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Targeting oxidative stress to treat endometriosis

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Introduction: Endometriosis affects 10% of women of reproductive age. It is defined as the presence of implanted active endometrial tissue outside the uterine cavity. The exact pathophysiology of endometriosis is still uncertain, although several optional etiological theories have been suggested. Being so common, a novel treatment for endometriosis is widely quested. Recent studies addressing the pathological characteristics of endometriosis have revealed a vicious cycle in which oxidative stress (OS) is generated, which in turn facilitates the implantation of the ectopic endometrium. At the same time, the generation of high amounts of reactive oxygen species further triggers a state of OS.

Areas covered: The author examined the evidence associating OS and endometriosis. After establishing an association, a search for antioxidant agents that were investigated specifically on endometriosis patients are described including Vitamins C and E, melatonin, resveratrol, xanthohumol and epigallocatechin-3-gallate. A significant effect of all the reviewed antioxidants on endometriosis is reported.

Expert opinion: Aiming for the reduction of OS as the treatment goal for endometriosis looks promising. However, since most of the studies are either *in vitro* or are animal based, further studies on human subjects are deemed necessary to elucidate the impact of OS reduction on patients with endometriosis.

Keywords: anti-angiogenesis treatment, anti-inflammatory, anti-oxidants, endometriosis pathophysiology, endometriosis treatment, oxidative stress, reactive oxygen species.

Expert Opin. Ther. Targets [Early Online]

1. Introduction

Endometriosis is an estrogen-dependent pelvic inflammatory disease characterized by the presence of endometrial glands and stroma outside the uterine cavity. The key presenting symptoms of the disease are pelvic pain and infertility [1]. It affects about 10% of all women within the reproductive age group. In fact, the prevalence of endometriosis in women with pelvic pain ranges from 30 to 45% of the infertile population [2]. Nevertheless, since diagnosis necessitates surgical confirmation, the factual prevalence of the disease is probably underestimated.

The etiology of endometriosis is still unclear. Several different theories have tried to designate the reason behind why endometriosis occurs only in certain women. Despite the lack of complete understanding of the pathophysiology of endometriosis, it is well-established that an endometriosis-related imbalance between reactive oxygen species (ROS) and antioxidants exists [3-5]. The elevated oxidative stress (OS) in endometriosis may either be a cause or a consequence of the pathophysiology of endometriosis. The unidentified etiology of endometriosis poses a major challenge in finding the right treatment for the disease. Unfortunately, as of today, most of the widely used pharmacological therapies of endometriosis aim to reduce the estrogen levels, which create a non-estrogen environment but achieve only partial success in reducing symptoms of the disease. Moreover, patients report of various side

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Article highlights.

- Endometriosis remains an enigmatic disease with a doubtful pathophysiology and a resultant debatable treatment.
- The current utilized treatment mainly aims to reduce the estrogenic environment thus providing only a limited relief of symptoms. Moreover, at the same time they yield in low estrogen side effects.
- Oxidative stress is not the underlying cause of endometriosis. However, it plays the major role in a pathologic vicious cycle which results in the ectopic endometrial tissue implantation, survival and the neovascularizton.
- Promising preliminary data indicate that although it is not the primary cause of endometriosis, oxidative stress may be targeted as the goal of endometriosis treatment with both a relief of symptoms and improved fertility.

This box summarizes key points contained in the article.

effects due to prolonged decreased estrogen levels and returning of symptoms upon the discontinuation of treatment.

Recent promising evidence indicates the effectiveness of targeting OS reduction as the goal of treatment regardless of its exact role in the pathophysiology of the disease. The aim of our current article is to review the association between OS and endometriosis and thereafter summarize the accumulating data regarding the effectiveness of targeting OS reduction as the goal for the endometriosis treatment.

2. Background on endometriosis

Endometriosis is defined as the presence of endometrial tissue exterior to the uterine cavity which induces a chronic inflammatory reaction. The disease affects young reproductive aged women, and its main outcomes are pelvic pain symptomatology and infertility [1]. Endometriosis is a debilitating disease which negatively affects social, occupational and psychological function [6]. It is one of the most widespread gynecological diseases with a reported prevalence of 6 – 10% in the general female population and up to 30 – 45% in patients with infertility or chronic pelvic pain [2,6]. The most common complaints from patients include significant cyclic pelvic pain that mountains shortly prior to the beginning of menses, and then lessens at the onset of flow, dysmenorrhea, dyspareunia and others. In contrast, some patients with endometriosis remain asymptomatic and therefore, frequently go undiagnosed [7]. The gold standard for diagnosing endometriosis is the classic appearance of lesions viewed during a surgical procedure such as laparoscopy or laparotomy with a later histological confirmation [8].

Several classification systems have been suggested to categorize endometriosis. Most of the classifications are based on the anatomic location and the severity of the disease. The most commonly used classification has been suggested by the American Society for Reproductive Medicine (ASRM) [8]. The major disadvantage of this system is the weak correlation of the classification with endometriosis symptoms such as pain and with infertility.

Nevertheless, the ASRM revised system enables an unvarying documentation of operative findings and it also allows for comparing of the results of various treatments. Another commonly used differentiation method to describe endometriosis relates mostly to its anatomical location. In the anatomical system, endometriosis is divided to (i) peritoneal or superficial disease, (ii) ovarian cysts or endometrioma and (iii) deeply infiltrating endometriosis [9]. This review will mostly relate to the impact of the treatment on superficial disease and some of it relates to ovarian endometriomas as most of the studies performed on the reviewed agents addressed these conditions.

Several risk factors for endometriosis have been identified including early menarche [10], short menstrual cycles [10,11], menorrhagia [12], nulliparity [13], low body weight [14] and obesity [14-16]. These risk factors reflect the estrogen dependence of the disease. The disease has a polygenic inheritance and genetic predisposition with an increased incidence in first-degree relatives and in monozygotic twins [17]. Environmental pollutants [18], immunological dysregulation [19], persistent inflammatory status [20] and epigenetic alterations [21] impose a risk of disease. Ovulation suppression decreases estrogen levels and hence reduces the risk of endometriosis and the severity of its symptoms. To achieve that state, parity [12] and prolonged breast feeding [22] are considered as protective factors.

Although endometriosis was first described in 1860 [23], its etiology and pathogenesis is still unclear setting a major obstacle to pursue a definitive treatment for the disease. In order to elucidate the pathophysiology of endometriosis, several theories have been suggested. As of today, none of these existing theories can be used to solely describe the pathophysiology of endometriosis in all its aspects.

Sampson's theory is the most commonly accepted theory of the origin of endometriosis. First published in 1927, Sampson described the required elements for endometriosis development: retrograde menstruation, presence of viable cells within the retrograde menstruation, and the implantation of viable endometrial tissue in the peritoneum [24]. Retrograde menstruation is the backflow of menstrual blood via the fallopian tubes into the peritoneal cavity. Interestingly, retrograde menstruation is not a unique phenomenon to endometriosis and occurs in most women [25]. Normally, the immune systems will eliminate these cells, preventing their implantation in the peritoneal cavity. In failing to do so, the patient develops endometriosis.

Some of the established data about endometriosis supports Sampson's theory. The location of occurrence of most of the observed peritoneal endometrial lesions favors the tubal reflux pathway. Likewise, the fact that these cells are characterized by integrins on their surface that allows them to attach to the peritoneal cavity, as well as their ability to implant, invade

the peritoneum, produce angiogenic factors and to create neoangiogenesis [25] supports the Sampson theory. Likewise, an obstructed cervix is known to induce endometriosis [26]. However, reports about observing endometriotic tissue outside the abdominal cavity [27] challenges Sampson's theory.

Other theories that have been suggested include the coelomic metaplasia, which basically claims that primitive parietal peritoneum has the capability to transform into endometrial tissue [28]. Since the ovaries and the Mullerian ducts are derived from the coelomic epithelium, a metaplastic transformation of these tissues may occur and result in endometrial tissue, another coelomic epithelium derivate. Moreover, this theory would explain why despite some degree of retrograde menstruation in most women, only a small minority will develop endometriosis [29]. The embryonic rest theory suggests of alternations in embryogenesis as the cause of endometriosis. Ectopic endometrial tissue observed in fetuses' autopsies [30] strengthens the theory. The Tissue Injury and Repair Theory postulates that endometriosis is caused by trauma. This auto-traumatization would enable the implantation of the endometrium in the peritoneal cavity [31]. The Quinn's 'denervation-reinnervation' theory assumes that endometrial cells are deposited outside the uterine cavity as a result of nerve injuries and denervation. The ectopic endometrial cells from the retrograde menstruation, adheres to the injured tissue in the peritoneal cavity. Pelvic pain is caused by re-innervation that later occurs [32]. The stem cell theory hypothesizes that stem cells present in shedding endometrium are the cause of early onset endometriosis [33]. These endometrial cells later implant and survive long term as endometrial stem cells/progenitor cells [34].

Every theory has its strengths and weaknesses and none of them provides a complete explanation to the pathogenesis and all different characteristics of endometriosis. Therefore, in the recent years, effort has been made to identify the pathologic features of the disease. By targeting these features as the treatment goal, a significant improvement of endometriosis symptoms and consequences can be achieved.

3. The association between oxidative stress and endometriosis

Oxidative stress occurs as a consequence of an imbalance between ROS and antioxidants. ROS are molecules that have an unpaired electron and that stabilize themselves by extracting electrons from different molecules in the body, like lipids, nucleic acids and proteins. Antioxidants are a defense mechanism created by the body to neutralize ROS. ROS have a physiological importance in the body with respect to reproduction. Serving as signaling molecules, they modify reproductive processes such as tubal function, oocyte maturation and folliculogenesis (**Figure 1**). Macrophages and apoptotic endometrial tissue that transplant into the peritoneal cavity possibly through retrograde menstruation are thought to be inducers of OS in women with endometriosis.

The initiative to seek an association between endometriosis and ROS originated in part from the similar characteristics shared by endometriosis and cancer, despite endometriosis being regarded as a benign disease. Nevertheless, endometriosis and cancer share some common characteristics such as the tendency to invade the tissues, an uncontrolled growth, angiogenesis capabilities, the ability to avoid apoptosis and distal spread [35]. The long-term survival and proliferation of both the endometriotic lesions and tumor cells are critically reliant on adequate blood supply through angiogenesis and protection from apoptosis. A well-established association between ROS and both the proliferation and metastatic potential of cancer cells has been reported in the literature [36,37]. In both endometriosis and cancer, augmented production of ROS is linked to increased proliferation rate. Likewise, OS-mediated damage in the pathogenesis of endometriosis and cancer cells are similar [36]. ROS actually serves as a second messenger of cell proliferation [38]. The elevated ROS is associated with cell proliferation through the activation of MAPK signaling pathways. As in cancer cells, the extracellular regulated kinase (ERK1/2) [39,40] is activated either directly through receptors which are known to induce the ERK signaling pathway [39] or indirectly through Src kinases [41]. The well-described linkage between ROS and proliferation of cancer cells, as well as elevated ROS production in endometriosis, points towards ROS as a major role player in the regulation of cell proliferation in endometriosis. Indeed, Ngô *et al.* [38] studied whether the dysregulation of ROS production in endometriotic cells correlates with a pro-proliferative phenotype in order to elucidate the spreading of endometriosis. Purified stromal and epithelial cells from ovarian endometrioma and eutopic endometrium of endometriosis patients were used to create cell lines. The endometriotic cells showed elevated OS levels with an increase in ROS production, alterations in ROS detoxification pathways, and a drop in catalase levels. As expected, the elevated ROS was associated with increased cellular proliferation and activation of ERK1/2 [38]. Nonetheless, while assessing the anti-proliferative effects of cannabinoid agonists on deep infiltrating endometriosis, Leconte *et al.* [42] observed a decreased proliferation of endometriotic cells due to the cannabinoid agonists while ERK1/2 activation remained stable in the same cells suggesting a different pathway for cannabinoid anti-proliferative effects in deep infiltrating endometriosis. Therefore, the alternative of mTOR/AKT pathway activation in deep infiltrating endometriosis was suggested [43]. There are several links that exist between endometriosis and the AKT pathway. First, the P3KCA mutation in ovarian clear cell carcinoma, a tumor that is linked with endometriosis, results in the AKT pathway activation [44]. Furthermore, AKT activity was reported to be elevated in ovarian endometriosis compared to the endometrium [45]. As in ovarian endometriosis, AKT activation was reported in endometriotic lesions from patients with DIE mostly in stromal cells [43]. However, despite the assumed association between AKT

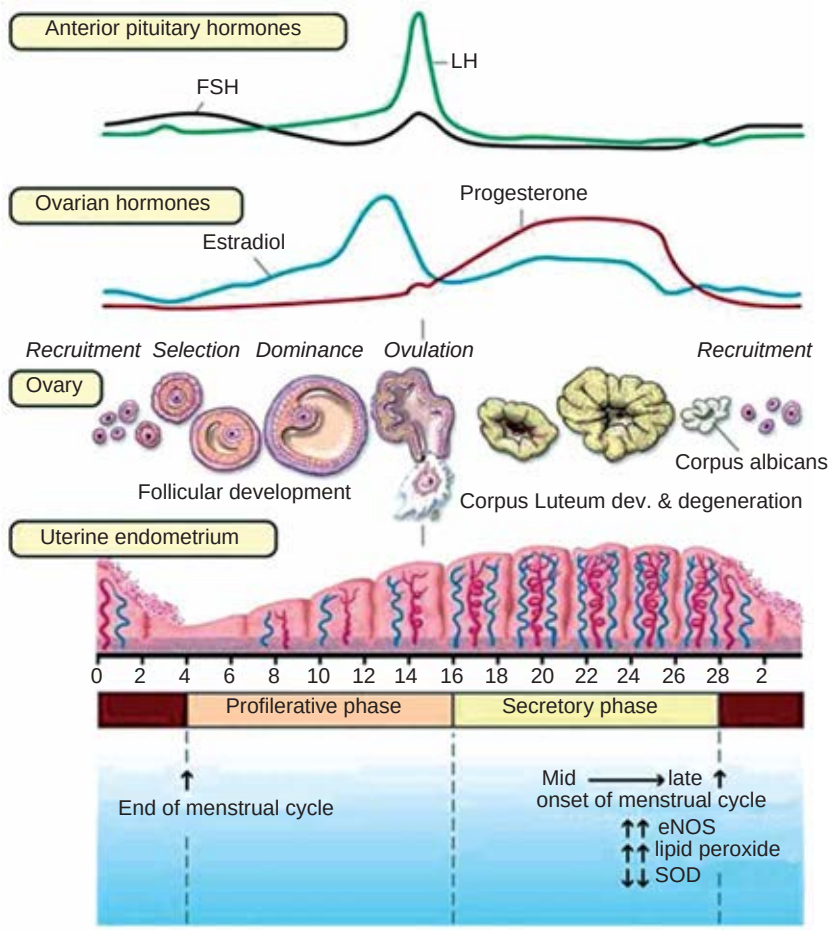

Figure 1. Modulation of redox balance throughout the menstrual cycle.
eNOS: Endothelial nitric oxide synthase; SOD: Superoxide dismutase.

activation and elevated estrogen levels, the AKT pathway activation can be elucidated by the overproduction of ROS, as previously reported in cancer cell lines [46]. Since the AKT pathway impacts cell proliferation and survival, the phenotype of stromal cells in DIE may be linked to the increased AKT activation [43].

Endometriosis is a chronic inflammatory process, which causes increased peritoneal macrophages recruitment and activation resulting in a release of pro-inflammatory cytokines such as interleukin (IL) 2, 4, 10, TNF-α and IFN-γ [47,48]. In cases of deeply infiltrating endometriosis, serum and peritoneal IL-33 were elevated compared to both endometriosis-free and superficial endometriosis patients [49]. Elevated macrophages and inflammatory activity are known to be major causes of increased OS [50]. The inflammatory process and the hyper-estrogenic stimulation in endometriosis are associated with a feed-forward cycle unremitted by the increased cyclooxygenase 2 (COX2) and CYP19A1levels, resulting in an unceasing production of prostaglandins and estrogen [51]. Interestingly, Zhao *et al.* [52] applied estrogen receptor (ER) ligands, chloroindazole (CLI) and oxabicycloheptene sulfonate (OBHS) – both characterized by strong ER-dependent anti-inflammatory activity on an animal model of immunocompetent mice and on human endometriotic stromal cell culture. All the well-known estrogen-dependent occurrences, including cell proliferation, cyst formation, vascularization and lesion growth, were halted by CLI or OBHS. The authors commented that these compounds have the anticipated features of both preventive and therapeutic agents for endometriosis using their dual suppression of estrogenic and inflammatory activities [52]. Despite the well-described association between the inflammatory process and endometriosis, no correlation between high-sensitivity C-reactive protein and endometriosis diagnosis or stage was observed [53].

The endometrial tissue and menstrual effluent found in the peritoneal cavity are seen as antigenic and activating macrophages. Macrophage number and activity in the peritoneal fluid (PF) have been found to be increased during endometriosis, which causes increased phagocytosis of the antigens as well as release of ROS [54]. Along with increased activity of macrophages, it has been found that transcription factor

NF-κB is up-regulated during the endometriosis process [55-57]. NF-κB can further increase the pro-inflammatory state and cause activation of many genes that induce the progression of the disease [58]. NF-κB elevation was found to be due to elevated iron levels, which will be detailed later in this chapter, and can also be released by macrophages, which were found by Lousse *et al.* to have the inert ability to secrete this transcription factor [59]. NF-κB can then bind to DNA and cause transcription of genes that code for cytokines, growth factors, angiogenic factors, adhesion molecules and inducible enzymes such as nitric oxide (NO) synthase and COX [58]. One of the main types of adhesion molecules that can be activated through this process is intercellular adhesion molecule-1 (ICAM-1). ICAM-1 mRNA and protein have been found to be elevated in ectopic endometrial cells [60]. In a study by Gonzalez-Ramos *et al.*, levels of ICAM-1 were studied in black and red endometriosis lesions, the latter being the implants that have the greatest proliferative capacity, and it was found that they had the largest expression of ICAM-1. Constitutive activation of the transcription factor NF-κB was also found in the red lesions [57]. Since red lesions are thought to occur at the earlier stages of endometriosis, measuring and targeting NF-κB levels may be an early diagnostic measure that could also be targeted by therapy to decrease the progression of the disease [57,61].

ROS have also been found to attack the fragile mesothelium which typically warrants against adhesion of the endometrial cells [62]. Mesothelial cells that were placed in culture with menstrual effluent or menstrual serum displayed morphologic changes that included retraction, shrinking and gap formation of the mesothelial cells. The creation of adhesion sites allows implantation of the endometrial cells and progression of the disease [62].

Another group of enzymes that play a role in the elevation of ROS and the progression of endometrial disease are the MMPs. Different types of MMPs are typically produced by endometrial stromal cells and play a role throughout the menstrual cycle [63]. The MMPs are proteolytic enzymes involved in remodeling and degradation of the extracellular matrix. In endometriosis, tissue remodeling is an essential component of its pathophysiology. Tissue inhibitors of matrix-metalloproteinases (TIMPs) are the regulators of the active form of the MMPs. In order for the implantation of ectopic cells to occur, the extracellular matrix of the peritoneal mesothelium must be degraded, which is facilitated by MMPs [64]. *In vitro* studies where the endometrium was displaced on chorioallantoic membranes have shown that MMPs may be necessary for endometriotic lesions to form and could therefore act as a therapeutic target.

Also, levels of angiogenic growth factor such as VEGF have been shown to be elevated in the PF of women with the disease [65]. Macrophages and other immune cells activated during the inflammatory process have the ability to produce increased amounts of VEGF [66].

The body's primary defenses against OS are antioxidants. These molecules donate electrons to ROS in order to inactivate them, limit their production or fix the damage they cause [67]. Antioxidants can be enzymatic such as catalase and glutathione peroxidase, or they can be non-enzymatic such as vitamins A, C and E. Women with endometriosis tend to have higher oxidative stress markers in the PF for both superficial and deep infiltrating endometriosis [68,69]. Various studies in the literature have demonstrated that OS plays a significant role in endometriosis because women with the disease have significantly lower levels of antioxidants, including superoxide dismutase and glutathione peroxidase, in their PF than healthy women do. Both these antioxidants are key components in the process of breaking down free radicals [70]. For this reason, it has been postulated that a high-antioxidant diet can help women with endometriosis. If the antioxidant system was somehow impaired or destroyed, the body would be unable to protect itself against ROS [71].

4. Nitric oxide and endometriosis

NO is an important molecule for normal reproductive biological processes such as maintenance of pregnancy at physiological levels [72]. However, at high concentrations, NO has a damaging effect on the gametes, embryos and oviductal function [73]. It has been hypothesized that IL-10, increased during earlier stages of endometriosis, may stimulate the release of NO by macrophages [74]. NO synthase, the enzyme that ultimately produces NO through conversion of L-arginine to L-citrulline, can be found in three forms. These three forms include the neuronal form, NOS1, the inducible form NOS2 (iNOS) and the endothelial form NOS3 (eNOS) (**Figure 2**). Macrophages isolated from women with endometriosis associated infertility had higher iNOS activity and subsequently released higher NO levels than endometriosis free controls [73]. The peritoneal macrophages have the ability to move from the peritoneal cavity to other parts of the female reproductive system including the fallopian tubes where fertilization takes place and where their increased capacity to produce NO can then cause greater risk of infertility [73].

The levels of NOS and NO are also correlated with the levels of the reproductive hormones estrogen and progesterone. When fasting blood samples were taken from women with endometriosis associated infertility, it was found that there was a positive correlation between the levels of estrogen and progesterone and eNOS protein levels [75]. NO activates cyclooxygenase 2; subsequently, prostaglandins such as prostaglandin E2 are increased and cause aromatase levels to rise as well [76]. The resultant estrogen increase stimulates further eNOS gene expression in a positive feedback loop [77].

NO is a well-known vasodilator and is one of the major mediators of the endothelium-dependent vasodilation [78]. Inflammation has been reported to reduce both endothelium-derived NO production and activation [79]. In endometriosis, NO is part of the blood flow regulation,

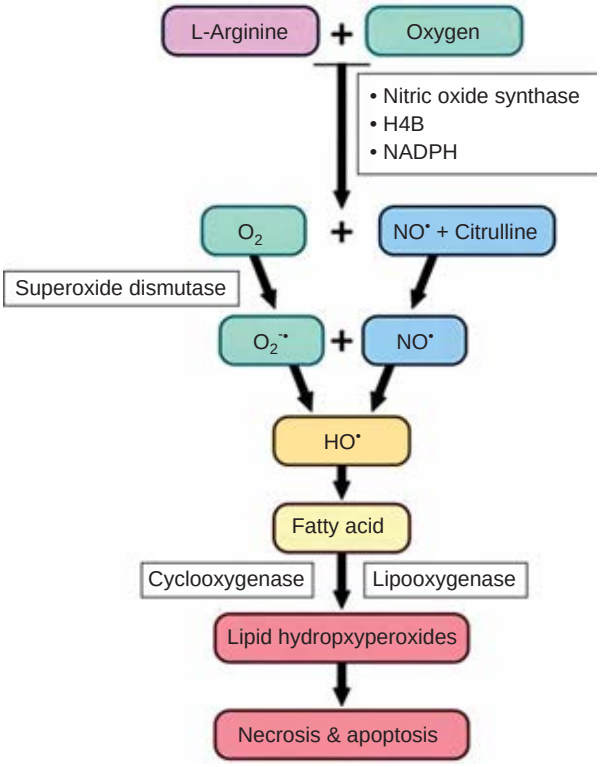

Figure 2. Pathologic pathway of apoptotic induction by ROS and NOS.

affecting angiogenesis abilities, a crucial component of the endometriotic lesion survival [79]. Assessing endothelium-dependent vasodilation in women with and without endometriosis, Kinugasa *et al.* [80], reported a significantly decreased flow-mediated vasodilation in endometriosis patients compared to non-endometriosis patients. Levels of asymmetric dimethylarginine, which is an inhibitor of endogenous NO synthase were significantly increased the endometriosis group. The authors concluded that both the elevated plasma asymmetric dimethylarginine levels and the enhanced inflammation in endometriosis are linked with the reduced endothelial function of endometriosis patients [80].

5. Iron and endometriosis

Iron is an essential element incorporated in a wide variety of molecules throughout the body, including hemoglobin (Hb). However, overproduction and increased levels of iron in the PF due to retrograde menstruation may be a cause of OS [81]. Endometrial cells have been quantitatively found in the peritoneal cavity of 59 – 79% of menstruating women, but only some develop endometriosis [82], which may be attributed to the impairment or efficiency of protective mechanisms against elevated iron levels. These include macrophage maintenance of iron homeostasis, haptoglobin sequestration of Hb and production of the hemopexin-heme complex.

Macrophages play a huge role in the homeostasis of iron levels and have the ability to phagocytize the erythrocytes, releasing Hb and heme. Subsequently, these molecules will be broken down into iron, ferritin, carbon monoxide and bilirubin through an enzyme called heme oxygenase 1 (HO-1) [83].

Hb released from erythrocytes can also be bound by haptoglobin (Hp), a scavenger protein [84]. The Hb-Hp complex is then recognized by a scavenger receptor CD163 on the surface of macrophages and phagocytized [84]. Finally, any free floating heme released during metabolism of Hb will be bound to hemopexin, which has antioxidant capacity.

In women with endometriosis, it has been found that there is an elevated level of iron within the PF compared to controls [85,86]. The increased levels of iron within the PF may occur in part due to increased amount of erythrocytes from retrograde menstruation or due to peritoneal lesion bleeding [87]. This, coupled with the decreased protective mechanisms, may account for the development of endometriosis.

Ferritin, an antioxidant, sequesters iron which allows for a decreased amount of the element to be available to produce OS via the Fenton reaction [88]. However, due to the elevated amount of erythrocytes found in the peritoneal cavity of women with endometriosis, the ferritin sequestration system becomes overwhelmed quickly, causing there to be release of iron into the peritoneal cavity, which can then participate in the Fenton reaction generating hydroxyl (OH) radical, and inducing OS [86]. Another cause of the ROS-antioxidant imbalance is the decreased amount of bilirubin, a potent antioxidant that is produced by heme oxygenase (HO) breakdown of heme [83]. HO-1 levels were found to be elevated in ectopic endometrium especially in the red lesions, but not in peritoneal mesothelium or in macrophages [89]. Decreased expression of the HO-1 enzyme does not allow the final byproduct of heme breakdown, bilirubin, to form, and its antioxidant capacity is missing in these women. Moreover, a recent study [90] proposed another role of OH-1 in endometriosis pathophysiology. Significantly increased OH-1 levels were observed in endometriomas compared to eutopic endometrial tissue of endometriosis and non-endometriosis patients. The increased OH-1 is proposed to induce autophagy. Autophagy is a catabolic defensive mechanism in which macromolecules are confiscated and subsequently degraded. Wide activation of autophagy is lethal to the cells, resulting in autophagic cell death [90].

Overall, iron overload causes increased proliferation of endometrial lesions and progression of the disease, which means that this mechanism could be a therapeutic target. By decreasing levels of iron, the levels of OS resulting from free iron in the peritoneal cavity can also be controlled [81].

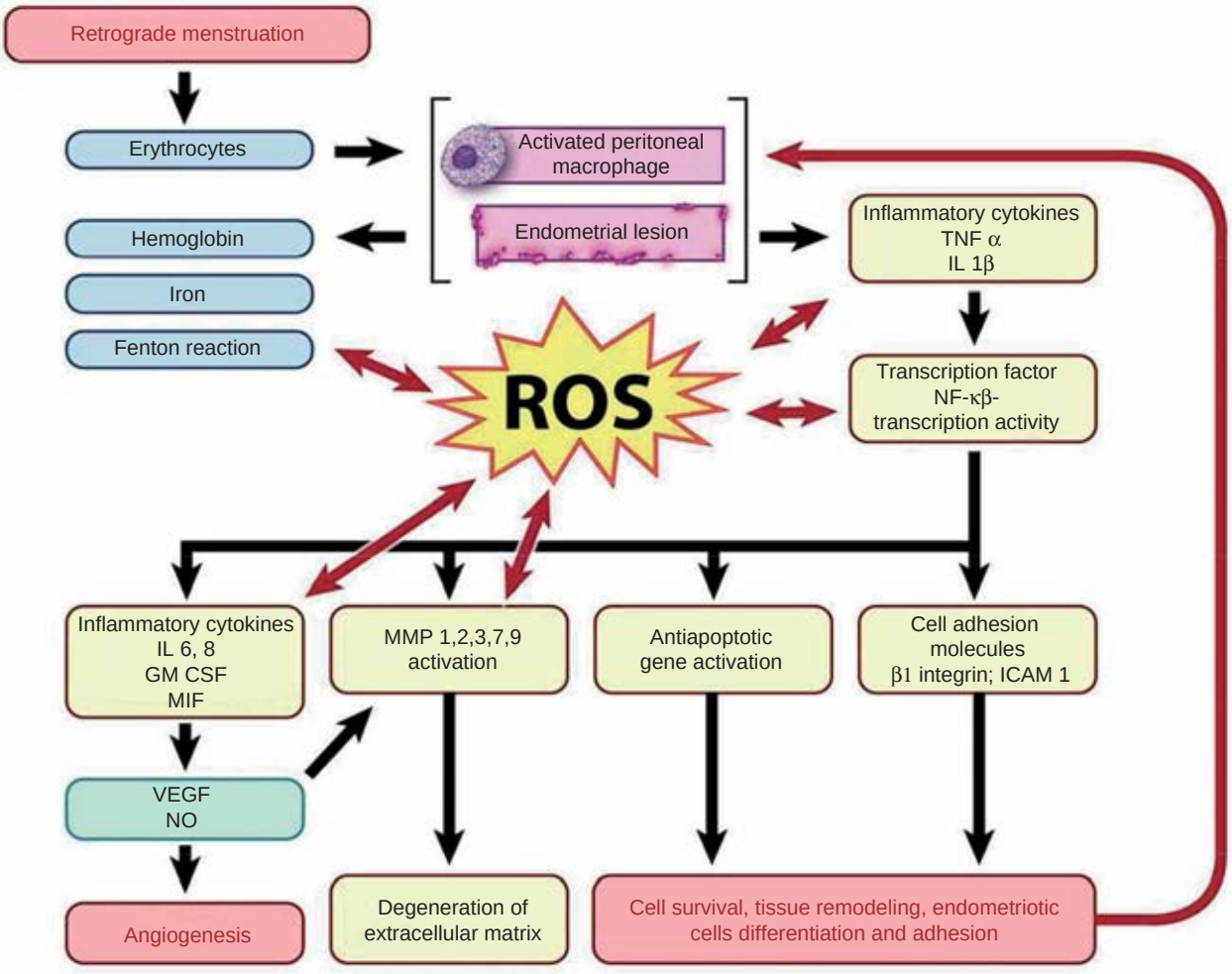

Figure 3. The vicious cyclical role of oxidative stress in the pathophysiology of endometriosis.

6. The role of antioxidative measures in the treatment of endometriosis

Despite the undiscovered enigmatic etiology of endometriosis, the significant association between OS and the endometriosis' development and progression is well established (**Figure 3**). The identification of OS as a major player in endometriosis pathophysiology has been directed to various studies that address the influence of OS reduction as the treatment goal.

7. Vitamins C and E

Vitamin C is an effective antioxidant. It is a water-soluble, chain-breaking antioxidant that acts directly with superoxide, OH radicals and singlet oxygen [91]. Vitamin E is a potent lipid-soluble, chain breaking antioxidant that acts as a peroxyl radical scavenger, inhibiting the effect of free radicals by forming a tocopheryl radical, which will then be reduced by a hydrogen donor (as Vitamin C) and so return to its reduced state [92]. The intake of these vitamins can be either through diet or through supplementation. The major advantage of these agents is the long period of time that they have been used in humans to yield study results unlike newer agents (**Table 1**).

In an animal study, Durak *et al.* [93] randomly assigned experimentally induced endometriotic cysts in rats to different dosages of vitamin C supplementation compared to a placebo group. The endometriotic cysts volume and weight as well as the natural killer cell content of the cysts were compared. The authors reported that a dose-dependent vitamin C supplementation significantly lowered both the volume and weight of the endometriotic cysts and the natural killer cell content.

The relation between vitamins C and E intake and thediagnosis of endometriosis was evaluated by Darling *et al.* [94] in a

Table 1. Studies investigating the effect of antioxidative stress agent on endometriosis and the suggested mechanism by the researchers.

Drug name	Author	Study type	Reported effect	Suggested mechanism of action
Vitamins C and E	Santanam *et al*. [96]	Human clinical study	Decrease in chronic pelvic pain, dysmenorrhea, dyspareunia, PF inflammatory markers, normal T-cell expressed and secreted, interleukin-6, and monocyte chemotactic protein-1	Reduction of oxidatively modified lipoproteins
	Darling AM *et al*. [94]	Human – prospective cohort study	Inverse associations of Vitamin C and Vitamin E intakes with laparoscopically confirmed endometriosis	Minimizing oxidative stress
	Durak Y *et al*. [93]	Animal study	Reduction of endometriotic cyst volume, weight, growth and natural killer cell content	Antioxidant and immune stimulator, stimulation of leukocyte functions as phagocytosis, enhanced NK function
	Mier-Cabrera J *et al*. [95]	Human clinical study	Decrease in OS markers: MDA, lipid hydroperoxides	Neutralizing ROS as OH, alkolyl, peroxyl, and superoxide anions, hydroperoxyl radicals, and RNS such as NO and peroxinitrite,
	Foyouzi N *et al*. [135]	*In vitro*	Inhibition of thymidine incorporation and proliferation of endometrial stromal cells	Decreased ROS activity
Resveratrol	Bayoglu Tekin Y *et al*. [105]	Animal study	Reduction of endometriotic lesion volume, histopathological grade, immunoreactivity to mmp2, mmp9 and VEGF, plasma and PF levels of IL-6, IL-8 and TNF-α	Down-regulation of pro-inflammatory cytokines induction of apoptosis and promotion of cellular proliferation and differentiation, decrease in mmp2 and mmp9 activities, suppressed microvessel activity
	Bruner-Tran KL *et al*. [101]	*In vitro* + *In vivo*	Reduction of number of endometrial implants, total volume of lesions (*in vivo*) and concentration-dependent reduction of invasiveness in human endometrial stromal cells (*in vitro*)	Reduction of proliferation of endometrial cells (through inhibition of AKT and MAPK1/3), increased apoptosis, and reduced ability of the endometrial tissue to attach and to implant
	Yavuz S *et al*. [104]	Animal study	Reduction of endometriotic implant volumes, proliferating cell nuclear antigen expression levels, increase in activities of superoxide dismutase and glutathione peroxidase in serum and tissue	Decreased lipid peroxidation inhibition of cyclooxygenase-2 (COX-2) expression and prostaglandin (PG) synthesis
	Ozcan Cenksoy P *et al*. [103]	Animal study	Reduction of areas of implants, mean histopathological scores, VEGF-staining scores of endometriotic implants, PF levels of VEGF and MCP-1, serum VEGF and MCP-1	Inhibiting angiogenesis through VEGF reduction and inflammation activity reduction
	Amaya SC *et al*. [97]	Animal study	Reduced expression of ESR1 and proliferative activity (Ki67), agonist and antagonist of estrogen in low and high concentrations, respectively	Reduction of ESR1 specifically in endometrial epithelium, action through non genomic action of alternative estrogen receptors such as GPR30
	Taguchi A *et al*. [141]	*In vitro*	Suppressed TNF-α-induced IL-8 release in a dose-dependent manner	Increased activity of SIRT1 (inhibitor of chronic inflammatory)
	Ergenoğlu AM *et al*. [102]	Animal study	Reduction of implant size, levels and expression of VEGF in the PF, plasma and endometriotic tissue, monocyte chemotactic protein 1 in the PF, histological changes in the endometriotic foci	Inhibitory activity on NF-κB, reduction of VEGF in both PF and plasma
Melatonin	Cetinkaya N *et al*. [113]	Animal study	Reduction of: endometriotic implant volumes, mean MDA levels lower in the control group	Anti-inflammatory through PGE2 inhibition due to COX-2 down-regulation

EGCG: Epigallocatechin-3-gallate; ERK: Extracellular regulated kinase; ESR1: Estrogen receptor α; MDA: Malondialdehyde; NAC: N-acetyl-l-cysteine; NO: Nitric oxide; OH: Hydroxyl; PF: Peritoneal fluid; TIMP: Tissue inhibitors of matrix-metalloproteinases.

Table 1. Studies investigating the effect of antioxidative stress agent on endometriosis and the suggested mechanism by the researchers (continued).

Drug name	Author	Study type	Reported effect	Suggested mechanism of action
	Güney M *et al* [114]	Animal study	Significant difference in spherical volumes, explant weights, COX-2 positivity, decreased levels of MDA in endometrial explants	Lipid peroxidation reduction, inhibitory effect on prostaglandin production through decreasing COX-2 enzyme activity
	Kocadal NÇ *et al*. [142]	Animal study	Reduction of: endometriotic lesions volumes, improved histopathological scores	Increased antioxidant activity, down-regulated proMMP-9 and MMP-3 expression and activity, enhanced expression of tissue inhibitors of metalloproteinases
	Paul S *et al*. [64]	Animal study	Protecting and regressing peritoneal endometriosis	Arrested lipid peroxidation and protein oxidation, downregulated proMMP-9activity and expression, elevation in the expression of TIMP-1
	Schwertner A *et al*. [115]	Human clinical study	Reduction of daily pain, dysmenorrhea, dysuria and dyschezia, risk of using an analgesic and improved sleep quality	Activation of supraspinal sites and the inhibition of ''spinal windup'', anti-inflammatory effects by inhibiting the release of proinflammatory cytokines, decrease of luteinizing hormone surge, inhibition of steroidogenesis by altering cyclic AMP levels through direct action on the theca or granulosa cells
	Koc O *et al*. [143]	Animal study	MDA was significantly lower and superoxide dismutase and catalase activity was significantly higher with melatonin	Stimulation of antioxidative enzymes, regulation of matrix MMP-9 via tissue inhibitors of MMP-1
	Paul S *et al*. [112]	Animal study	MMP-3 activity reduction, significant regression of glandular epithelium, increased apoptotic cells	Diminished activator protein (AP)-1 DNA-binding activity, reduced Bcl-2 expression along with increased Bax expression andcaspase-9 activation
Xanthohumol	Rudzitis-Auth J *et al*. [120]	Animal study	Reduction of implant size, lesions vascularization	Reduced level of phosphoinositide 3-kinase protein
EGCG drug and pro-drug	Wang *et al*. [127]	Animal study	Decrease in growth of endometrial implants, lesions size and weight, inhibition of functional and structural microvessels, enhanced lesion apoptosis	Anti-angiogenesis through inhibitory effects on VEGF expression and receptor activity, anti-oxidation capacities
	Xu H *et al*. [124]	Animal study	Inhibited microvessels in endometriotic implants, suppressed VEGF and VEGF receptor expression	c-JUN, interferon-γ, matrix metalloproteinase 9, and chemokine (C-X-C motif) ligand 3 pathways for endothelial proliferation, inflammatory response, and mobility
	Matsuzaki S *et al*. [125]	*In vitro* + *In vivo*	Inhibited cell proliferation, migration and invasion of endometrial tissue, decreased fibrotic markers, prevented progression of fibrosis	Reduction of the transforming growth factor -β1-dependent increase in the mRNA expression of fibrotic markers, inhibited activation of MAPK and Smad signaling pathways in endometrial and endometriotic stromal cells
	Laschke MW *et al*. [123]	*In vitro* + *In vivo*	Decrease in E(2)-stimulated activation, proliferation and VEGF expression of endometrial cells *in vitro*, inhibited angiogenesis and blood perfusion, induces regression of the endometriotic lesions	Effect on VEGF expression, VEGF receptor binding, VEGF receptor phosphorylation, interleukin-8 production and matrix metalloproteinase activity, mitogenesis and ephrin-A1-mediated migration of endothelial cells, endothelial cell apoptosis induction, blocks of E2-induced activation of endometrial cells

EGCG: Epigallocatechin-3-gallate; ERK: Extracellular regulated kinase; ESR1: Estrogen receptor α; MDA: Malondialdehyde; NAC: N-acetyl-l-cysteine; NO: Nitric oxide; OH: Hydroxyl; PF: Peritoneal fluid; TIMP: Tissue inhibitors of matrix-metalloproteinases.

Table 1. Studies investigating the effect of antioxidative stress agent on endometriosis and the suggested mechanism by the researchers (continued).

Drug name	Author	Study type	Reported effect	Suggested mechanism of action
NAC	Ngô *et al*. [38]	*In vitro* + *In vivo*	Endometriotic cells displayed higher endogenous oxidative stress with an increase in ROS production, alteration in ROS detoxification pathways, and a drop in catalase levels. ROS elevation correlated with increased activation of ERK1/2. All phenomena were repealed by NAC both *in vitro* and *in vivo* in a mouse model.	Inhibition of the intracellular level of ROS by antioxidant molecules abrogates ERK phosphorylation and cellular proliferation
	Onalan G *et al* [138]	Animal study	Significant decreases in the mean implant areas and significant decreases in serum and peritoneal TNF-α levels	NAC elevates GSH levels and improves the toxic effects of ROS, a protection that is related to scavenging of free radicals
	Ray K *et al*. [139]	*In vitro* + Animal study (samples obtained from endometriosis patients)	An abundance of oxidatively modified lipoproteins in the PF of women with endometriosis. Antioxidant supplementation lessened endometriosis-related pain	Oxidized lipoproteins (L1–L2) generated in the presence of NAC.
	Foyouzi N *et al*. [135]	*In vitro*	Inhibition of thymidine Incorp and proliferation of endometrial stromal cells	Decreased ROS activity
	Wu *et al*. [136]	*In vitro*	Cell proliferation assay demonstrated an anti-proliferative effect.PR-A, PR-B, AR, and FasL expression were all increased as compared with untreated cells	Decreased ROS activity mainly H_2H_2 reduction
	Pittaluga *et al*. [137]	*In vitro* + Animal study	NAC reduced endometrioma mass, reduced the immunohistochemical staining of the inflammation-related COX-2 protein and decreased MMP-9 expression and activity	Switching cell behavior from proliferation toward differentiation, and decreased both tissue inflammation and cell invasiveness
	Porpora *et al*. [140]	Clinical study	Reduction in endometrioma size, pain reduction, decrease in cell invasive behavior and a decrease in the inflammatory COX-2	Increase in proteins of cell-cell junction complex such as E-cadherin and β-catenin

EGCG: Epigallocatechin-3-gallate; ERK: Extracellular regulated kinase; ESR1: Estrogen receptor α; MDA: Malondialdehyde; NAC: N-acetyl-l-cysteine; NO: Nitric oxide; OH: Hydroxyl; PF: Peritoneal fluid; TIMP: Tissue inhibitors of matrix-metalloproteinases.

prospective cohort study of 1383 patients. The assumption was that if these agents effectively influence the pathophysiology of endometriosis, the prevalence of endometriosis in the Vitamins C and E consuming population would be lower. Indeed, the authors reported that Vitamins C and E obtained through food sources were inversely related to the diagnosis of endometriosis. Nevertheless, no association between the intake of these nutrients from supplements alone and endometriosis was observed.

The effect of Vitamins C and E supplementation on peripheral OS markers of endometriosis patients was evaluated by Mier-Cabrera *et al.* [95] in a randomized, double-blind trial by treating endometriosis patients with Vitamins C and E or placebo for 6 months and further evaluating the levels of malondialdehyde (MDA) and lipid hydroperoxides (LOOHs) as peripheral OS markers. Significantly decreased levels of MDA and LOOHs were observed after 4 months and 6 months, respectively, associating Vitamins C and E supplementation with a reduction in OS markers of women diagnosed with endometriosis. However, despite noting the OS markers' reduction, pregnancy rate, did not improve during or after the intervention [95].

The actual clinical effects of Vitamins C and E on endometriosis patient's symptoms were studied in 59 patients, all of whom were diagnosed with chronic endometriosis-related pelvic pain and were planned for surgical intervention. Patients were randomly assigned to either oral supplements of Vitamins C and E or a placebo. After 8 weeks of treatment, a significant decrease in PF stress markers, interleukin-6 and monocyte chemotactic protein-1 was detected. Moreover, a significant reduction in chronic pelvic pain was reported in the study group as compared to the placebo group. Clinical improvement in dyspareunia was observed, although it was not statistically significant ($p = 0.09$) [96].

8. Resveratrol

Resveratrol (trans-3,5,40-trihydoxystilbene) is a natural polyphenolic flavonoid synthesized by plants subsequent to ultraviolet radiation. Resveratrol is found plentifully in seeds and the skin of grapes, in mulberries and in red wine. The antineoplastic, anti-inflammatory and antioxidant effects of Resveratrol are well established [97]. Resveratrol is already used in the treatment of several clinical conditions such as cardiovascular diseases, cancer, type-2 diabetes mellitus and neurodegenerative diseases.

Resveratrol has several mechanisms of action that are applicable to endometriosis. Its anti-inflammatory effect is achieved through inhibition of cytokine (tumor necrosis factor-α, IL-6, IL-8), VEGF and monocyte chemotactic protein 1 as well as the inhibition of ROS production in monocytes, macrophages and lymphocytes (**Figure 3**). Moreover, resveratrol was found to affect cell proliferation and apoptosis by inhibiting the NF-κB [98]. As endometriosis is an estrogen-dependent disease the effect of resveratrol on the endometrium is highly relevant. Both eutopic and ectopic endometrium in women with endometriosis overexpress estrogen receptor α (ERα; ESR1) and estrogen receptor β (ERβ; ESR2) [99]. Resveratrol is recognized to have various actions, both agonist and antagonist, in different tissues [97]. Another relevant effect of resveratrol is anti-angiogenesis by reducing VEGF levels [100]. Hence, since resveratrol's mechanisms of action may overlay the pathophysiology of endometriosis (**Figure 3**), several studies have been conducted to explore the impact of resveratrol on endometriosis (**Table 1**).

The influence of resveratrol on the endometrium both *in vitro* and *in vivo* was studied by Amaya *et al.* [97]. Using a well-differentiated endometrial cancer cell line (Ishikawa), the comparative estrogenicity of resveratrol was studied *in vitro* by an alkaline phosphatase assay. The activity of the proliferative marker Ki-67 as well as the expression of ESR1 was examined *in vivo* on xenograft implants of human endometrial tissue in ovariectomized immunodeficient RAG-2-g(c) mice, after 30 days of treatment with subcutaneous E2, E2 plus progesterone, or E2 plus different doses of resveratrol. The authors reported that when combined with E2, low concentrations of resveratrol acted as an estrogen agonist as opposed to inspected antagonist activity when high doses of resveratrol were combined with of E2. Moreover, in the high-dose resveratrol group, the endometrial epithelial cells had a significant reduced expression of ESR1 and proliferative activity. The authors concluded that at high doses, resveratrol probably has the ability to decrease proliferation of human endometrium through ESR1 [97].

In continuation to the positive reported effect of resveratrol on endometriosis [101], similar encouraging results of reduction in endometrial lesions size, cell proliferation, vascularization, inflammation, OS and lipid peroxidation markers were recently reported in several animal studies [102-106]. Furthermore, a clinical benefit of resveratrol in a group of 12 patients who failed to obtain pain relief during the use of combined oral contraceptives was reported. The addition of resveratrol to the treatment milieu resulted in a significant decrease in pain scores, with 82% of patients reporting complete resolution of dysmenorrhea and pelvic pain after 2 months [107]. Despite the impressive results of this study, the inherent limitation of the small sample size warrants further investigation to validate the described effect.

9. Melatonin

Melatonin, *N*-acetyl-5-methoxytryptamine, is a main secretory product of the pineal gland synthesized from tryptophan. It is predominantly secreted during the night, and its potent antioxidant effects are well-established. Melatonin was shown to be both a powerful free-radicals scavenger and an antioxidant enzymes stimulator with potent anti-inflammatory attributes [108]. Melatonin's mechanisms of action as an antioxidant that can be implied on endometriosis include a direct scavenging effect on the toxic *OH, hydrogen peroxide and NO* radicals [109].

A significant mechanism of melatonin to reduce the OS is through the down-regulation of MMPs. Previous studies demonstrated elevated MMP-3 levels in the ectopic endometrium [110] and imbalanced MMP-9/TIMP levels in the eutopic endometrial tissue of women with endometriosis [111,112]. Inspecting the mechanism of action of melatonin in endometriosis, Paul *et al.* [64] reported of an arrest of lipid peroxidation and protein oxidation as well as a down regulated proMMP-9 activity and expression after applying melatonin to rats with endometrial lesions (**Figure 3**). Furthermore, the lessened activity of proMMP-9 was related to an elevation in TIMP-1 expression [64].

While inspecting the effect of melatonin on endometrial lesions several animal studies reported positive results with a significant reduction in lesion size and other endometriosis-related markers [113,114]. Moreover, compared to other new antioxidant agents suggested for the treatment of endometriosis, melatonin is relatively ahead with recent results of its effect on endometriosis (**Table 1**). Investigating the efficacy of melatonin in the treatment of endometriosis on human subjects, Schwertner *et al.* recently reported the results of a Phase II, randomized, double-blind, placebo-controlled trial in which the effects of melatonin on 20 endometriosis patients was compared with 20 controls using placebo [115]. A significant reduction of 38 – 39% in the reported dysmenorrhea and dysuria was observed. Sleep quality was improved, the use of analgesic agents was reduced by 80% and brain-derived neurotrophic factor levels were reduced autonomously of its effect on pain.

10. Xanthohumol

Xanthohumol (2′,4′,4-trihydroxy-6′-methoxy-3′-prenylchalcone, Xn) is a polyphenol chalcone from hops (*Humulus*

lupulus) and is an active component in beer [116]. It is known for its antioxidant and anti-inflammatory effects and is therefore widely studied as an anticarcinogenic agent. The antioxidative and anti-inflammatory effects are achieved by the inhibition of NF-κB signaling pathway [117]. Also, it is known to inhibit NO production by suppressing the expression of NO synthase [118]. Since endometriosis is an estrogen-dependent disease, the affinity of the previously described hop derivatives to human estrogen receptors would limit the potential use of these agents. Fortunately, previous studies in the cancer field showed that xanthohumol does not have this affinity and what is more, it decreases estrogen production by inhibiting aromatase activity [119].

The effect of Xanthohumol on endometriotic lesions was investigated by Rudzitis-Auth *et al.* (**Table 1**) [120]. Endometriotic peritoneal lesions were surgically induced in mice. Xanthohumol was administered through drinking water 3 days prior to the tissue transplantation. Xanthohumol significantly reduced the size of these lesions, unrelated to their localization within the peritoneal cavity. Furthermore, in the Xanthohumol-treated group, a significant suppression of vascularization of the lesions was observed as indicated by a significantly lesser microvessel density compared with the control group. The authors concluded that Xanthohumol is a promising treatment option through diet intake that may be considered in the future for selective treatment of endometriotic lesions [120].

11. Epigallocatechin-3-gallate

Epigallocatechin-3-gallate (EGCG) is the most abundant polyphenol found in green tea. It has strong antioxidative, antimitotic and antiangiogenic properties [121]. Leaves of the tea plant *Camellia sinensis* which contains high nutraceutical values are used to prepare the green tea. EGCG was demonstrated to affect several carcinogenesis mechanisms such as mutation, cell proliferation, cell invasion and apoptosis [122]. Some of these mechanisms are common to endometriosis, and thus, its effect on endometriosis has been studied both *in vitro* and *in vivo*.

The main relevant mechanism of action of EGCG acts by reducing OS via inhibition of angiogenesis through VEGF reduction (**Figure 3**). Laschke *et al.* [123] investigated the effect of EGCG on estrogen-induced activation of endometrial cells *in vitro* and its effect on endometriotic lesions *in vivo* (**Table 1**). EGCG suppresses estrogen-stimulated activation, proliferation and VEGF expression of endometrial cells. Likewise, blood perfusion and angiogenesis were inhibited *in vivo* without affecting blood vessel development in ovarian follicles. The regression of the endometriotic lesions was further histologically confirmed [123]. Moreover, comparing the effect of EGCG and Vitamin E on angiogenesis in an animal study, EGCG, but not Vitamin E was found to inhibit angiogenesis in the endometriotic implants [124].

One of the consequences of endometriosis is the formation of fibrosis due to the increased inflammatory and OS. In order to assess the effect of EGCG on fibrosis, Matsuzaki *et al.* reported that treatment with EGCG significantly inhibited cell proliferation, migration and invasion of endometrial cells from patients with endometriosis. Moreover, the treatment reduced mRNA expression of fibrotic markers in both endometriotic and endometrial stromal cells. Animal experiments showed that EGCG prevented the progression of fibrosis in endometriosis [125].

The major disadvantage of EGCG is marked unstableness and poor bioavailability [126]. A synthetic derivative of EGCG, obtained by acetylation of EGCG, was suggested to be used as a pro-drug of EGCG with improved stability and better bioavailability [127]. The researchers compared the effect of saline, Vitamin E, EGCG or pro-EGCG on mice with subcutaneously transplanted endometrium. Histological, microvessel and apoptosis examinations were performed. The authors reported a significant decrease in the growth of endometrial implants, reduced lesion size and weight, inhibited functional and structural microvessels in the lesions, and enhanced lesion apoptosis at the end of interventions in the EGCG and pro-EGCG group. The pro-EGCG caused a significantly greater inhibition in all the angiogenesis parameters compared to EGCG. Furthermore, a better bioavailability, greater antioxidation and anti-angiogenesis capacities were noted in the pro-EGCG compared to the EGCG group. Vitamin E had no effect on the inspected endometriosis parameters. In their conclusion, the authors stated that pro-EGCG has a high efficacy, bioavailability, antioxidation and anti-angiogenesis capacities and could be a potent anti-angiogenesis agent for endometriosis [127].

12. *N*-acetyl-L-cysteine

N-acetyl-L-cysteine (NAC) is a thiol antioxidant and the precursor of glutathione [128]. It is involved in several metabolic pathways. Apart from its ability to protect against damage from both hydroperoxides [129] and other alkylating agents [130], it also has anticarcinogenic properties [131] and the ability to limit tissue invasion by cancerous cells [131]. The mechanism of action of NAC is mostly based on activation of the immune system. NAC has been reported to increase IL-2 levels and the expression of CD25 on T cells [132]. By increasing glutathione levels, it suppresses the activation of NF-κB, an important transcriptional factor [132]. NF-κB plays a major role in endometriosis pathophysiology (**Figure 3**). Both NAC and the glutathione systems synergistically upsurge the cytotoxic effect of the lymphokine-activated killer cells and the natural killer cells by the elevation of IL-2 [133,134]. Another reported mechanism in which NAC inhibits cancer cells is through sustained increase of membrane TNF-α expression. It was reported to increase membrane TNF-RI and TNF-RII in cancer cell lines and in T cells after stimulation [128].

The similar characteristics of cancer and endometriosis have led to the investigation of the use of NAC, which had been experimentally used for cancer treatments, for endometriosis treatment. In a combined *in vitro* and *in vivo* study described earlier, Ngô *et al.* [38] demonstrated a higher endogenous OS with an evidently increased ROS production along with decreased catalase levels, similar to that of cancer cells. These higher ROS levels correlated with activation of the ERK1/2 system and in turn an increase in cellular proliferation. Applying NAC abolished those findings in both the *in vitro* and animal model of endometriosis [38].

NAC was reported to inhibit proliferation of endometrial cells [135,136]. It was also reported to reduce proliferative capabilities through MMP-9 reduction [137]. In a prospective randomized animal study [138], an autologous transplantation of endometrial tissue to the abdominal cavity was performed on 40 rats. After 3 weeks of growth, laparotomies were performed. Treatment with several agents including NAC was initiated and 3 weeks later, re-laparotomies were performed. Also, levels of TNF-α from serum and PF were evaluated. NAC was reported to significantly decrease endometriotic lesions areas and to significantly reduce serum and peritoneal TNF-α levels [138].

The impact of NAC on endometriosis-associated pain was studied in a trial involving samples from endometriosis patients and controls undergoing a surgical procedure. PF from 43 women with endometriosis and a control group was tested for lipoprotein-derived oxidation-sensitive pain molecules and for the ability of antioxidants to suppress this lipoprotein-induced nociception. A higher amount of modified lipoproteins was observed in the PF of women with endometriosis. An animal model proved that the oxidatively modified lipoproteins did induce a pain-related behavior. The authors reported that treatment with NAC as well as other anti-inflammatory and antioxidant drugs suppressed the pain-inducing capability of the lipoproteins. The proposed mechanism was that of an inhibition of oxidation of LDL by the treatment agents [139].

In an observational clinical study, Porpora *et al.* [140] examined the effect of NAC treatment on women scheduled for surgery due to endometriomas. A total of 47 patients were treated with NAC compared to 45 women in the untreated group. A significant reduction in cyst size was observed in the NAC treatment group compared to an increase in endometrioma diameters in the control group. Moreover, 24 women of the NAC-treated group versus 1 in the control group cancelled their scheduled laparoscopy by reason of endometrioma decrease and/or relevant pain reduction. The authors concluded that NAC should be considered as an effective treatment for endometriosis, without side effects [140].

13. Conclusion

Endometriosis is still an enigmatic disease in term of pathophysiology and as a consequence its treatment remains controversial. Being recognized as an estrogen-dependent disease, most of the current treatment modalities aim estrogen reduction as goal of treatment. The outcomes of the treatment are menopause like side effects and symptom return upon discontinuation of the treatment. The evolving data about targeting the reduction of OS as the treatment goal in these patients looks promising. All the reviewed antioxidative stress agents showed a significant inhibitory effect on different studied aspects of the development and progression of endometriosis in both *in vitro* and *in vivo* studies. Since OS reduction in endometriosis is relatively new, only the minority of the studies were performed on human subjects. Nonetheless, the studies that did involve human patients reported significant results.

In conclusion, the endometriosis treatment by targeting a reduction in OS appears to be a promising strategy; however, this approach requires additional human clinical trials.

14. Expert opinion

Endometriosis is a debilitating gynecologic disease which occurs with a relatively high prevalence and which is described by the presence of implanted endometrial tissue outside the uterine cavity. Despite endometriosis having been first described a long time ago, its etiology remains unclear. Over the course of time, several optional theories have been suggested as the pathophysiological basis of the disease. However, as yet, no single suggested theory can provide a comprehensive clarification for the pathophysiology of this enigmatic disease. The lack of its clear pathophysiology challenges the detection of a proper curative treatment for endometriosis. Nevertheless, in recent years, a vicious cycle in which: a) OS is produced and thus facilitates the pathways that enable implantation and survival of the endometrium in the peritoneal cavity, and which b) on the other hand, augments further OS production was reported.

Accumulating evidence indicates the effectiveness of targeting OS reduction as the primary goal of treating endometriosis regardless of its exact role in the disease pathophysiology – whether it is the cause or the consequence. Fortunately, some of the shared common pathways between endometriosis and endometrial cancer has led to a vast advancement of research regarding endometriosis.

In this review, we focused on antioxidative treatment options beginning with long-standing antioxidative agents such as vitamins C and E to more recently studied agents such as melatonin, resveratrol, xanthohumol and EGCG. As explained in this review, all these agents have already been reported to negatively affect endometriosis including reduction in OS markers, reduction in lesion size and implantation, enhanced apoptosis while some of the older agents elicited a clinical improvement.

However, there are weaknesses in the research studies that have been performed so far. The first is that, most of studies investigating the antioxidant agents were performed either

in vitro or on animal subjects. Endometriosis studies in humans are challenging to perform as the measured effect of the treatment on the actual peritoneal lesion will necessitate surgical interventions. Instead, measurement of the efficacy of endometriosis treatment can be achieved through secondary peripheral markers or the subjective responses of the patients, in terms of clinical symptoms such as pain, thus weakening the acquaintance of the direct effect of the treatment. Clearly, for future studies a crucial need is to develop innovative non-surgical techniques to measure the treatment efficacy on humans.

The second weakness of the current data is the knowledge regarding the administration route of the treatment for human patients. While studying animal models, the treatment can be given relatively easily using a parenteral route, unlike administration in chronically ill human patients. Therefore, in our opinion, easily administered drugs like EGCG pro-drug – that can be consumed orally and have a greater bio-availability – will have a weighty advantage over other drugs that were not yet tested for the route of administration in human subjects.

The biggest achieved research discovery so far is the understanding that reducing the OS will break the vicious cycle of endometriosis, thus improving the quality of life of the patient, although it will not result in a complete cure. Obviously, as stated above, further studies on human subjects are needed to enforce the findings of the *in vivo* and animal studies.

Nonetheless, some additional questions should be addressed. The first is to find a method to use the peripheral OS markers to diagnose and to evaluate the treatment efficacy in endometriosis patients. As of today, diagnosis of endometriosis is achieved through laparoscopic surgical procedure and the efficacy of the treatment is measured by the patient's subjectively reported symptoms. If a way can be found to achieve this critical information in a non-surgical way (for diagnosis) and an objective measure (for treatment efficacy), then a great advancement would have been achieved. The second issue to address is whether targeting OS as the treatment goal will improve fertilization. Endometriosis, as part of its pathophysiology will cause infertility in some of the patients. As for now, many of the treatment agents used aim to eliminate the estrogenic environment in endometriosis patients and therefore must be ceased as and when the patient desires pregnancy. Treating OS will not contraindicate the ability to conceive during treatment and furthermore, may resolve some of the pathologic pathways leading to infertility.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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