Medical management of endometriosis

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Current approved medical therapies for endometriosis rely on drugs that suppress ovarian steroids and induce a hypoestrogenic state, which determines the atrophy of the ectopic endometrium. Gonadotropin-releasing hormone analogs such as danazol, progestogens and estrogen–progestin combinations have all proven effective in relieving pain and reducing the extent of endometriotic implants. However, symptoms often recur after discontinuation of therapy and hypoestrogenism-related side effects limit the long-term use of most medications. Recently, knowledge of the pathogenesis of endometriosis, particularly at the molecular level, has grown substantially, providing a rational basis for the development of new drugs with precise targets that may be safely administered over the long term.

Endometriosis is a condition in which functional endometrial glands and stroma are present outside the uterine cavity; it is believed to be a polygenetically inherited disease with a multifactorial origin. Ectopic endometrium contains estrogen receptors (ERs) and progesterone receptors and is able to respond to the physiological fluctuations of sex steroids [1].

Although it is difficult to estimate the prevalence of endometriosis in the general population, the largest epidemiological studies report a prevalence of 8% (range 1–43%) in asymptomatic women undergoing tubal sterilization and 23% (range 4–65%) in women with pelvic pain [2–6]. In addition, female relatives of a patient with endometriosis may be at a higher risk of developing this disease [7]. Endometriosis may be asymptomatic, but is frequently associated with dysmenorrhea, dyspareunia and chronic pelvic pain. Endometriosis is observed in 30–40% of patients who are infertile when no other causes of infertility are present [2]. The high prevalence of the disease, its frequent recurrence after conservative treatment and the associated symptoms have made endometriosis one of the leading causes of hysterectomy in women of fertile age [8].

Keywords: amenorrhea, dysmenorrhea, dyspareunia, endometriosis, hormonal treatment, medical treatment, pelvic pain

Rationale of hormonal treatment

The medical treatment of endometriosis has long played a major role in the therapeutic approach to this disorder. The original development of medications to treat endometriosis was built upon several observations:

- Endometriosis is infrequently encountered in parous women, whereas it is found much more often in the nulliparous female, suggesting a protective effect of the hormonal milieu of pregnancy
- The endometrium is known to be estrogen-dependent and ectopic endometrium is assumed to behave similarly
- Endometriosis tends to occur almost exclusively in menstruating women of reproductive age, thus suggesting hormonal dependence

These findings suggested the potential benefits of hormonal therapy in altering the normal menstrual cycling of the reproductive years as the mainstay of medical treatment for endometriosis [9].

Heterogeneous classes of drugs used in the medical treatment of endometriosis have the biological capacity to suppress ovarian steroids and induce a hypoestrogenic state. The hormonal effects of the different therapies vary substantially: suppression of ovarian steroidogenic activity and serum estradiol levels is extremely variable, as are as the direct actions on endometrial tissue. However, the different hormonal treatments for endometriosis share a common effect, most likely the true mechanism of action; amenorrhea. Amenorrhea induces atrophy of the ectopic endometrium and, eventually, regression of the lesions; indeed, bleeding during treatment is frequently associated with pain, independently of the degree of hormonal suppression. As Brosens recently proposed, endometriosis appears to be an ovarian steroid hormone-dependent

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Box 1. Medical options for endometriosis.

Traditional

- Oral contraceptives
- Danazol
- Gestrinone
- Progestins
- Gonadotropin-releasing hormone agonists
- Gonadotropin-releasing hormone plus add-back therapy
- Levonorgestrel-releasing intrauterine device

New

- Aromatase inhibitors
- Antiprogestogens
- Gonadotropin-releasing hormone antagonists
- Matrix metalloproteinase inhibitors
- Angiogenesis inhibitors
- Anti-inflammatory agents
- Immunomodulators
- Cytokines
- Estrogen-receptor modulators

process that is physiological as long as no bleeding occurs in the ectopic implants [10]. Medical therapies that abolish the recurrent ectopic bleeding lead to the regression of endometriotic implants, as observed at repeat laparoscopy immediately after suspension of treatment, and have proven their efficacy in ameliorating pelvic pain in women suffering from endometriosis.

However, in light of the former considerations, it is clear that available medical therapies cannot eradicate endometriosis, but might only induce regression of pain and reduce the extent of endometriotic implants (Box 1). Moreover, the fact that existing treatments for endometriosis cause anovulation and amenorrhea carries major limitations. As demonstrated by biopsies of lesions taken during treatment, inactive hypotrophic endometrium persists [11,12] and it is unlikely that, at resumption of ovulation, ectopic endometrium should degenerate or remain in persistent atrophy, while proliferation occurs promptly in the eutopic endometrium [13]; as a consequence, the efficacy of treatment is temporary and symptoms often recur after it is discontinued. In addition, amenorrhea is associated with side effects, mostly those related to hypoestrogenism, which limit the long-term use of some medications (Box 2). Finally, these drugs are of limited value in patients with a desire to become pregnant, as they inhibit ovulation.

Existing treatment

Oral contraceptives

Oral contraceptives (OCs) in high or low dosages, both monophasic and tricyclic, have been used for the reduction of dysmenorrhea and pelvic pain associated with endometriosis. Although their use in the treatment of endometriosis is not licensed within the UK and EU, they are widely used in clinical practice for treating endometriosis-associated dysmenorrhea. Only a limited number of studies have evaluated or compared them with other treatments for this indication. Parazzini and colleagues, in a randomized clinical trial, compared an OC (gestodene 0.75 mg and ethinylestradiol 0.03 mg; 47 patients) administered for 12 months versus tryptorelin 3.75 mg slow-release every 28 days for 4 months, followed by OC treatment for 8 months (55 patients), for the relief of endometriosis-related pelvic pain [14]. After 1 year, they observed a comparable improvement in both dysmenorrhea and menstrual pain in the two groups. Similar results were reported in a previous study that compared goserelin depot with a low-dose cyclic OC in women with pelvic pain associated with endometriosis [15]. When used cyclically, OCs are the only treatment for endometriosis that permit monthly uterine bleeding; dysmenorrhea may therefore not subside completely during the administration of OCs. Recently, Vercellini and colleagues administered continuous OCs for 2 years to 50 patients experiencing recurrent dysmenorrhea during cyclic assumption of OCs after conservative surgery for endometriosis [16]. In this series, mean scores in both the visual analog and verbal rating scale for the quantification of dysmenorrhea were significantly decreased at the end of the 2-year treatment period, as compared with baseline values. Moderate or severe side effects were reported by 14% of patients. Overall, 80% of patients were satisfied or very satisfied with continuous OC treatment. OCs represent an effective, safe and well-tolerated long-term treatment for endometriosis-associated dysmenorrhea in women who do not want children. Continuous administration can be proposed to women with persistent or recurrent dysmenorrhea during cyclic OC administration.

Danazol

Danazol is a synthetic derivative of 17-ethinyltestosterone that inhibits pituitary Gonadotropin production and ovarian steroid release. This results in the suppression of ovulation and endometrial atrophy with amenorrhea.

Danazol is administered orally at doses ranging from 400 to 800 mg daily and the standard duration of treatment is 6 months. Numerous studies have definitively shown that the drug is unable to restore fertility compared with expectant management in the early stages of the disease [17]. Danazol

Box 2. Side effects reported with the main hormonal medical therapies.

Gonadotropin-releasing hormone agonists

- Hot flushes
- Vaginal dryness
- Mood changes
- Bone loss

Progestins

- Bleeding abnormalities
- Weight gain
- Mood changes
- Breast tenderness
- Headache
- Depot bone loss

Gestrinone

- Hirsutism/acne

Danazol

- Increased liver transaminase
- Weight gain
- Mood changes
- Hot flushes

induces amenorrhea in most patients and, after 6 months of therapy, up to 90% of women with mild-to-moderate endometriosis reported a total or partial relief of pain. The amelioration of symptoms is less marked in patients with extensive adhesions. Early studies reported a significant regression of disease during treatment with danazol, but further experience has failed to confirm these observations [11]. Pain symptoms return and disease recurs in most symptomatic patients in the first 6–12 months after the end of treatment. Therapies longer than 6 months have been administered, but no specific study has addressed this issue and, hence, no scientific data on the safety and efficacy of long-term treatment with danazol are available. The main problems are the unwanted side effects, which include hirsutism, acne, hot flushes, weight gain, mood changes, alteration of plasma lipoprotein levels and increase in liver transaminase. These frequent side effects are a limitation to the prolonged use of this drug. A new administration route for danazol has been proposed. Igarashi and colleagues administered danazol using a vaginal ring-delivery system containing 1500 mg of the drug, and found it to be effective in relieving dysmenorrhea and dyspareunia, especially in women with deeply infiltrating endometriosis of the cul-de-sac [18]. Pregnancy occurred in 17 out of 31 patients during treatment. Androgenic side effects, typically observed during oral therapy, were not reported. This new administration route appears to allow significant pain relief without substantial side effects. However, the experience is confined to a small number of patients and the efficacy of this new delivery system must be proven in women with ovarian and peritoneal endometriosis localizations.

Gestrinone

Gestrinone is derived from 19-nortestosterone and causes endometrial and endometriosis atrophy. It reduces serum progesterone, and has androgenic side effects and negative effects on the lipid profile similar to those of danazol. Oral gestrinone administration 2.5 or 1.25 mg twice-weekly induces amenorrhea in 50–100% of women, and causes cellular activation and degeneration of endometriotic implants [11]. Atrophy is the prevailing feature of eutopic endometrium after 3–6 months of treatment [19]. A 6-month course of therapy allows a recovery of fertility similar to danazol and, hence, probably not greater than expectant management [20].

Although marked relief of pain symptoms associated with endometriosis has been reported in most symptomatic patients [21], pelvic pain recurred in up to 60% of the patients within 6 months of the end of treatment [20,21]. Unfortunately, only scant experience exists regarding prolonged administration of gestrinone in women with pain and endometriosis, and any comment on this subject lacks scientific evidence.

Progestins

Progestins have been used for the treatment of endometriosis since the 1960s, but in recent years their role has regained popularity. They may induce anovulation and hypoestrogenism according to their dosage, and they provoke marked decidualization and atrophy of eutopic and ectopic endometrium. Their effectiveness is also partly due to their proven anti-inflammatory effect. Most pelvic lesions associated with endometriosis are secondary to the strong inflammatory state caused by the metabolic activity of ectopic endometrium and the resulting immune response; progestins cause a reduction in peritoneal fluid volume and number of leukocytes. Progestins inhibit the expression of metalloproteinases [22], enzymes that contribute to the capacity of endometrial fragments for invading the peritoneal surface and establishing endometriotic implants.

These drugs might have the ideal characteristics for long-term treatment: they are effective, inexpensive and generally well tolerated. Depot

formulations are not recommended for patients wishing to conceive due to the variable and often prolonged delay in resumption of ovulation following discontinuation of therapy [23]; moreover, in women using depot formulations, bone mineral density (BMD) tends to decline [24]. Several compounds have been evaluated in the treatment of endometriosis, but there is no evidence that any one is preferable to any other. Vercellini and colleagues recently reviewed the studies published on this topic from 1993 to 2003 in which the following compounds were used [25]: medroxyprogesterone acetate [23,26,27], cyproterone acetate [28,29], norethisterone acetate [30,31], dydrogesterone [32], dienogest [33], lynestrenol [33], tibolone [35] and nestorone [36]. All treatments were given orally, with the exception of nestorone, which was administered with subdermal implants; the duration of therapy was 6 or 12 months in most studies, with one study only considering a period of 3 months, and the study on subdermal nestorone considering 7 months. Dydrogesterone was given for 12 days/cycle in the postovulatory phase in 43 women wanting children and did not prove more effective than placebo in reducing pain symptoms, confirming the essential role of ovulation suppression in the control of pain in women with endometriosis. In all other studies, progestins were given continuously and, when considering the most effective schedule of drugs for which different dosages were compared in the same study, the overall mean pain relief rate was 85% (range: 50–100%). Many women treated with progestins experienced side effects, with bleeding abnormalities, bloating and weight gain being by far the most frequent disturbances. Pain recurrence was reported a few months after treatment in a half of the patients. The main advantages of progestins are a lower cost and a reduced metabolic impact compared with other drugs available for the treatment of endometriosis, such as Gonadotropin-releasing hormone (GnRH) analogs and danazol [37]. The availability of the levonorgestrel-releasing intrauterine device (IUD) (Mirena®) has represented a new opportunity for the medical treatment of endometriosis. This device delivers the 19-norethisterone derivative, progestin, directly into the uterine cavity, achieving amenorrhea through a direct effect on the endometrium. This particular way of delivering the progestin succeeded in all the women treated [38]. The efficacy of this treatment has also been demonstrated in patients with symptomatic endometriosis of the rectovaginal septum who had not been treated with previous surgery [39]. A great improvement in deep dyspareunia and tenesmus was also observed. Moreover, sonographic evaluation showed a significant reduction of endometriotic lesions. Although levonorgestrel administered directly inside the uterus is supposed to act predominantly locally at the level of endometrial tissue, the side effects associated with this treatment confirm that there is significant systemic absorption of the drug. Indeed, irregular periods, breast tenderness, mood changes, acne, seborrhea, weight gain, abdominal bloating and headache were observed during treatment [38,39]. Recently, Lockard and colleagues demonstrated that Mirena is effective in symptom control throughout a 3-year therapy [40]. The continuation rate was 36% at 3 years; most patients stopped the therapy in the first 12 months due to irregular bleeding and/or persistent pelvic pain. These results confirmed that the levonorgestrel IUD may play an important role in the long-term treatment of dysmenorrhea associated with endometriosis, as well as in the long-term management of women with endometriosis of the rectovaginal septum.

Gonadotropin-releasing hormone agonists

GnRH agonists are widely used in the treatment of endometriosis-associated symptoms. These preparations induce an artificial menopause; they bind to pituitary GnRH receptors and induce a continuous instead of pulsatile stimulation. Several GnRH agonists have been introduced in clinical practice and different routes of administration are available; namely depot injection, nasal spray and subcutaneous pellet. These compounds are all extremely effective in achieving a profound but reversible hypoestrogenic state [41]. There are no published trials evaluating the theoretical differences among the various analogs; therefore, choice seems to depend mainly on preferences in the route of administration. Hypoestrogenism and amenorrhea induced by a 6-month course of GnRH agonists lead to macroscopic regression of implants and nearly complete relief of pain symptoms associated with endometriosis in over 90% of patients [40,42]. This efficacy has also been demonstrated in women with more extensive disease and severe symptoms not responding to previous treatments. In one randomized study, a 3-month course of GnRH agonists showed similar efficacy to the classic 6-month course [43]. However, all studies demonstrated that pain symptoms recur within

6–12 months in most patients who were symptomatic prior to therapy [44,45]. Pain recurrence appears independent of the stage of disease, whereas older patients have a longer pain-free period [46]. The median time to pain recurrence from discontinuation of therapy was found to be 5.2 months [46]. Several randomized trials have compared the efficacy of GnRH agonists and danazol in the treatment of endometriosis, but failed to demonstrate any significant difference in the relief of pain and regression of disease between the two therapies [47–49]. As with danazol, GnRH agonists do not improve fertility in women with endometriosis [17,45]. However, side effects associated with the two treatments differ substantially. Side effects induced by GnRH agonists are the obvious consequence of the profound hypoestrogenism established during their administration, and resemble those associated with the physiological menopause. Hot flushes, vaginal dryness, mood changes and insomnia are the most frequent. Prolonged hypoestrogenism, however, can induce bone loss and unfavorably modify lipoprotein levels. These long-term effects have led clinicians to limit the duration of therapy to no more than 6 months, since it has been demonstrated that trabecular bone loss induced by GnRH agonists may be reversible if therapy is limited over this period [48]. However, symptoms of the disease usually recur within 6–12 months; thus, the medical-treatment strategy in endometriosis is either intermittent reinduction therapy or long-term therapy, which requires add-back therapy with low-dose progestins and estrogens to prevent bone loss. The efficacy of GnRH agonist-induced hypogonadism plus steroid add-back therapy has been confirmed by a number of randomized, controlled clinical trials. The efficacy of GnRH-agonist treatment has not been diminished by the addition of low doses of exogenous steroids and it has been suggested that estradiol levels of less than 40 pg/ml over a longer time period are required to hinder the proliferation of ectopic endometrium, while 20–40 pg/ml estradiol serum levels might be sufficient to reduce the risk of bone loss and avoid postmenopausal symptoms [50–51]. The efficacy of GnRH agonists on pain symptoms has also been demonstrated in patients in whom add-back therapy begun concurrently with GnRH agonist administration [49,52,53]. However, the issue of the ideal add-back regimen remains unresolved. Recently, Hornstein and colleagues demonstrated in a randomized study that the use of leuprolide acetate depot in combination with norethindrone acetate 5 mg alone, or with norethindrone acetate 5 mg and conjugated equine estrogens 0.625 mg is effective in the suppression of pelvic pain symptoms associated with endometriosis while protecting against bone loss [52]; while Zupi and colleagues, in a recent randomized study, demonstrated a higher reduction of pelvic pain, dysmenorrhea and dyspareunia in patients treated with the GnRH agonist (leuprolide acetate) plus transdermal E2 25 μg and daily oral norethindrone 5 mg [54].

New medical treatments

Aromatase inhibitors

Aromatase is a cytochrome P450 enzyme that catalyzes the conversion of androstenedione to estrone. Aromatase is not expressed in the endometrial tissue of women without endometriosis. Recent studies have shown that patients with endometriosis have high levels of aromatase cytochrome P450 expression in eutopic endometrial tissue. Ectopic implants are also found to express high levels of aromatase. Aromatase expression may induce a significant estrogenic production within the endometrial lesion itself, thus contributing to the development of the endometriotic implant and associated symptoms [9]. Consequently, the rational basis for the use of aromatase inhibitors (AIs) as a treatment for endometriosis is to block the production of estrogens, both at the level of the ovaries and ectopic implants [55]. AIs such as anastrozole are currently used in postmenopausal women to treat hormone receptor-positive breast cancer refractory to tamoxifen [56].

The most commonly experienced side effects include hot flushes, headaches, nausea, diarrhea and mood changes. The long-term effect on bone density and serum lipids is unknown [56–58].

Since AIs are not potent enough to completely block ovarian production of estrogens, they have been proposed for the treatment of endometriosis in postmenopausal patients or in association with other hormonal suppressive therapies. Zeitou and colleagues reported a case of symptomatic recurrent endometriosis in an overweight postmenopausal patient who had already had total abdominal hysterectomy and bilateral salpingo-oophorectomy, in which the endometriotic lesion disappeared after 9 months of treatment with anastrozole 1 mg/day, with relief of pain symptoms [55–56]. In a recent study, AIs were given with a progestin for the treatment of endometriosis in ten premenopausal patients

with pain symptoms refractory to previous surgical and medical treatment, most with stage III or IV disease; the patients received letrozole 2.5 mg, norethindrone acetate 2.5 mg, elemental calcium 1.25 mg and vitamin D 800 IU, daily for 6 months [59]. At the end of treatment, a highly significant reduction of pain was achieved. Moreover, second-look laparoscopy showed a significant reduction of visible endometriosis, while histologically demonstrable endometriosis was absent. Gonadotropin and estrogens levels were not modified by the treatment and there was a significant improvement in hip-bone density. The most frequent side effect was the presence of hot flushes, which occurred in nine out of ten women. Ovulation was blocked, as shown by serial ultrasound evaluations; however, as the authors state, further studies are needed to clarify the mechanism responsible for this finding.

Soysal and colleagues conducted a prospective, randomized trial comparing a combination of anastrozole and goserelin for 6 months with goserelin alone for 6 months after conservative surgery for severe endometriosis [60]. They observed a significantly longer median time to symptom recurrence (>24 vs 17 months; log-rank test; $p = 0.0089$) and a lower recurrence rate at 24 months follow-up (3/40 vs 14/40) in the goserelin plus anastrozole group than in the goserelin-only group. Although suppression of estradiol concentrations and bone loss were higher in the group where anastrozole was added, this did not adversely affect menopausal quality of life, and BMD reduction at 2 years after medical therapy was not significantly different in the two groups.

A recent prospective study proposed anastrozole and OCs (20 μg ethinylestradiol, 0.1 mg levonorgestrel) for a 6-month treatment of endometriosis in 15 patients with chronic pelvic pain refractory to multiple medical and surgical treatment. A total of 14 out of 15 patients achieved significant pain relief using this therapy [61].

If an increased level of aromatase in eutopic or ectopic endometrium is demonstrated to be one of the pathogenetic factors of endometriosis, then AIs will constitute the first treatment with a specific molecular target for the disease.

Antiprogestogens

Numerous progesterone antagonists have been synthesized. Their biological activity ranges from pure antagonists (e.g., onapristone and ZK137,316) to steroids with mixed agonist–antagonist activity (e.g., RU-486; J-compounds: J867, J956, J912 and J1042, known as mesoprogestins) [62]. Mifepristone (RU-486) has been investigated in the treatment of endometriosis. Mifepristone has both antiprogesterone and antiglucocorticoid activity [62], and has been shown to inhibit ovulation and cause endometrial atrophy in a series of normally cycling women [63]. In primates, the long-term administration of mifepristone led to a decrease in endometriotic lesions and endometrial atrophy [63]. Three small, pilot studies have been published on the use of mifepristone in women with endometriosis and pain symptoms. Three different doses have been used: 100 [64], 50 [65] and 5 mg/day [66]. With all schedules, there was an improvement in symptoms; with the 50 mg dose, there was a 55% mean regression of visible endometriosis after 6 months of treatment. Furthermore, mifepristone therapy induced a significant decrease in visible endometriotic implants [65]. Side effects reported were minimal and no patients suspended the treatment; however, the authors expressed some concerns regarding the effects on eutopic endometrium, although overt hyperplasia was not identified.

The efficacy and side-effect profile of mifepristone point to a promising use for antiprogesterones in the treatment of endometriosis; however, large clinical trials are needed to better define their efficacy and safety profiles. Asoprisnil (J867) shows partial progesterone agonist and antagonist activities in animals and humans. A recent double-blind, placebo-controlled, dose-escalation study evaluated the effects of asoprisnil administered for 28 days on mestrual and ovarian cyclicity. A total of 60 women were enrolled. Asoprisnil was well tolerated; it reversibly suppressed menstruation at doses over 10 mg/day, since it induced amenorrhea primarily by targeting the endometrium in the absence of estrogen deprivation. It is considered a candidate for further investigation in the treatment of endometriosis [67].

Gonadotropin-releasing hormone antagonists

Only recently, GnRH antagonists with acceptable pharmacokinetic, safety and commercial profiles have become available. They exert an antagonistic effect on endogenous GnRH by competing for pituitary binding sites. Due to the lack of any intrinsic agonistic activity of these compounds, the characteristic initial

flare-up is absent. An immediate decrease in estrogen levels can be expected after administration and therefore, an earlier onset of the therapeutic effect (pain relief) can be hypothesized [68]. A single study on cetrorelix has been published; a total of 15 patients were treated with 3 mg cetrorelix every 7 days over a period of 8 weeks. All patients were free of pain during the course of treatment. After 8 days, a laparoscopy was performed to evaluate the number, size and location of the lesion(s); macroscopically, improvement of the disease was observed in 60% of cases. For abarelix depot, preliminary data are available on the treatment of endometriosis from an as yet unpublished Phase II trial [69]. Side effects are very similar to those of GnRH-agonists and their use for long-term treatment is questionable.

Matrix metalloproteinase inhibitors

Matrix metalloproteinases (MMPs) are a large family of zinc-dependent structurally-related endoproteases that play a role in the degradation and turnover of extracellular matrix proteins [70–71]. There are specific tissue inhibitors of metalloproteinases that regulate the action of these proteins. Steroid hormones regulate the activity of these enzymes; estrogens increase endometrial MMPs, while progesterone suppresses MMP activity. In patients with endometriosis, the activity of MMP proteins is increased and these enzymes seem to contribute to the implantation and growth of ectopic endometrium in the peritoneal cavity. Inhibition of these proteins should be effective in inhibiting the development of endometriosis [72]. With the nude mouse model, Osteen's group demonstrated that estrogen treatment of human endometrial tissue in organ culture maintains secretion of MMPs and promotes the establishment of ectopic peritoneal lesions when injected into recipient animals [70–71]. In contrast, suppressing MMP secretion *in vitro* with progesterone treatment or blocking enzyme activity with a natural inhibitor of MMPs inhibits the formation of ectopic lesions in the experimental model. Another trial has been conducted: the MMP inhibitor ONO4817 was used in the mouse model to deter the development of experimental adenomyosis [73]. This study demonstrated that treatment with ONO4817 inhibited the development and progression of uterine adenomyosis in mice. The practicability of this treatment in endometriosis has yet to be tested.

Angiogenesis inhibitors

Angiogenesis is required for the development of endometriosis [74]. Once implanted, ectopic endometrium produces angiogenetic factors, which induce the formation of new capillaries from existing vessels. The steps include proliferation, migration and extension of endothelial cells, adherence of these cells to the extracellular matrix, remodeling of the matrix and formation of a lumen. The vascular endothelial growth factor (VEGF) is one of the most prominently studied angiogenic factors. This molecule has been described in the peritoneal fluid and endometriotic lesions of patients with endometriosis [74].

Smith and colleagues group tested, in the nude mouse model, the effect of two antagonists of the angiogenic growth factor VEGF A: soluble truncated receptor (flt-1) and an affinity-purified antibody to human VEGF A. These antiangiogenic agents inhibited the growth of lesions in the *in vivo* model of endometriosis by disrupting the vascular supply [75].

A recent prospective study was performed to determine the effects of the angiostatic compounds anti-VEGF antibody, TNP-470, endostatin and anginex (a synthetic 33-amino acid peptide modeled to reproduce the structure of antiangiogenic proteins) on vascularization and endometriosis-like implant formation in the chicken chorioallantoic membrane model [76,77]. Endometrium was taken from 15 women undergoing laparoscopy for benign indications and the tissue was transplanted into the model. Endometriosis-like lesion formation was impaired after treatment with angiostatic agents, which was associated with decreased vessel densities in the chorioallantoic membrane and increased necrosis in the endometriosis-like lesions. This *in vivo* study demonstrated that a proper endometrium-induced angiogenic response in the ectopic environment is critical for implantation and survival of the tissue [78]. Angiostatic therapy may provide a suitable alternative to prevent endometriosis recurring after surgical or hormonal therapy.

Anti-inflammatory agents & immunomodulators

The direct and indirect production of cytokines and other mediators of the immune response associated with the peritoneal implantation of endometrial cells causes a chronic inflammatory state in the peritoneal environment. This inflammatory condition may contribute to the

development or progression of endometriotic lesions and the pathogenesis of pelvic pain. Peritoneal fluid macrophages are increased in patients with endometriosis and have higher levels of cyclooxygenase (COX)-2 than those of women without endometriosis [79]; in addition, the concentrations of cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-8 and -10 are augmented. The peritoneum itself, ovaries and ectopic endometrium may all contribute to the peritoneal pool of prostaglandins (PGs) [80]. Increased PG exposure has been associated with pelvic pain, at least in a subgroup of patients with endometriosis [81]. Increased levels of IL-1, one of the foremost mediators of acute inflammation, have been demonstrated in the peritoneal fluid of women with endometriosis [82]. In these patients, it has also been demonstrated that ectopic endometrium lacks specific receptors for IL-1 (IL-1ra), whereas eutopic endometrial cells stained for IL-1ra. The deficiency of IL-1ra in endometriotic implants may lead to insufficient control of the inflammatory reaction and subsequent adhesion formation. Such modifications suggest that endometriosis may be considered as an inflammatory disease and, therefore, anti-inflammatory agents could be helpful for its treatment. It has been hypothesized that a reduction in PG levels secondary to COX-2 inhibition can disrupt the inflammatory process evident in women with endometriosis, but a recent study demonstrated that treatment with COX-2 inhibitors, such as nimesulide, was not able to reduce the size or number of endometriotic lesions in the nude mouse model of endometriosis [83]. The methylxanthine derivative pentoxifylline is an inhibitor of phosphodiesterase that reduces the production of cytokines, the activation of B and T-lymphocytes and the activity of natural killer cells [84]. It is not an inhibitor of ovulation, so it can be administered throughout the time period of attempting conception, and it has been investigated as a treatment for endometriosis-associated infertility. A clinical study was published in which 56 infertile women with stage I or II endometriosis were randomly assigned to receive either pentoxifylline (400 mg per oral, twice daily) (n = 29) or placebo (n = 27) for 12 months: the pregnancy rate was higher, although not significantly, in the pentoxifylline group (25 vs 19%) [85]. Additional trials will further investigate this approach to help clarify the advantages of this and other similar drugs.

Since TNF-α, secreted by macrophages, is known to be overproduced in the peritoneal fluid of women with endometriosis, a specific anti-TNF-α therapy has been proposed [86]. Animal models have been established to assess the efficacy of TNF-binding protein-1, the soluble form of the type 1 receptor for TNF-α, which, by determining high affinity complexes with TNF-α, prevents its adhesion to membrane receptors [87]. Recombinant human TNF-binding protein-1 at a dose of 10 mg/kg subcutaneously for 1 week significantly reduced endometriotric implants in a rat model [88].

In baboons, recombinant human TNF-binding protein-1 (1 mg/kg subcutaneously on days 0, 2, 4, 6 and 8 after seeding of endometrial tissue in the pelvis) as well as a GnRH antagonist (antide, 2 mg/kg on days 0, 3, 6 and 9) proved effective, compared with placebo, in reducing the American Fertility Society stage of endometriosis at a laparoscopic assessment performed 25 days after induction of the disease [89,90].

In another randomized study using the baboon endometriosis model, Barrier tested the efficacy of anti-TNF therapy (etanercept) for the treatment of endometriosis. Treatment and control groups were randomly assigned to receive either etanercept or placebo, administered as a subcutaneous injection, three-times/week for 8 weeks. After 8 weeks, the animals were screened by laparoscopy and biopsies of the endometriotic lesions were performed for histological confirmation of endometriosis. The results showed that etanercept effectively reduced the amount of active endometriosis in the baboon model [91]. While this drug seems to be effective in treating the physical manifestations of endometriosis in baboons, it has not yet been tested in humans.

Cytokines

It has been proposed that the pathogenesis of endometriosis involves the incapacity of the immune system to clear the endometrial cells refluxed with menstrual flow from the pelvic cavity. The immune systems of normal women have the ability to recognize errant endometrial tissue as foreign and remove it. This process does not happen efficiently in women with endometriosis. This theory is supported by numerous studies showing that peritoneal immunosurveillance might be altered in patients with endometriosis [92,93].

On the basis of these observations, cytokines that have the property of enhancing cell-mediated immunity have been studied as a potential

therapy for endometriosis. In particular, interferon (IF)-α-2β [94] and IL-12 [95] were shown to significantly reduce endometriotic implants in a rat model when treated animals were compared with untreated controls. In a clinical study, IF-α-2β was administered intraperitoneally by laparoscopy to 25 infertile women with endometriosis and the dose was modulated according to the severity of disease: 3 million U for stage II, 6 million U for stage III and 12 million U for stage IV endometriosis. In this series, a significant reduction of symptoms, cancer antigen (CA) 125 levels and extent of the disease was observed at second-look laparoscopy after 3 months [96].

The possibility of treating endometriosis by modulating the immune system is supported by these preliminary data.

Estrogen-receptor modulators

Endometriosis is an estrogen-dependent disease. It has recently been discovered that there are two ERs: ER-α and ER-β. Although ER-α has clearly been related to proliferation of the endometrium, the function of ER-β is not completely understood. This receptor is expressed in a wide range of tissues, including the immune system. ER-β mRNA has been found in endometrial stroma epithelium and endometriomas, but the actual receptor protein has not been found in these tissues. In a recent paper, Harris and colleagues used the nude mouse model with explants of human endometriosis to prove that a selective ER-β agonist, ERB-041, was able to cause regression of human endometrial xenografts, especially when the endometrial lesions were intraperitoneal [97]. In six studies, ERB-041 caused complete lesion regression in 40–75% of the mice studied. ERB-041, and possibly other ER-β selective agonists, may be a useful approach to treating endometriosis [98].

Conclusion

Approved medical treatments of endometriosis rely on hormonal manipulation of the ovarian cycle and exert their effect by inducing amenorrhea. Medical treatments are effective in reducing endometriosis-associated pain, but are unable to eradicate the disease. OCs or progestins are the first-line medical treatment options. If unsuccessful, second-line treatments, on a long-term basis, would include GnRH agonists with or without add-back therapy. Biological research in endometriosis has recently given new molecular targets for future medical treatments. Aromase inhibitors are probably the most promising hormonal drugs, whereas larger studies are needed to confirm the first reports on the efficacy and safety of the numerous different approaches in the field of angiogenesis inhibition, immunomodulation and ER activity regulation.

Future perspective

Primary research on endometriosis should soon allow the identification of one or more safe and effective treatments with a specific biological target in the pathogenic process of the disease.

Executive summary

- Currently-approved medical therapies for endometriosis rely on drugs that suppress ovarian steroids and induce a hypoestrogenic state, which determines the atrophy of ectopic endometrium.
- Gonadotropin-releasing hormone (GnRH) analogs, danazol, progestogens and estrogen–progestin combinations have all proven effective in relieving pain and reducing the extent of endometriotic implants. However, symptoms often recur after discontinuation of therapy and hypoestrogenism-related side effects limit the long-term use of most medications. Furthermore, these therapies are of limited value in patients seeking a pregnancy, as they inhibit ovulation.
- Molecular biology identifies new targets for the treatment of endometriosis:
  - Aromatase inhibitors
  - GnRH antagonists
  - Matrix metalloproteinase inhibitors
    - Angiogenesis inhibitors
    - Anti-inflammatory agents and immunomodulators
    - Estrogen-receptor modulators
- These new medical treatments are currently being evaluated and have given encouraging preliminary results for potential clinical application.

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