potential of these agents for the treatment of endometriosis.

Women with endometriosis tend to have elevated levels of VEGF-A in their peritoneal fluid [41]. VEGF-A is a potent stimulator of angiogenesis that operates by signaling through VEGFR2, a kinase receptor. This ligand-receptor interaction results in migration, proliferation, and differentiation of endothelial cells that initiates the neova-scularization from existing blood vessels [41]. These elevated VEGF-A levels are most likely related to the abnormally high concentrations of estrogen and PGE2 also seen in endometriosis patients. Both of these molecules upregulate the expression of VEGF; in turn, VEGF positively regulates COX-2 production of PGE2 [41]. Additionally, activated macrophages in the peritoneum produce IL-1β that leads to the production of VEGF-A and other cytokines [42].

To date, no human clinical trials have been conducted using antiangiogenics to treat endometriosis, but several studies report interesting findings using the mouse model. Hull et al. used mice intraperitoneally injected with human endometrial fragments and initiated treatment directly after disease induction. After 9 days of subcutaneous injections, the group treated with sflt-1, a soluble competitive inhibitor of VEGF-A, demonstrated fewer endometriotic lesions compared with the control group (P=0.002) [42]. Administration of sflt-1 effectively inhibited the formation of lesions in the peritoneal cavity. In 2004, Nap et al. [43] used nude mice injected with human endometrial fragments, but waited three weeks after endometrial tissue introduction to begin treatment. According to these investigators, this study protocol more closely recreates endometriosis in humans because patients present with established disease, whereas Hull et al. initiated treatment before the tissue could implant. After 2 weeks of treatment, mice treated with humanized antibody against VEGF (anti-hVEGF), TNP-470, endostatin, and anginex all showed reduced number of lesions compared with control mice (P<0.05) [43]. More specifically, those treated with anti-hVEGF, endostatin and anginex displayed reduced microvessel density (P<0.05) and fewer new vessels formed (P<0.05), but no change in mature vessel number. In the case of established disease, anti-hVEGF, endostatin, and anginex effectively impaired the formation of new vessel formation, but did not affect the number of mature vessels.

The Hull and Nap studies imply these antiangiogenic agents would not be likely candidates for single-agent therapy as they are able only to block new vessel formation and not induce regression of established vessels. However, Nap et al. point out that they represent a potential adjuvant therapy after laparoscopic lesion removal to prevent disease recurrence [43].

While the aforementioned antiangiogenic agents seem to be more beneficial in treating early-stage disease, some research has been done on vascular disrupting agents (VDAs) to potentially treat advanced endometriosis. Vascular disrupting agents, currently in phase I and II clinical trials for cancer treatments, operate by inducing apoptosis of endothelial cells in existing blood vessels [44]. In theory, elimination of the blood supply would cause ischemia and necrosis of the endometriotic lesions. A small-molecule VDA called DMXAA, which depends on local production of TNF-α, induces apoptosis in endothelial cells compared to other VDAs that impair tubulin function in rapidly proliferation tissues [44]. Ligand-based VDAs can target either inflammatory molecules that are overexpressed in endometriosis or specific molecules on the surface of endometriotic tissue. For example, deep-infiltrating endometriosis has abnormally high expression of I-CAM and selectin molecules to attract leukocytes. Eniola and Hammer proposed using microspheres coated with selectin ligand, antibody against I-CAM, and an anti-inflammatory drug to selectively target inflamed tissue [45].

Because angiogenesis plays a role in normal female reproductive function in the proliferative endometrium during the menstrual cycle and embryo implantation during early pregnancy, human clinical trials must be conducted to ensure these treatments do not upset the physiological balance between proangiogenic and antiangiogenic factors, especially if fertility is to be preserved [44].

Tumor necrosis factor-α

Previous experiments have demonstrated abnormally elevated levels of tumor necrosis factor-α

(TNF-α) in the peritoneal fluid, peripheral blood, and endometrial tissue of women with endometriosis compared to women without endometriosis [46, 47]. TNF-α appears to play an important signaling role in the inflammatory cascade by inducing expression of IL-8 and RANTES, which in turn recruit T cells, macrophages, and eosinophils [48]. Moreover, TNF-α functions to upregulate the expression of matrix metalloproteinases and other inflammatory cytokines integral to endometriotic tissue invasion and angiogenesis [49]. The level of TNF-α also varies directly with the severity of the disease. Thus far, rat and baboon model studies have shown that targeting TNF-α signaling may represent a viable point of inhibition in the pathogenesis of endome-triosis.

Animal studies using recombinant human TNF-binding protein-1 (r-hTBP-1) and monoclonal antibodies against TNF-α demonstrated significant reductions in lesion and rAFS score. D'Antonio et al. tested this protein in rats with surgically induced disease and found a 64% reduction in lesion size 9 days after treatment ($P<0.05$) [50]. Using the established baboon model, D'Hooghe et al. reported r-hTBP-1 to be as effective as the GnRH antagonist antide in reducing rAFS scores ($P=0.002$) and lesion size ($P=0.003$) compared with control [30]. In addition to finding reduced surface area (by 32%) and volume (by 44%), Falconer et al. also observed a reduction in active red lesion surface area, volume, and number in baboons with induced endometriosis treated with c5N monoclonal anti-TNF-α antibody ($P<0.05$) [48].

While studies have found anti-TNF-α agents effective in reducing lesion size, specifically red lesion size, their effect on established lesions is not as promising. In a study on baboons with spontaneous endometriosis, Barrier et al. observed that treatment with the recombinant fusion protein TNF-α scavenger etanercept resulted in red lesion surface area reduction by 69% compared with placebo ($P=0.018$) [49]. However, no statistically significant change was observed in healed/latent white or established black lesions, which may suggest that treatment with etanercept would not be effective in the case of established lesions.

To date, no human trials have been conducted using anti-TNF-α agents. One case reported on a 35-year-old G0P0 woman with rheumatic arthritis and comorbid infertility due to stage IV endometriosis [46]. Despite four years of continuous etanercept treatment for arthritis and another previous four-year treatment with leflumide, the patient had an American Fertility Society (AFS) score of 42 at her initial laparoscopic diagnosis. Despite evidence in the baboon model showing the effectiveness of recombinant TNF-binding proteins in reducing lesion size and AFS score, the patient's AFS score did not improve upon second laparoscopy even after increasing the doses of etanercept. In accordance with Barrier's observation that etanercept did not change the size of white or black lesions, Shakiba and Falcone noted that treatment with etanercept was not effective in reducing lesion number in this patient with advanced disease. However, they could not rule out its potential use in the treatment of early disease, as afore-mentioned studies have demonstrated anti-TNF-α agents effective in reducing active lesion size [46].

Of particular interest, after eight years of anti-TNF-α treatment and subsequently increasing her dose of etanercept, the patient from the case study was able to conceive on her first *in vitro* fertilization (IVF) cycle (may need to cite some studies on the success rates of IVF with stage endo). Elevated levels of TNF-α are associated with infertility, although the exact relationship between has yet to be elucidated. TNF-α may have negative effects on sperm motility, function and/or development [46]. Although not able to conceive naturally, it is likely that the etanercept treatment played some role in the ability of the patient to conceive on the first IVF cycle. Thus, anti-TNF agents may have a potential usage in infertility treatment if not the treatment of endometriosis.

Despite promising results in animal studies demonstrating the ability to reduce lesion size and number, treatment with immunomodulators may not be as effective in humans because it is unclear whether the inflammatory cascade is a cause or a product of the disease [46]. These agents are unlikely to be potential treatments in patients with established disease, but more studies need to be performed in human trials to determine whether they can be used in early stages of endometriosis and for infertility treatments.

Matrix metalloproteinase inhibitors

Under normal physiological conditions, the endometrium tissue undergoes extracellular matrix (ECM) remodeling. Matrix metalloproteinases are proteolytic enzymes that function to remodel and degrade the ECM. To ensure proper physiological functioning, MMPs are under tight regulatory control. Studies have demonstrated that disturbances in MMP regulation contribute to the development of endometriosis [31, 51].

Matrix metalloproteinases have two forms: latent and active. Matrix metalloproteinases are secreted in the latent, pro-enzyme form. The latent enzyme undergoes a thiol linkage cleavage to produce the active form. Regulation of the active forms of MMPs is accomplished by binding with tissue inhibitors of MMPs (TIMPs); TIMP inhibits the majority of MMPs [52, 53]. A MMP-TIMP balance needs to persist; an imbalance has been shown to

be associated with rheumatoid arthritis, gastric ulceration, and endometriosis [54-56]. In particular, studies demonstrate that MMP-9 levels, a specific active enzyme, are increased in eutopic endometrium of women with endometriosis [57]. As a result, investigations focus on inhibiting MMPs as pharmacological targets in managing endometriosis.

Matrix metalloproteinase activity can be further controlled by oxidative stress (OS). Reactive oxygen species (ROS) are responsible for converting MMPs from the latent to active form, in particular MMP-2 and MMP-9. Numerous studies have documented the role of ROS in increasing growth and adhesion of endometrial cells in peritoneal cavity of women with endometriosis [58, 59]. Furthermore, rabbit studies have demonstrated the use of antioxidant enzymes as a protective factor in preventing endometriosis development [60]. Consequently, studies aim to evaluate the role of antioxidants in maintaining an oxidant stress-free environment, thereby decreasing MMP activity.

Melatonin is a documented antioxidant and serves to protect damage from free radicals. Paul et al. conducted a novel study in mice to demonstrate the antioxidant role of melatonin in healing endometriosis. In the study, endometriotic tissue samples were collected from women undergoing laparoscopy for infertility or pelvic pain. The samples were then categorized into the following groups: (1) severe endometriosis (stage III/IV), (2) moderate endometriosis (stage II), and (3) mild endometriosis (stage I). Eutopic endometrium from normal women served as the control group [61].

Using these samples, mice were induced with peritoneal endometriosis. Post-induction, mice were killed on days 7, 15, and 21; control mice were killed immediately after induction. Tissue homogenate was created from both human tissue samples and peritoneal endometriosis in mice. These were used to measure the levels of thiobarbituric acid-reactive species (TBARS) as an indicator of lipid peroxidation and protein carbonyl content as an indicator for protein oxidation. Finally, an assay of MMP-9 activity was measured [61].

Results of the study demonstrated the levels of secretory pro-MMP-9 increased gradually in mice with peritoneal endometriosis from the 7th day onwards until day 21 when compared with the control group. Likewise, synthesized pro-MMP-9 activity elevated as time progressed; a 10-fold increase was seen on day 15 and a 14-fold increase on day 21. The results strongly correlated duration of disease and pro-MMP-9 activity, at the level of secretion and synthesis [61].

As discussed, TIMPs are responsible for regulation of MMPs by means of inhibition. The study further established this relationship by analyzing the activity of MMP-9 and TIMP-1. Mouse endometriotic tissue exhibited a decreased TIMP-1 expression with time. The corresponding upregulation of pro-MMP-9 activity supports the regulatory role of TIMPs on MMPs [61].

To assess the relationship between pro-MMP-9 and severity of endometriosis, human endometriotic biopsies were examined. The three different categorized groups containing mild, moderate, and severe endometriosis were assessed for pro-MMP-9 activity. Results demonstrated a gradual increase in pro-MMP-9 activity (secreted and synthesized) from mild to severe endometriosis compared to the control group. Based on the results, a severity dependent upregulation in pro-MMP-9 activity was suggested. Furthermore, the expression ratio of pro-MMP-9 vs. TIMP-1 can serve as a marker for assessing severity and progression of endometriosis [61].

As mentioned, OS has been documented to play a critical role in the development of endometriosis. The role was analyzed in the study by measuring protein oxidation and lipid peroxidation in control, endometriotic, and melatonin-pretreated endometriotic mouse tissues. Results showed significant increase of protein carbonylation and lipid peroxidation in endometriotic tissue compared to control group. Similarly, the same observation was seen in human endometriotic tissue with moderate and severe endometriosis [61].

Melatonin has been documented as having antioxidant activity. In endometriotic tissue, melatonin inhibits protein oxidation by 80% and lipid peroxidation by 90%. This suggests that melatonin serves as a protective mechanism against oxidative stress. Since OS enhances the activity of MMPs, the study observed the role of melatonin on pro-MMP-9 activity; melatonin downregulated the activity and expression of pro-MMP-9. For the first time, the study revealed melatonin's protective role in arresting peritoneal endometriosis, warranting future studies on melatonin's role in endometriotic lesion regression *via* MMP regulation [61].

Nap et al. [62] conducted a study on a chicken chorioallantoic membrane (CAM) model to further suggest the role of MMP in endometriosis development. Endometrium was transplanted onto CAM, resulting in endometriosis-like formation. The process required extensive tissue remodeling conducted by MMPs. Expression of MMPs was evaluated in menstrual endometrium, endometriosis-like lesions in CAMs, in peritoneal endometriosis, and in endometriosis in the rectovaginal space. Furthermore, the role of MMP in early lesion formation in the CAM model was studied. The results of the study demonstrated the presence of MMPs in all studied tissue samples, CAM models and human

endometriosis alike. Moreover, the study demonstrated impaired endometriosis-like lesion formation in CAMs in the presence of MMP inhibition. The results of the study demonstrated similarities in MMP expression observed in experimentally induced endometriosis in CAMs and human endometriosis; therefore the CAM model served as a suitable model to study MMP expression. It is suggested that since endometriosis-like lesion formation is prevented by inhibiting MMPs in CAMs, MMPs have a critical function in early development of endometriotic lesions [62].

Matrix metalloproteinases expression and regulation need further evaluation in defining its precise role in the development of endometriosis. A relationship needs to be established to focus efforts on creating new avenues for development of potential treatment modalities for endometriosis.

Selective progesterone receptor modulators

Progesterone has a critical role in female reproduction. Under normal physiologic circumstances, progesterone is involved in control of ovulation, prepares the endometrium for implantation, regulates the process of implantation, and maintains the later stages of pregnancy by suppressing uterine contractility [63]. The multiple roles of progesterone in the female reproduction system have led to studies evaluating the pharmacological applications of progesterone. Numerous compounds have been synthesized containing a wide array of properties: agonists (progestins), antagonist, and mixed agonists/antagonists.

Synthesis of progestin compounds is marked as an important medical breakthrough, especially in the development of oral contraceptives. Progestins are used in numerous gynecological conditions, including endometriosis, due to their antiproliferative effects on the endometrium. However, chronic use is associated with adverse side effects: mood change, depression, bloating, and breakthrough bleeding; these notably limit their use [64].

Research in the potential use of progesterone as pharmacological agents heightened worldwide after the synthesis of mifepristone, the first glucocorticoid and progesterone receptor (PR) antagonist [65]. Initially, progesterone antagonist (PA) use was indicated in fertility control and treatment of breast cancer. With time, continued research led to the development of modified PAs, more specific to the progesterone receptor [66-72]. Although PAs showed promising potential in treating gynecological disorders, especially endometriosis, PAs continued to be indicated for termination of pregnancy and post-coital contraception [73, 74].

Unopposed estrogenic properties proved to be the most notable adverse effect preventing use of PAs in endometriosis [75, 76]. Selective progesterone receptor modulators (SPRMs) have the potential to provide the beneficial effects of progestins and PAs in endometriosis patients while avoiding this adverse effect. Selective progesterone receptor modulators are characterized as a class of PR ligands providing clinically relative tissue-selective progesterone agonist, antagonist, or mixed agonist/antagonist effects on various progesterone target tissues [77]. Asoprisnil is the first SPRM to reach an advanced stage of clinical development for treatment of women with endometriosis.

In assessing the available treatment options for endometriosis, SPRMS are more remarkable than traditional treatments due to greater efficacy and flexibility. Current traditional medical therapies used in endometriosis produce serious side-effects, namely a hypoestrogenic environment and breakthrough bleeding. In regards to endometriosis treatment, SPRMs demonstrate the following properties and thereby ameliorate these undesirable side effects: (1) selective inhibition of endometrial proliferation without systemic side effects of estrogen deprivation and (2) reversible suppression of endometrial bleeding *via* a direct effect on endometrial vessels [78].

Moreover, SPRMs, along with all other treatment modalities used in endometriosis, focus primarily on alleviating endometriosis-associated pain. It is believed that endometriosis-associated pain is caused by a local inflammatory reaction in response to recurrent bleeding from ectopic implants [79]. The pain is a result of prostaglandin production. Numerous studies demonstrate increased levels of pro-inflammatory cytokines in the peritoneal fluid of endometriosis patients. This, in turn, leads to an upregualtion of COX-2 [80-82]. Selective progesterone receptor modulators have the potential to suppress endometrial prostaglandin production in a tissue-specific manner [78].

Asoprisnil's ability to suppress uterine endometrial prostaglandins was demonstrated in a study conducted in a guinea pig model [83]. In guinea pigs, endometrial prostaglandins are produced in a pulsatile manner during the late luteal phase of the menstrual cycle. The prostaglandins produced are tightly regulated by progesterone and PR to function in the process of luteolysis. The study demonstrated asoprisnil's ability to suppress uterine prostaglandin F2α and downregulate COX-2 expression in guinea pigs, without producing the adverse unopposed estrogenic effect. Although the corpus luteum in humans is regulated by an ovarian mechanism, the mechanism of prostaglandin synthesis by the endometrium might be similar in humans and guinea pigs. This was suggested by two different studies: one conducted in non-human primates [84] and the other conducted on mifepristone in

humans [85]. Ongoing clinical studies are currently evaluating this relationship.

Studies are promising in demonstrating asoprisnil's potential as a new, tissue-selective approach to control endometriosis-associated pain. However, current studies have demonstrated only early, short-term safety and tolerability. Further studies are warranted on the long-term safety of asoprisnil and other related SPRMs.

Conclusions

Advances in the understanding of the pathophysiology of endometriosis have facilitated the development of newer treatment modalities for endometriosis. Novel approaches in management focus on targeting ectopic endometrium to provide maximal therapeutic benefit. This approach considerably minimizes the adverse effects and risks observed with traditional treatment modalities. Substantial ongoing research is under way to develop selective estrogen and progesterone receptor modulators, ligands, vascular growth factor inhibitors, and immunomodulators. Although promising research is moving in the direction of developing new therapeutic regimens, more studies are warranted before we can implement these regimens as the standard of care. Until then, the standard of care continues to be the use of traditional treatment modalities, including GnRH agonist, progestins, and danazol.

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