Review

Women's Health

Experimental treatments of endometriosis

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Endometriosis is defined as the presence of endometrial gland and stroma outside the uterine cavity. It is an estrogen-dependent disease and is associated with chronic pelvic pain, dysmenorrhea, dyspareunia and infertility. The treatment of endometriosis is conservative or radical surgery, medical therapies or their combination. All currently used hormonally active treatments are effective in the treatment of endometriosis; however, the adverse effects of these hormonal treatments limit their long-term use. Moreover, recurrence rates are high after cessation of therapy, and the treatments have no benefit in endometriosis-associated infertility. Therefore, researchers are working on new treatment modalities with improved side effects, mainly focusing on the molecular targets involved in etiopathogenesis of endometriosis. Here we summarized these novel treatments modalities.

Keywords: antiangiogenic agents • antioxidant agents • apoptotic agent • aromatase • aromatase inhibitors • dopamine • endometriosis • treatment

Endometriosis is defined as the presence of endometrial gland and stroma outside the uterine cavity. The prevalence of pelvic endometriosis is 6–10% in the general female population and 35–50% in women with pelvic pain, infertility or both [1,2]. Although endometriosis is primarily a pathology of the reproductive years, it is rarely observed in premenarchal girls and postmenopausal women, and in men treated with high-dose estrogen therapy for prostate cancer [3]. It is an estrogen-dependent disease and is associated with chronic pelvic pain, dysmenorrhea, dyspareunia and infertility [2,4].

The goal of the treatment of endometriosis is to relieve pain and/or achieve successful pregnancy in infertile patients. The treatment involves conservative or radical surgery, or medical therapies. Most of the current medical treatments modify the hormonal environment, because endometriosis is an estrogen-dependent gynecological disease. These drugs act through hypothalamo–pituitary–ovarian axis or act directly on hormone receptors in the lesions and cause regression of active lesions. Commonly used drugs are: combined oral contraceptive pill (COC), GnRH agonists, progestins, danazol. They are all effective in the treatment of endometriosis; however, the adverse effects of these hormonal treatments limit their long-term use. Also, recurrence rates are high after cessation of therapy, and the treatments have no benefit in endometriosis-associated infertility [5]. Therefore, new medical treatments with an improved side effect profile are desired.

Researchers are working on new therapies focusing on the molecular targets involved in etiopathogenesis of endometriosis including hormone-based regimens, selective progesterone receptor modulators (SPRMs), selective estrogen receptor modulators (SERMs), antiangiogenic substances, metalloproteinases inhibitors, antioxidants and stem cells [6–9]. Some of these new drugs act on reducing estrogenic activity such as GnRH antagonist, aromatase inhibitors (AIs) and SERMs, or cause a pseudopregnancy such as progestins and SPRMs or acts on more downstream

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pathogenetic cascade such as anti-inflammatory, anti-angiogenic and proapoptotic medications. In this article we summarized these novel treatments modalities for endometriosis.

Aromatase inhibitors

Aromatase (estrogen synthetase) is the key enzyme in the synthesis of estrogens. It mediates the conversion of androstenedione and testosterone (T) to estrone and estradiol (E2) [2]. Aromatase is expressed in a number of cells including the ovarian granulosa cell, the placental syncytiotrophoblasts and the testicular Leydig cells, as well as various extraglandular sites including the brain and skin fibroblasts [10]. The highest levels of aromatase are in the ovarian granulosa cells in premenopausal women, whereas adipose tissue becomes a major site of aromatase expression in postmenopausal women [11].

Cyp19A1 encodes aromatase in mice and humans [12]. Recent studies indicate that the transcription of the aromatase gene in human tissues is regulated by at least 10 distinct promoters each of which is regulated by a distinct signaling pathway in a tissue. Follicle-stimulating hormone (FSH) induces aromatase expression via cAMP and promoter II in the ovary while cytokines, TNF-α and glucocorticoids induce its expression via promoter I.4 in adipose and bone tissues. Extraovarian endometriotic tissue and cells derived from ovarian endometrioma use promoter II and aberrant aromatase expresion is mediated through promoter II [2]. Aromatase expression in endometriotic and peripheral tissues is shown to be critical as it is one of the rate limiting steps in E2 synthesis [13].

E2 is produced from cholesterol through six serial enzymatic conversions in two cell types that cooperate in a paracrine fashion in the ovary where there are two rate-limiting steps. The first rate-limiting step is the entry of cholesterol into the mitochondrion facilitated by the StAR protein in theca cells and the second one is the conversion of androstenedione to estrone by aromatase in granulose cells [2]. High levels of StAR and aromatase activity are seen in ectopic and eutopic endometrium of the endometriosis patients. They are most potently induced by PGE2. PGE2 induces aromatase expression via the transcriptional factor SF1 which is also aberrantly expressed in endometriosis patients. SF-1 binds to steroidogenic promoters in endometriotic tissues and mediates PGE2–cyclic AMP-dependent co-activation of multiple steroidogenic genes, most notably StAR and aromatase in a synchronous fashion. İn this way PGE2 stimulates *de novo* synthesis of E2 from cholesterol in endometriotic cells [2]. Therefore, endometriotic aromatase is not solely dependent for substrate on adrenal or ovarian secretion. StAR and/or aromatase expression is either absent or barely detectable in the endometrium of disease-free women.

Estrogen synthesis by aromatase occurs not only in the ovary but also in a number of tissues throughout the body [2]. In adipose tissue, aromatase residing in peripheral adipose and skin tissue catalyzes the conversion of circulating androstenedione of adrenal origin to estrone in relatively large quantities. At least one-half of this estrone is eventually converted to E2 in extraovarian tissues.

The aromatase inhibitors (AIs) have the ability to reduce estrogen production through inhibition of aromatase. They are classified into type I inhibitors (non-competitive) and type II inhibitors (competitive) [2]. Type I inhibitors binds to the enzyme irreversibly and permanently block the enzyme activity while type II inhibitors bind reversibly and can disassociate from the binding site, allowing renewed competition between the inhibitor and the substrate for binding to the site.

There are three generations of AIs. The first-generation of AI, aminoglutethimide was used to block estrogen synthesis in postmenopausal women with breast cancer [14]. Its action is not specific and its inhibition is not complete. It inhibits many other enzymes in steroid biosynthesis and induces a medical adrenalectomy with side effects such as lethargy, skin rashes and nausea [15]. The second-generation of AIs include fadrozole and formestane, show higher specifity for aromatase, and have fewer side effects. They are defined as the type I group. They are mainly steroidal inhibitors. They are androstenodione derivates and bind aromatase as substrate analogue [15]. The third-generation AIs, including letrozole, anastrazole and examestande, are triazole derivatives. They are more potent than aminoglutethimide. They are more specific for aromatase and have fewer side effects. Their inhibitory effect last longer. Therefore, daily administration is not necessary [15]. Third-generation AIs belong to the type II group of nonsteroidal inhibitors.

The first report describing the use of an aromatase inhibitor in the treatment of endometriosis was by Takayama *et al.* in 1998 [16]. They presented the treatment of a 57-year-old woman with recurrent severe endometriosis after hysterectomy and bilateral salpingooophorectomy with oral anastrozole for 9 months. They reported a significant reduction in pelvic pain and lesion size. They suggested that aromatase inhibitors may be successful candidate drugs in the treatment of endometriosis [16].

They were followed by another report confirming that an aromatase inhibitor is the medical treatment of choice in postmenopausal endometriosis [17]. In the same year another study reported the treatment of two premenopausal women with severe endometriosis and

pain. They were treated with anastrozole combined with progesterone, calcitriol and rofecoxib [18]. Treatment resulted in a rapid, progressive elimination of symptoms over 3 months with the maintenance of remission of symptoms for over a year.

Verma *et al.* [19] reported the treatment of three premenopausal patients with refractory endometriosis and chronic pelvic pain with AI. The treatment resulted in a significant reduction in pelvic pain.

In 2002, Fang *et al.* [20] showed that mice with experimentally-induced endometriosis in which the expression of aromatase has been genetically disrupted exhibit significantly smaller endometriotic implants. Other animal studies also suggest that AIs are effective in reducing endometriotic implant size in rodents with experimentally-induced endometriosis [21–24].

Stating that some form of ovarian suppression needs to be added to the currently available doses of aromatase inhibitors in premenopausal women as estrogen depletion in the hypothalamus may cause FSH secretion and ovarian stimulation if the ovary is not suppressed concomitantly, aromatase inhibitors were administered together with a GnRH agonist, a progestin, progesterone or a combination oral contraceptive in four Phase II trials [18,25–27]. These studies showed a significant benefit of an aromatase inhibitor in reducing pelvic pain.

Amsterdam *et al.* [25] treated 15 premenopausal patients presenting with documented refractory endometriosis and chronic pelvic pain with anastrozole in combination with oral contraceptive for 6 months. Significant reduction in pelvic pain scores were noted in 14 of 15 patients and occurred as early as 1 month after treatment initiation.

Ailawadi *et al.* [26] showed laparoscopic evidence of eradicating visible pelvic endometriotic implants and significantly decreasing pain with letrozole treatment. In another study it was demonstrated that combination of an aromatase inhibitor with a GnRH agonist significantly increased the pain-free interval and decreased symptom recurrence rates [27]. Sasson and Taylor [28] reported the case of a postmenopausal woman with a large, recurrent abdominal wall endometrioma who was successfully treated with letrozole and medroxyprogesterone acetate. Other case reports also suggest that letrozole either alone or in combination with steroids is effective in treatment of endometriosis-associated pelvic pain [29–31].

In another study the effect of anastrazole with a GNRH-agonist on endometriomal volume, CA125 levels and standard fertility parameters was evaluated in women with endometriosis who underwent IVF procedure [32]. This study demonstrated that combined anastrazole with goserelin markedly reduced endometriomal volume and disease activity. It was supported by another report in which 159 infertile women undergoing controlled ovarian stimulation and artificial insemination treated with the aromatase inhibitor, letrozole in combination with FSH, exhibited comparable pregnancy rates with less cancelled cycles and less FSH required for stimulation compared with FSH-treated patients alone [33].

These studies show that AIs are effective in the treatment of endometriosis. However, the use of AI could be limited because of its adverse effects such as hot flush, joint and muscle pain, headaches and its negative impact on bone mineral density, especially when associated with GnRH agonists. Therefore, future studies should be done to clarify if the long-term administration of AIs is superior to currently available medical therapies in terms of pain relief, adverse effects and patient satisfaction.

GnRH antagonists

GnRH antagonists induce competitive receptor occupancy of GnRH receptor, leading to a rapid and reversible suppression of gonadotropin secretion. They have the advantage of having an immediate blocking action on the GnRH receptor without the 'flare-up' effect. GNRH antagonists (cetorelix, ganirelix) are currently approved for use in assisted in reproductive technology (ART) as an alternative to GnRH agonists to prevent a premature LH surge.

These drugs are effective in suppressing endometriosis-associated pelvic pain when treatment is continued for 3–6 months; although combined oral contraceptive, danazol, gestrinone, medroxyprogesterone acetate and GnRH agonists are equally effective, their side effects and cost profiles greatly differ [34].

Currently available GnRH antagonist formulations require subcutaneous administration at least once a week. Their oral administration in healthy premenopausal women was shown to immediately suppress LH and FSH levels associated with a dose-related suppression in estrogen levels [35]. They may also inhibit the growth of endometriotic cells while maintaining sufficient estradiol production avoiding bone loss and other hypoestrogenic side effects such as vasomotor symptoms, vaginal atrophy. However, this has to be confirmed by further studies.

Many Phase I/II and few Phase III studies on the use of oral forms of GnRH antagonists (Elagolix, Abarelix, Cetorelix, Ozarelix, TAK-385) have been done for the treatment of endometriosis-related pain [36]. Although the pharmaceutical companies report good tolerability and efficacy of these compounds with no adverse impacts on bone mineral density, none of these studies have been published in peer-reviewed medical journals [14,36].

GnRH antagonists should be used as second or third line of treatment in endometriosis because of their limiting side effects such as vasomotor symptoms, vaginal dryness, decreased libido, irritability and loss of bone mineral density due to the hypoestrogenism. This group of drugs is not US FDA-approved for treatment of endometriosis pain, and the therapy should be supplemented with estrogen–progestin add-back therapy to reduce loss of bone mineral density [37].

Selective estrogen receptor modulators

SERMs are nonsteroid molecules that exert selective agonist or antagonist effects in different estrogen target tissues. They directly bind to estrogen receptor (ER)-α and/or ER-β in target cells and exert estrogen- or antiestrogen-like actions in various tissues. They may have tissue-selective effects, acting as ER agonists in the bone, but as ER antagonists in the breast and uterus. As endometriosis is a hormone-dependent disease, certain SERMs have been evaluated as therapeutic options.

Raloxifene is a nonsteroidal SERM with a high affinity for both ER-α and ER-β and acts as a partial estrogen agonist in bone, preserving BMD, but stimulates neither the endometrium nor the breast tissue in postmenopausal women. Raloxifene has been shown to reduce the size of endometriotic lesions in rats and nonhuman primates [22,38]. In a Phase II trial, Stratton *et al.* evaluated whether postoperative treatment with raloxifene was more effective than placebo in women with endometriosis [36]. Women in the raloxifene group experienced more pain and had an earlier second surgery than women in the placebo group leading to an early study termination [39].

A recent study showed that bazedoxifene antagonizes estrogen-induced uterine endometrial stimulation in a mice model of endometriosis [40]. Bazedoxifene may be an effective new candidate for the treatment of endometriosis because of its endometrial specific estrogen antagonism compared with other SERMs [40]. Another new SERM, TZE-5323, reduced the size of endometriotic implants in a rat experimental model without decreasing bone mineral density [41].

Several studies have shown a downregulation of ER-α and an upregulation of ER-β in endometriotic lesions that could be related to an aberrant DNA methylation of the ER-β promoter [42,43]. ER-β has been suggested to play an anti-inflammatory role and to antagonize the development of endometriotic lesions [44]. In a murine model, ERB 041, a selective ER-β agonist, has been shown to cause the regression of endometriotic lesions [45]. A Phase II trial has evaluated the safety and efficacy of ERB-041 (75 and 150 mg) versus placebo on the reduction of symptoms associated with endometriosis [36].

SERMs seem to be effective in treatment of endometriosis; however, the majority of findings were obtained in animal models and these drugs can also be associated with an increased incidence of venous thromboembolic events, vasomotor symptoms and sometimes stroke. Therefore, randomized clinical studies should be done before they are used as alternatives drugs in the treatment of endometriosis.

Selective progesterone receptor modulators

Selective progesterone receptor modulators (SPRM) are a class of molecules that act as ligands on the progesterone receptor (PR) ligands. They can have a differential effect on different tissues and can have an activity that ranges from pure agonist, mixed agonist/antagonist of pure antagonist [36]. SPRMs selectively inhibit endometrial proliferation without the systemic effects of estrogen deprivation and induce amenorrhea. They also suppress endometrial prostaglandin production and in this way cause relief of endometriosis-related pain [46]. SPRMs bind minimally to ER and have an antiproliferative effect.

Mifepristone, a progesterone antagonist (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 [36,46–47]. Mifepristone and other SPRMs antagonize estrogen effects in the endometrium in nonhuman primates [48]. They cause endometrial atrophy and amenorrhea in ovariectomized, estrogen-substituted monkeys [48]. Grow *et al.* showed that they decrease the size of endometriotic implants in a primate model [49]. However, long-term safety of mifepristone treatment should be studied as it has antiglucocorticoid properties [46].

A number of studies in nonhuman primates showed that onapristone and ulipristal also inhibit endometrial proliferation leading to endometrial atrophy [50,51].

Asoprisnil is a steroidal SPRM with a partial agonist/antagonist activity. Asoprisnil suppresses endometrial proliferation and induces amenorrhea in nonhuman primates. It may suppress pain associated with endometriosis through an endometrial-specific mechanism [36,52]. Also it may be effective in treating dysmenorrheal [46]. However, more studies are necessary.

Antiangiogenic agents

It is well established that the survival of endometriotic implants at ectopic locations is highly dependent on the development of an adequate blood supply [53]. Early developing lesions, which are the most

active ones, have a typically pink-red appearance and exhibit an increased number of immature pericyte-free microvessels when compared with the black lesions of later stages. Also the peritoneal fluid of women with endometriosis contains a variety of proangiogenic factors such as VEGF, IL-8 and placental growth factor and reduced concentrations of antiangiogenic compounds [54,55]. These proangiogenic factors are either secreted by the endometriotic lesions themselves or by peritoneal macrophages. Also, endometriotic lesions and peritoneal macrophages have been shown to secrete proangiogenic factors, in particular VEGF [56].

VEGF is one of the most important angiogenic factors. It acts as a potent selective endothelial mitogen and survival factor. Highly active red endometriotic lesions contain the highest VEGF concentrations when compared with other lesion types [57]. Also, peritoneal fluid concentrations of VEGF correlate significantly with the stage of endometriosis. It was demonstrated that vascularization, VEGF and its receptor expression are abundant in deeply infiltrating endometriosis [58].

Studies showed that treatment with both a soluble truncated fms-related tyrosine kinase (Flt-1)- receptor and an affinity-purified VEGF-antibody significantly inhibits the growth of developing endometriotic lesions in nude mice by disrupting their immature microvasculature [59,60]. Similar results were found by Nap *et al.* [61,62] who treated endometriotic lesions with an anti-VEGF antibody in the nude mouse model and the chicken chorioallantoic membrane (CAM) assay. These findings indicate that blockade of VEGF signaling prevents the establishment of endometriotic lesions. In the future, this may be achieved by VEGF-targeted gene therapy.

Ricci *et al.* [63] demonstrated that treatment with bevacizumab (Avastin) which is an anti-VEGF antibody significantly diminishes vascular density and cell proliferation and increases apoptotic cell death in surgically induced endometriotic lesions of BALB/c mice. It also reduces VEGF levels in the peritoneal fluid. However it has severe side effects, such as hypertension, proteinuria, impaired wound healing, gastrointestinal perforation, thrombosis and bleeding.

Dopamine and its agonists, such as cabergoline, promote VEGF receptor-2 endocytosis in endothelial cells, preventing VEGF-receptor binding and reducing angiogenesis. The effect of cabergoline on growth and vascularization of endometriotic lesions were analyzed in the nude mouse model [64]. They found that daily oral treatment with cabergoline over 14 days causes the regression of endometriotic lesions by suppression of cell proliferation and VEGF-mediated angiogenesis. They also demonstrated that cabergoline treatment resulted in a significantly lower expression of VEGF and VEGFR-2 in endometriotic lesions [65].

Vascular-disrupting agents (VDAs) are promising new drugs for the treatment of tumors. They target established blood vessels and may be more efficient against advanced disease. The encouraging advantage of these drugs is that they seem to note the physiological differences between tumor and normal endothelium inducing acute vascular shutdown only in the disease [66].

In addition to these classic angiogenic agents, tissue factor (TF) was shown to be aberrantly expressed in pathologically growing endothelium [67]. It is the key initiator of the hemostatic cascade. It mediates angiogenesis using a variety of distinct intracellular signaling pathways. Krikun *et al.* [67] examined the therapeutic utility of immunoconjugate (ICON), a novel compound which binds with high affinity and specificity to this aberrant tissue factor and demonstrated that it promoted devascularization, and prompted regression of well-established disease in their experimental model. Treatment with Icon did not interfere with subsequent fertility nor did it have any teratogenic effects.

Parecoxib, Rofecoxib and Celecoxib are selective COX-2 inhibitors. They were also found to be effective in animal studies [58,68–69]. Parecoxib was shown to decrease the implant size and led to atrophy and regression of endometrial implants in a rat model of peritoneal endometriosis. It also reduced the microvessel density, the number of macrophages and the expression of VEGF, showing an antiangiogenic effect [58]. Rofecoxib led to a significant relief of both pelvic pain and dyspareunia after 6 months, persisting after the end of the treatment [68]. It was shown that celecoxib and rosiglitazone reduced the mean number of lesions established per mouse, diminished the vascularized area in the lesion and diminished the implant volume in experimental endometriosis mouse model [69].

Epigallocatechin gallate (EGCG), the major component of green tea, seems to have antiangiogenic properties. It was shown to decrease endometriotic lesions, glandular epithelium, angiogenesis, microvessel size and density and mRNA expression for VEGF-A in the implants in an experimental endometriosis mouse model [70]. Moreover, the product from green tea increases apoptosis in endometriotic lesions [70].

Many other antiangiogenic treatments have been tested in animal models, but there are only scarce data in humans. Angiogenesis inhibitors such as endostatin, romidepsin-a histone deacetylase inhibitor, anginex and lodamin-an oral nontoxic formulation of TNP-470 have been shown to decrease endometriosis lesions and their vascularization in animal models [36,60,63,70].

Antioxidant agents

Endometriosis is a condition associated with imbalanced oxidative stress. Reactive oxygen species (ROS)

are upregulated and antioxidants depleted in the peritoneal fluid of affected women [24,71]. Stating that antioxidant therapy can be effective in the treatment of endometriosis, many studies focused on antioxidant agents such melatonin, omega-3 fatty acids, statins and pentoxifylline [24,36,46,70–71].

Melatonin is an indole mainly produced in the mammalian pineal gland during the dark phase. It is an important analgesic, anti-inflammatory agent, antioxidant and a free radical scavenger. It directly neutralizes a number of free radicals and reactive oxygen and nitrogen species. It stimulates several antioxidative enzymes which increase its efficiency as an antioxidant. Therefore, it may interfere with the oxidative stress seen in endometriosis. It may also have an impact on the extracellular matrix remodeling seen in this disease, through the regulation of the zinc-requiring proteolytic enzymes matrix metalloproteinases (MMPs) [24,71].

In animal studies melatonin led to a significant reduction in the volume and weight of endometriosis-like lesions [24,71]. In a Phase II trial Schwertner *et al.* [72] investigated the effects of melatonin compared with a placebo on endometriosis-associated chronic pelvic pain (EACPP), brain-derived neurotrophic factor (BDNF) level and sleep quality. They showed that melatonin improved sleep quality, reduced the risk of using an analgesic by 80% and reduced BNDF levels independently of its effect on pain. This study provided additional evidence regarding the analgesic effects of melatonin on EACPP and melatonin's ability to improve sleep quality. It also revealed that melatonin modulates the secretion of BDNF and pain through distinct mechanisms.

Omega-3 fatty acids inhibit the release of inflammatory mediators like IL-8 and prostaglandins in human endometrial stromal cells (HESC) [70]. A possible effect of these fatty acids in endometriosis has also been suggested by evidence that omega-3 fatty acid supplementation decreased prostaglandin E2 concentrations and endometriotic implant diameter in a rabbit model with surgically induced endometriosis [73]. Another study demonstrated that eicosapentaenoic acid (EPA) dietary supplementation caused a reduction in the thickness of the interstitium of the endometriotic tissue in a rat model of endometriosis [74]. EPA also suppresses some of the genes involved in the pathogenesis of endometriosis, like IL-1b, IL-1r2 and IL-10, which are downregulated after the oral administration of EPA [70].

Experimental animal models have shown that statins could also play a therapeutic role on endometriotic lesions through an effect on oxidative stress, VEGF and metalloproteinases [70,75–76]. Statins are inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase with intrinsic antioxidant activity [70]. The proliferation of endometrial stroma is stimulated by oxidative stress and inhibited by an assortment of antioxidants. Statins inhibit the growth of endometrial stromal cells in a concentration-dependent manner. It was shown that lovastatin reduced the proliferation of stromal cells and abolished angiogenesis in an *in vitro* model of endometriosis. Atorvastatin caused significant reduction in adhesion formation in rats [70]. Also it was demonstrated that atorvastatin significantly reduced the size of experimentally induced endometriotic implants and VEGF concentrations in peritoneal fluids of rats [76]. Simvastatin induced a dose-dependent reduction in the number and volume of endometriotic implants in a nude mouse model of endometriosis and reduce levels of matrix metalloproteinase (MMP)-3 expressions in cultures of endometrial stromal cells [75].

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 a pleiotropic immunomodulating agent. It influences both the production of inflammatory mediators, such as tumour necrosis factor-a, and the responsiveness of immunocompetent cells to inflammatory stimuli [77]. Vlahos *et al.* [78] reported that pentoxifylline exerts an antiangiogenic effect on developing endometriotic lesions in rats. Pentoxifylline has been tested in clinical trials as a potential drug for patients with endometriosis [79]. A major advantage of this drug is the fact that it does not inhibit ovulation. Therefore it can be administered throughout the time period of attempting conception [77]. Also, it is well tolerated with only minor side effects, such as gastric discomfort and dizziness.

A Cochrane database publication recently reviewed four clinical trials on the use of pentoxifylline treatment in women suffering from infertility [79]. However, the results of this review showed that pentoxifylline was not efficient on pain symptoms and did not improve the chances of spontaneous pregnancies.

TNF-alpha blockers

There is increasing evidence that immune dysfunction plays a role in the development of endometriosis. In the peritoneal fluid of affected women, decreases in natural killer (NK) cell activity, dysfunction of T lymphocytes, infiltration of macrophages and aberrant concentrations of immune-related cytokines are observed [80,81]. Women with endometriosis display increased TNF-α levels in their peritoneal fluid and its levels correlate with the stage of the disease [80,81].

TNF-α is a pro-inflammatory cytokine able to initiate inflammatory cascades. TNF-α, produced by activated macrophages and by endometriotic cells themselves, are increased in the peritoneal fluid of women with endometriosis [80,81]. It is implicated in various processes favoring the development of endometriosis such as the adhesion of stromal cells to the peritoneum, proliferation of endometriosis stromal cells, extracellular matrix degradation and invasion.

There are currently scarce data in humans regarding the use of immunomodulators acting on TNF-α in the treatment of endometriosis. In a small study of 21 women, Koninckx *et al.* [82] studied the effect of infliximab (a monoclonal anti-TNF-α antibody) versus placebo on endometriosis-related pain in women with nodules of deep infiltrating endometriosis and found an improvement of pain symptoms in both the treatment and placebo group. However, the difference was not statistically significant.

Immunomodulators

It was shown that abnormalities in the immune system play an important role in the aetiology and pathogenesis of endometriosis. Therefore, 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 [83]. These compounds have been shown to be effective in eradicating endometriosis in animal models; however, contradictory or ineffective results have been reported in humans.

Other immunomodulators like leflunomide and imiquimod were studied in the rat endometriosis model and were shown to be effective [14]. Immunomodulators agents with specific antiangiogenic effects such as LXA4, rapamycin, pentoxifylline, anginex, romidepsin, quinalizarin and the immunoconjugate molecule Icon were also studied [60–61,66,78,84–86].

LXA4 is an endogenous eicosanoid, which is involved in the regulation of various inflammatory processes. It has been shown to inhibit VEGF-stimulated endothelial proliferation and angiogenesis [84]. The effect of LXA4 on angiogenesis in mouse endometriotic lesions was analyzed and it was found that treatment with LXA4 inhibits the activity of MMP-9 and decreases mRNA levels of VEGF in endometriotic lesions, resulting in a significant growth suppression and atrophy of their glands [84].

Rapamycin (Sirolimus) is a mTOR inhibitor. It is widely used as an immunosuppressive drug to prevent rejection in organ transplantation. It inhibits tumor angiogenesis by decreasing VEGF production. It was demonstrated that rapamycin induces regression on endometriotic lesions [85]. This was associated with an inhibition of VEGF-mediated angiogenesis. Also, rapamycin suppresses the proliferation of endometrial and endothelial cells.

Anginex is a synthetic b-sheet-forming peptide. It mimics b-sheet domains of several antiangiogenic agents, such as platelet factor-4, IL-8 and bactericidal permeability increasing protein-1. It inhibits endothelial cell proliferation, adhesion and migration and induce apoptosis in these cells. It was demonstrated to suppress the formation of endometriotic lesions and to reduce the number of established lesions in the peritoneal cavity of nude mice [60,61].

Romidepsin belongs to the group of HDAC inhibitors. HDAC inhibitors influence gene expression by enhancing acetylation of histones in specific chromatin domains. Romidepsin has antifungal, immunosuppressant and antiangiogenic effects. It was shown to suppress the transcription, expression and secretion of VEGF in human epithelial endometriotic cells [70,85]. It causes regression of endometriotic lesions by inhibiting neovascularization and cell proliferation in an *in vitro* model.

Quinalizarin is a selective inhibitor of protein kinase CK2, which is a serine/threonine kinase. It regulates various biological processes, including angiogenesis. It was demonstrated that quinalizarin inhibite the vascularization of endometriotic lesions and cause regression in the lesions [86].

Peroxisome proliferator-activated receptors (PPARs) are a new class of immunomodulators found in adipose tissue, liver, spleen, colon, adrenal gland, muscle tissue, macrophages and endometrial epithelial and stromal cells. PPAR g-ligands modulate cell growth and angiogenesis, probably by downregulating proinflammatory mediators in macrophages [87]. In experimental models, they have been shown to inhibit proliferation and reduce vascularisation of endometriotic lesions by an effect on the expression of the angiogenic factor VEGF [14]. It was also shown that they decrease aromatase CYP450 activity in cultured human granulosa cells [88].

Ciglitazone, pioglitazone and rosiglitazone are thiazolidinediones, a new class of antidiabetic drugs with high affinity for PPAR-g. They inhibit the expression of various inflammatory molecules in chronic inflammatory diseases and can be used to treat pain associated with endometriosis. Rosiglitazone and pioglitazone have anti-inflammatory, antiangiogenic and antioxidant effects in an *in vitro* study [70,85]. It was demonstrated that ciglitazone and rosiglitazone reduce the volume and weight of lesions in the murine model of endometriosis compared with controls [70,85]. Rosiglitazone was shown to inhibit cell proliferation and

vascularization and augment apoptosis in experimental endometriosis models [68,70,85]. In baboons, pioglitazone was used in the treatment of induced endometriosis [68]. The surface area and the volume of endometriosis-like lesions and specifically the number and surface area of red lesions were statistically significantly lower in pioglitazone-treated baboons compared with controls [87]. Animal studies demonstrated that rosiglitazone, ciglitazone or pioglitazone reduced endometriotic growth and caused regression of established implants, reduced adhesions and the size of experimentally induced endometriotic lesions and provoked implant regression [70,85,87]. They seem to be effective but clinical studies are necessary to support the clinical use of thiazolidinediones for the treatment of endometriosis.

Oral fenofibrate, a PPAR-a ligand, also produced a statistically significant reduction in the mean area of implants in a rat model of endometriosis [85].

The cannabinoids play a role in the regulation of inflammation and immunological responses [70]. Cannabinoid agonists can also be used for endometriosis treatment. They have antiproliferative, antifibrotic and analgesic properties. The effects of WIN 55212-2, a cannabinoid agonist, were evaluated in *in vitro* and *in vivo* experiments. It was demonstrated that it has antiproliferative effects on endometriosis. *In vitro*, WIN 55212-2 reduced cell proliferation in endometriotic and endometrial stromal cells from women with or without deep infiltrating endometriosis.

Matrix metalloproteinase inhibitors

MMPs are a family of endopeptidases that are involved in the degradation and reconstruction of the extracellular matrix. It is important for many physiological and pathological processes such as embryo implantation, cyclic endometrial breakdown and endometriosis. It is regulated by its natural occurring inhibitor, tissue inhibitors of matrix metalloproteinase (TIMPs) [46]. Derangement of MMP regulation is considered to be a critical factor in the development of pathologic conditions such as endometriosis [71,85].

A mouse model of adenomyosis was used to test the tissue inhibitor of matrix metalloproteinase (TIPM) known as ONO-4817 [89]. Oral administration of the compound determined a lower grade of progression of adenomyosis than in control mice. Other drugs such as statins, melatonin, MAPK inhibitors, and metformin, have also been reported to interfere with the pathophysiology of endometriosis by inhibiting MMP expression [71,85].

MMP inhibitors seem to be effective in the prevention and treatment of endometriosis; however, the side effects of excessive TIMP activity on reproduction should be taken into account as it was shown that recombinant TIMP-1 administration was associated with reproductive abnormalities such as fewer ovarian follicles and fewer and altered zygotes [85].

Apoptotic agents

Endometrial cells from women with endometriosis overexpress antiapoptotic genes such as *Bcl-2*, and strongly inhibit proapoptotic genes such as *Bax* [85]. This general antiapoptotic gene profile seems to be important for the survival and proliferation of endometrial cells when they reach the peritoneal cavity.

Curcumin is a natural polyphenolic compound used in popular medicine as an anti inflammatory agent. It is also reported to be a NF-κB inhibitor and to induce p53-mediated apoptosis [85,90]. It was shown to be effective both in preventing occurrence of endometriotic lesions and in treating established lesions in endometriosis induced in rodents [85]. Other apoptotic agents that have been tested are bufalin and b-hydroxyisovalerylshikonin (b-HIVS). They have been shown to reduce the number of viable cells, inhibite DNA synthesis, and increased DNA internucleosomal fragmentation in hESCs cultures, significantly.

Metformin

Metformin, is an insulin sensitizer from the family of the biguanides. It is widely used in the treatment of diabetes and polycystic ovary syndrome. It has a modulator effect on the inflammatory response and on sex-steroid production. Metformin used on abdominal endometriosis induced in rats resulted in a statistically significant reduction of volume, weight and mean surface area of established lesions [23,85].

Others treatment modalities

Many other treatment modalities are investigated in endometriosis. These include nuclear factor κB inhibitors, histone deacetylase inhibitors, tanezumab – an anti-NGF inhibitor antibody, P38 MAPK, hyaluronic acid (HA), Chinese herbal mixtures, resveratrol – a nonflavonoid polyphenolic compound found in grapes and red wine with anti-inflammatory properties, vitamin E, ebselen and *N*-acetylcysteine (NAC), vitamin C and b-carotene and elocalcitol – a selective vitamin D receptor agonist and lignocaine – a local anesthetic (sodium channel agonist, potassium channel antagonist, membrane integrity inhibitor) [14,36,70,85].

Several lines of experimental evidence suggest that endometrial stem/progenitor cells function in the development of endometriosis [9]. Therefore, researchers are focusing also on targeting stem cells for the treatment of endometriosis. They are studying on

deregulated signaling pathways by these cells and their local microenvironment, blocking key self-renewal pathways and prosurvival-signaling pathways, preventing cell recruitment, flux and adhesion through interference with chemokines, and adhesion molecules that regulate these processes, as well as inhibiting abnormally activated pathways within the cells or surrounding niche cells [91].

Conclusion

Endometriosis is a multifactor disease which affects the quality of life of the patients. It causes chronic pelvic pain, dysmenorrhea, dyspareunia, multiple surgeries and also infertility. The treatment of endometriosis aims at relieving pain and/or achieving successful pregnancy in infertile patients. All currently used hormonally active treatments are effective but they have severe side effects which limit their long-term use, and the recurrences are high. Moreover, they do not have any effect on infertility. Due to the high prevalence and relevance of the disease, there is urgent need to introduce new therapeutic agents in this field. Many progresses were done in the past 10 years on research in the pathogenesis of endometriosis, new treatments and possible adjuvant therapies. These treatments were shown to be effective. However, most of the studies were done on experimental models of endometriosis. Therefore further studies are necessary to support the clinical use of these novel agents in clinical practice.

Future perspective

Many progresses were done on research in the pathogenesis and treatment of endometriosis. In the near future targeted therapies will be possible for the treatment of endometriosis.

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Executive summary

- It is an estrogen-dependent disease and is associated with chronic pelvic pain, dysmenorrhea, dyspareunia and infertility.
- Treatment of endometriosis aims at relieving pain and/or achieving successful pregnancy in infertile patients.
- All currently used hormonally active treatments are effective but they have severe side effects that limit their long-term use, and the recurrences are high.
- New treatments modalities such as aromatase inhibitors were shown to be effective in animal models of endometriosis.
- Further studies are necessary to support the clinical use of these novel agents in clinical practice.

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