The molecular connections between the cannabinoid system and endometriosis

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ABSTRACT: The endocannabinoid system consists of an array of endogenously produced bioactive lipids that activate cannabinoid 1 (CB1) and 2 (CB2) receptors. Alterations of this system have been described in almost every category of disease. These changes can be protective or maladaptive, making the endocannabinoid network an attractive therapeutic target. Little is known about the potential role of endocannabinoids in endometriosis development although this is a topic worthy of further investigation since endocannabinoid modulators have recently been shown to affect specific mechanisms critical to endometriosis establishment and maintenance. A literature review was herein performed with the aim of defining the regulation and function of the endocannabinoid signaling in in vitro and animal models of endometriosis. The components of the endocannabinoid system, CB1 and CB2 receptors and the enzymes N-acylphosphatidylethanolamine-phospholipase D and fatty acid amide hydrolase are differentially regulated throughout the menstrual cycle in the endometrium and are expressed in deep endometriotic nodules and in sensory and sympathetic neurons innervating the lesions. Selective cannabinoid receptor agonists, such as WIN 55212-2, appear to have a favorable action in limiting cell proliferation and in controlling pain symptoms. Conversely, endometrial cell migration tends to be stimulated by receptor agonists. The phosphatidylinositol 3-kinase/Akt and extracellular signal-regulated kinase 1/2 pathways seem to be involved in these processes. However, the underlying mechanisms of action are only just beginning to unfold. Given the complexity of the system, further studies are needed to clarify whether the endocannabinoid system might represent a promising target for endometriosis.

Key words: endometriosis / endometrium / cannabinoid / anandamide

Introduction

Endometriosis is defined as the presence of endometrial-like tissue outside the uterus, which induces a chronic inflammatory reaction. Population-based studies estimated the annual incidence of endometriosis to be between 0.1 and 0.3%. This equates to 176 million women worldwide who have to deal with the symptoms of endometriosis during the reproductive years of their lives. As for today, endometriosis is still an unmet clinical need since an optimal drug that allows both pain management and continued attempts to conceive does not exist. The current medical treatments are effective in relieving pain, often with relatively short-term effect (Vercellini et al., 2009). Yet, they have many undesirable and sometimes severe side effects that limit the long-term management of the disease. An ideal medical treatment would eliminate endometriotic lesions, prevent recurrence and not impede ovulation. Consequently, novel therapeutics capable of accomplishing these needs are surely required (Guo et al., 2009).

The recent understanding of the strong mutual interaction between the local inflammatory process and the hormonal system at molecular level has led to consideration of the enzyme aromatase as a potential therapeutic target. However, results with this target were less clinically promising than previously envisioned, mostly because of the need to use other compounds in association with aromatase inhibitors during treatment (Vercellini et al., 2011). Other therapeutic targets derived from the molecular analysis of the endometriotic lesions have received some attention but limited subsequent development (Lebovic et al., 2010) or have failed in preliminary clinical trials (Koninckx et al., 2008).

Cannabinoids and modulators of the endocannabinoid system have recently been shown to affect specific mechanisms (inflammation, cell proliferation and cell survival) that are critical to endometriosis establishment and maintenance (Pertwee, 2009).

The endocannabinoid system, composed mainly of bioactive lipids and their receptors, is uniquely poised to respond locally to a
disease status since alterations in endocannabinoid levels and cannabinoi
d receptors expression appear to be a widespread response to the pathologic process. In some diseases such as multiple sclerosis, the up-regulation of the endocannabinoid system is thought to have a protective role by inhibiting the progression of the disease; conversely, in other diseases such as liver fibrosis and colorectal cancer, modulation of the receptor expression is maladaptive (Miller and Devi, 2011). The study of the mechanisms of the cannabinoid system regulation not only sheds light on the pathophysiology of a disease but is also interesting from a therapeutic perspective. Cannabinoid receptor agonists might be beneficial in diseases in which receptor activation has a protective effect; alternatively, antagonists might be useful in those situations in which receptor activation has been shown to be associated with the pathogenesis of the disease (De Petrocellis et al., 2004).

Given the potential interest but the paucity of available data on the role of the endocannabinoid system in endometriosis, the present review aims at uncovering molecular connections between the disease pathogenesis and the endocannabinoid signaling. This with the ultimate purpose of understanding whether pharmacological therapies targeting the endocannabinoid system might be considered for the treatment of endometriosis.

Materials and Methods

We searched the electronic databases MEDLINE (1966 to July 2012) and EMBASE (1985 to July 2012) using the Medical Subject Heading (MeSH) term ‘endocannabinoid’ OR ‘cannabinoid’ OR ‘anandamide’ OR ‘methan-
andamide’ combined with ‘endometriosis’ OR ‘endometrium’. Furthermore, we reviewed reference lists of retrieved articles to search for more studies.

Results

A total of 21 studies were identified in the search. Thirteen referred to pregnant uterus, peripheral levels or endometrial cancer and were not considered. Three studies have evaluated the expression of the com-
ponents of the endocannabinoid system in normal human endometrium (Lec
tone et al., 2010; Taylor et al., 2010a; Resuehr et al., 2012) and one in the eutopic endometrium of patients affected by endometriosis (Resuehr et al., 2012). Four studies addressed the effect of the system on specific pathways, functions and symptoms associated with the disease (Table 1). These studies will be herein described in a broad context that includes what is it currently known about the endocannabinoid system-based therapies in other diseases as well as an overview of the various molecular events leading to endometriosis establishment on which cannabinoids might actually play a role. Special attention will also be directed to unex-
plored areas requiring further investigation.

The endocannabinoid system

Endocannabinoids are implicated in a variety of physiological and pathological conditions including inflammation, immunomodulation, analgesia and cancer (Guindon and Hohmann, 2011).

The main active ingredient of cannabis, Δ⁹-tetrahydrocannabinol (Δ⁹-THC), produces its effects through activation of G-protein-

Table I Effects of cannabinoids on endometrial functions and in endometriosis.

<table>
<thead>
<tr>
<th>Cannabinoid ligands</th>
<th>Action</th>
<th>Measured effect</th>
<th>Reference</th>
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</table>
| WIN 55212-2        | Agonist CB1/CB2 | Decreased cell proliferation in vitro and in vivo | Lec
tone et al. (2010) |
| AM251              | Antagonist/inverse agonist CB1 | Increased endometriosis associated hyperalgesia | Dmitrieva et al. (2010) |
| Methanandamide     | Agonist CB1 | Induced endometrial stromal cell migration | Gentilini et al. (2010) |
| Δ⁹-THC N-arachidonyl glycin anandamide | Agonist CB1/CB2 | Induced migration of HEC-1B (human endometrial cell line) | McHugh et al. (2011) |

coupled cannabinoid 1 (CB1) and 2 (CB2) receptors (Muccioli, 2010). The CB1 receptor is expressed mainly in the brain, particularly in areas that are involved in control of motor activity (cerebellum and basal ganglia), memory and cognition (cortex and hippocampus), emotion (amygdala), sensory perception (thalamus) and in the hypo-
thalamus, pons and medulla but also in other tissues such as testis, eye, vascular endothelium, spleen and uterus (Howlett et al., 2004; Venance et al., 2004). The CB2 receptor is expressed abundantly in the immune system, both by circulating cells and in tissues, as spleen and lymph nodes (Pertwee, 1997; Gardner et al., 2002). Other tissues expressing the CB2 receptor are intestine, lung, uterus, pancreas and skin (Onaivi et al., 2002; Casanova et al., 2003).

The discovery of the receptors that mediate the effects of the can-

nabinoiids prompted a search for the endogenous ligands activating them under physiological conditions, the so-called endocannabinoids. Endocannabinoids are endogenous lipid-signaling molecules that are generated in the cell membrane from phospholipid precursors. The most important is the N-arachidonylethanolamide or anandamide, which is the amide of arachidonic acid and ethanolamine (Devane et al., 1992). Anandamide is an agonist with affinity for both CB1 and CB2 receptor, and it is present in those regions where the CB1 receptor is expressed (Felder et al., 1996). A second arachidonic-acid derivative is the 2-arachidonoylglycerol with similar affinity for both CB1 and CB2 receptor with a tissue distribution similar to anandamide (Kondo et al., 1998).

The discovery of the endocannabinoid system stimulated the develop-

ment of CB1- and CB2-selective receptor agonists and antagonists. There is an extensive pharmacopoeia of cannabinoid receptor ligands and the complete list can be found elsewhere (Greeneisen and Turner, 2010). Classic CB1/CB2 receptor agonists include Δ⁹-THC, derived from the plant Cannabis sativa, while synthetic agonists include HU-210 and methanandamide. Other non-classical agonists are represented by CP55940 and aminoalkylindole derivatives, like WIN 55212-2.
Among the antagonists, the group of SR141716A (SR1), AM251, AM281 and AM630 act as inverse agonists with a stronger effect compared with neutral antagonists (Pertwee, 2005).

Unlike cannabis, which has been used as a medicine over many centuries, individual cannabinoid receptor agonists have entered into the clinical practice only in the last 30 years. Nabilone (Cesamet®), a CB1/CB2 receptor agonist, was licensed in 1981 for chemotherapy-associated nausea. Δ⁹-THC (Marinol®, dronabinol) was approved in 1985 as an antiemetic drug and for excessive weight loss in acquired immune deficiency syndrome patients. In 2005, nabilone and dronabinol were joined by the cannabis-based medicine Sativex® for the treatment of oncological pain and for neuropathic pain in patients with multiple sclerosis (Pertwee, 2009).

CB1 receptor antagonists have also been developed and have been tested in several clinical trials. Rimonabant successfully completed four Phase III trials and was put on the market in the European Union and in several other countries between 2006 and 2008 as a complement to diet and exercise for body-weight reduction in obese patients (Scheen et al., 2006). Approval of the drug was officially withdrawn by the European Medicines Agency on 2009 for its side effects on the central nervous system (www.emea.europa.eu). Indeed, at present, the use of some modulators of the endocannabinoid system still have safety restrictions as they might raise serious concerns because of their psychoactive effects.

Finally, in the specific context of endometriosis, the safety profile of these compounds should also be verified in relation to the maintenance of fertility potential. There are several reviews that have extensively addressed this issue and dealing with this topic is well beyond the aim of the present review (Wang et al., 2006; Maccarrone, 2009). It should be considered that the preimplantation embryo, oviductal embryo transport, blastocyst development and implantation and placentation are all potential targets of cannabinoid ligand-receptor signaling. Interestingly, lower levels of anandamide are beneficial to embryo implantation, whereas non-receptive uterine areas have high anandamide levels. Therefore, safety issues with respect to implantation and pregnancy should be taken into consideration for potential future therapeutic developments in this area.

**Metabolism of cannabinoids**

The endocannabinoids, unlike neurotransmitters, are synthesized and secreted ‘on demand’. Owing to their lipidic structure, they are liberated into the extracellular space immediately after their synthesis, as no evidence exists for their storage in secretory vesicles (De Petrocellis et al., 2004). Anandamide is synthesized from its precursor N-acylphosphatidylethanolamine (NAPE) by the NAPE-phospholipase D (NAPE-PLD) (Di Marzo et al., 2004).

Once in the cytosol, endocannabinoids are degraded by different enzymatic pathways. Anandamide is degraded mainly by the fatty acid amide hydrolase (FAAH) (Ueda et al., 2000), an enzyme of the amidase family, localized in the membrane of diverse organelles and highly expressed in the brain, liver, kidney, intestine, testis, lungs and spleen (Cravatt et al., 1996).

**Endocannabinoid system expression in normal and ectopic endometrium**

The expression of the individual components of the endocannabinoid system was initially demonstrated in mouse uteri (Das et al., 1995; Paria et al., 1999, 2001) and then also in humans (Taylor et al., 2010a). Moreover, several studies since 1975 have evaluated the effect of cannabinoids on the uterus in animal models (Chakravarty et al., 1975; Paria et al., 1992, 1994, 1999; Das et al., 1993, 1995). In general, these studies tend to support the idea, albeit not consistently (Bloch et al., 1978), that cannabinoids have a weak estrogenic effect on endometrium and uterus (Paria et al., 1992, 1994). They also found that, within the reproductive tract, the uterus contains the highest concentrations of anandamide (Das et al., 1995).

The main components of the endocannabinoid system (CB1 and CB2 receptors and the enzymes NAPE-PLD and FAAH) coexist in many cell types in the human endometrium (Taylor et al., 2010a):

- According to Taylor et al. (2010a), CB1 receptor immunoreactivity was more intense in the glandular epithelium compared with the stroma and its expression was not regulated throughout the menstrual cycle. These findings are in contrast with the recent observations by Resuehr et al. (2012), who recently reported a dramatic increase of CB1 receptor mRNA and protein in normal endometrial samples in the secretory phase due to the ability of progesterone to regulate this receptor expression (Resuehr et al., 2012).
- CB2 receptor immunoreactivity was found in both glands and stroma; its expression was shown to be minimal at the beginning of the cycle with the most intense expression during the late-proliferative phase (Taylor et al., 2010a).
- The enzyme NAPE-PLD was shown to be intensively expressed in the menstrual, early-proliferative and late secretory glands with its lowest levels in the early-secretory phase. This enzyme was also found in the stroma (Taylor et al., 2010a).
- Glandular expression of the enzyme FAAH was intense during the menstrual phase, was reduced to a significant degree in the early-proliferative phase to the mid-secretory phase and then it tended to increase during the late-secretory phase. Similar FAAH expression was found in the stroma (Taylor et al., 2010a).

The expressions of these two enzymes in the endometrium suggest their critical role in controlling anandamide concentration during the menstrual cycle which is lower in the mid-luteal phase, consistent with the idea that low levels are beneficial and high levels detrimental, to blastocyst development (Taylor et al., 2010b).

The single study that has evaluated the expression of CB1 receptor in eutopic endometrium of patients affected by endometriosis found minimal CB1 receptor mRNA and protein levels in these samples compared with those of control healthy women independently from the phase of the cycle. This reduced expression has been attributed to the ability of persistent environmental toxicants and interleukin (IL)-1α to disrupt normal endometrial CB1 receptor expression via the induction of a progesterone resistance phenotype in patients affected by the disease (Resuehr et al., 2012).

Data from ectopic endometrium are very scanty and poorly detailed. Leconte et al. (2010) showed that CB1 and CB2 receptors are present in epithelial and stromal cell lines derived from deep-infiltrating endometriotic nodules from women with endometriosis (Leconte et al., 2010). Dmitrieva et al. (2010), using a rat model of endometriosis, showed that the CB1 receptor was expressed in both somata and fibers of sensory and sympathetic neurons that innervate endometriosis lesions (Dmitrieva et al., 2010). Therefore, in order to target the endocannabinoid system as a possible therapeutic...
Proliferation/apoptosis in endometriosis and the cannabinoid system

Endometriosis is being recognized as a condition in which ectopic endometrial cells exhibit abnormal proliferative and apoptotic regulation in response to appropriate stimuli. Diverse signaling pathways have been studied in endometriosis that correlate with the abnormal proliferation or growth of the endometrial cells. Cannabinoids are indeed involved in the regulation of these same pathways.

One of these pathways is represented by the phosphatidylinositol 3-kinase (PI3K)/Akt pathway. PI3Ks are enzymes primarily involved in the phosphorylation of membrane inositol lipids, mediating cellular signal transduction. The activation of PI3K generates the second messenger phosphatidylinositol (3–5)-trisphosphate from phosphatidylinositol 4,5-bisphosphate, which in turn recruits proteins to the cell membrane, including Akt, a serine-threonine kinase, resulting in its phosphorylation. Upon activation, Akt can phosphorylate more than 50 different substrates including mediators of apoptosis such as Bax, Bad and caspase 9 that are inhibited upon phosphorylation. This signaling pathway can be negatively regulated by the phosphatase and tensin homolog (PTEN), a major negative regulator of the PI3K pathway and a tumor suppressor gene associated with many carcinomas (Myers and Tonks, 1997).

A decreased PTEN expression has been shown in eutopic and ectopic endometria of patients with endometriosis (Fig. 1). In normal endometrium, PTEN immunostaining was strong and homogeneous in glands; conversely, in eutopic and ectopic endometrium of patients affected by the disease, PTEN immunostaining was weak or negative, and this correlated with a high activity of mitogen-activated protein kinase family (MAPK)/extracellular signal-regulated kinase (ERK) signaling, PI3K/Akt signaling and nuclear factor κB signaling (Zhang et al., 2010) (Fig. 1).

More recently, constitutive activation of the Akt/mTOR pathway (mammalian target of rapamycin) has been demonstrated in deep-infiltrating endometriotic lesions and this was related to the high proliferation rate of both eutopic endometrial cells from endometriosis patients and deep-infiltrating endometriotic cells. Accordingly, Akt/mTOR pathways inhibition using temsirolimus could decrease endometriotic cell proliferation both in vitro and in vivo in a mouse model of deep-infiltrating endometriosis (Leconte et al., 2011).

Aberrant apoptosis of endometrial and endometriotic cells has been also associated with endometriosis. Endometrium from affected women was shown to be resistant to apoptosis due to an increased expression of the anti-apoptotic factor Bcl-2 and a reduced expression of the pro-apoptotic factor Bax (Meresman et al., 2000). Survivin, a member of mammalian inhibitors of apoptosis family, was also found to be up-regulated in endometriotic lesions (Ueda et al., 2002).

Over the last decade, cannabinoids and derivates have been suggested as useful inhibitors of tumor cell growth by modulating several survival pathways. The activation of the CB1 receptor has been shown to regulate members of MAPK and ERK families, the PI3K/Akt signaling pathway, c-Jun N-terminal kinase and p38.

The role of CB2 receptor was extensively studied as well. Methanandamide, as well as JWH-015, a synthetic CB2 receptor agonist, exerted anti-proliferative effects, induced cadherine synthesis and inhibited Akt pathway in the PC-3 prostate cancer cells (Olea-Herrero et al., 2009). Although the general consensus in the current literature indicates that cannabinoids have anti-proliferative effects, there are a few studies that have shown that Δ⁹-THC has a biphasic effect in cancer cells, as lower (nanomolar range) concentrations result in an increased proliferation while higher concentrations (micromolar range) cause a decreased cell proliferation. For example, Δ⁹-THC at 100–300 nM elicited a 1.2 and 2-fold increase in the proliferation rate of the lung cancer cell line NCI-H292 and the glioblastoma cell line U373-MG (Hart et al., 2004), respectively. Moreover, Sanchez et al. (2003) demonstrated that Δ⁹-THC (50–100 nM) increases the proliferation and viability of androgen-independent PC-3 cells.

Several studies also confirmed the pro-apoptotic effects of cannabinoids in different cancer cells (Freimuth et al., 2010). In hepatocellular carcinoma-2 cells, a receptor agonist induced apoptosis that was accompanied by the up-regulation of the death-signaling factors Bax, Bcl-X(S) and t-Bid and by the down-regulation of the survival factors survivin, phospho-Akt, Hsp72 and Bcl-2 (Giuliano et al., 2009).

There is only a single study that addressed the role of cannabinoids in the proliferation of endometriosis cells (Table I). Leconte et al. (2010) have shown that CB1 and CB2 receptors are expressed by epithelial and stromal cells derived from the endometrium and by deep-infiltrating endometriosis nodules. In vitro treatment with the agonist WIN 55212-2 at 40 μmol/l had an anti-proliferative effect, which was stronger on stromal cells deriving from eutopic samples from affected women and...
from deep-infiltrating nodules than on stromal cells deriving from healthy controls. This anti-proliferative effect was mediated through a mechanism involving a reduction in reactive oxygen species production and the inactivation of the Akt pathway. The effectiveness of WIN 55212-2 in vivo was tested in a mouse model of deep-infiltrating endometriosis. Nude mice were grafted with fragments deriving from a human deep nodule, and after treatment with WIN 55212-2 (3 mg/kg, 5 days a week for 2 weeks), implant volumes were reduced compared with those that developed in untreated mice (Leconte et al., 2010).

Therefore, although the evidence is scanty, cannabinoid receptor agonists seem to affect cellular proliferation and apoptosis both in vitro and in vivo and via the same growth and survival pathways involved in endometriotic cell growth.

The migratory capacity of endometrial cells and the cannabinoid system

The invasive properties of endometrial tissue play an important role in the pathogenesis of the disease.

In endometriosis, a deregulation of the expression of specific matrix metalloproteinases (MMPs) by ectopic and eutopic endometrium has been suggested to be involved in the development of the disease. An increase in the expression of MMP-1 (Kokorine et al., 1997), MMP-2 (Wenzl and Heinzl, 1998), MMP-3 (Cox et al., 2001), MMP-7 (Rodgers et al., 1993) and MMP-9 (Chung et al., 2001; Liu et al., 2002) in endometriotic tissue has been reported. Eutopic endometrium from patients with endometriosis is characterized by a higher expression of MMP-2 and lower expression of tissue inhibitor of MMP (TIMP)-2 and TIMP-3, compared with endometrium from women without endometriosis (Chung et al., 2002).

As in endometriosis, increased expression and activation of MMPs, thought to favor cell migration and spreading, are found in almost every type of human cancer compared with normal tissue, and this has been associated with poor patient prognosis. Cannabinoids are able to reduce MMP2 levels in cells from murine gliomas (Blazquez et al., 2003) and to block the stimulatory effect of cannabinoid receptor agonists on migration of endometrial cells (Barriga et al., 2009). The effect of cannabinoid receptor agonists on migration of endometrial cells deserves further investigation. The effect on this specific process could depend on the type and concentration of cannabinoids used and/or the nature of the disease. In vivo models of endometriosis spreading might shed light on this peculiar mechanism.

Immunity/inflammation in endometriosis and the cannabinoid system

Endometriosis is a chronic inflammatory disease.

According to the results of the experiments performed in a mouse model of endometriosis, both macrophages in the peritoneal fluid and macrophages infiltrating ectopic lesions display features of alternative activation. They are actively involved in both (i) the phagocytic clearance of aged red cells and endometrial cell debris and (ii) the secretion of trophic and neo-angiogenic mediators (Capobianco et al., 2011).

Various cytokines and growth factors are increased in peripheral blood and in peritoneal fluid of women with endometriosis and in the setting of a chronic local tissue destruction, it is also likely that autoreactivity develops (Gentilini et al., 2011).

CB1 and CB2 receptor mRNAs are expressed in human and mouse immune cells in the following order: B cells > natural killer cells > monocytes > neutrophils > CD8 leukocytes > CD4 leukocytes. However, both human and mouse immune cells express the CB2 receptor at higher levels than CB1 (Parolari, 1999; Lee et al., 2001).

Psychoactive and non-psychoactive receptor ligands have demonstrated either in vivo or in vitro effects on the production and function of a variety of cytokines via both cannabinoid receptor-dependent and independent mechanisms. In a murine model, Δ⁹-THC was shown to inhibit the chemotactic response of murine peritoneal macrophages to RANTES/CC15 during an early inflammatory process via the CB2 receptor (Raborn et al., 2008). Rimonabant was shown to be able to reduce plasma levels of monocyte chemoattractant protein-1 and of IL-12 in low-density lipoprotein receptor knock out (LDLR−/−) mice, a model of atherosclerotic lesion development (Dol-Gleizes et al., 2009). The effect of Rimonabant has been proved also in in vitro models; in human umbilical vein endothelial cells, the drug has been shown to exert an anti-inflammatory effect by inhibiting tumor necrosis factor-α-induced IκB kinase α/β phosphorylation, inhibitor of kappa B-α degradation and IL-6 production (Huang et al., 2010).

Although the majority of the studies tend to support the hypothesis that the immunomodulation mediated by cannabinoids has suppressive effects, under specific conditions they may also exacerbate ongoing immune responses (Greineisen and Turner, 2010). For
example, ligation of the CB1 receptor on mast cells actually stimulates the release of inflammatory mediators and activates a pro-inflammatory transcriptional program. The promise of cannabinoid receptor agonists as a novel class of anti-inflammatory drugs has led to intensive investigation in this area particularly for the treatment of asthma, chronic obstructive pulmonary disease and Crohn’s disease. On the other hand, since the activation of CB2 receptors seems to be central to the immunosuppressive effects of cannabinoids while apparently most of their pro-inflammatory effects seem to be attributable to the expression of CB1 receptors, it is likely that the clinical promise of cannabinoids as anti-inflammatory drugs may be fulfilled only with the development of highly selective CB2 agonists (Nagarkatti et al., 2009).

Notwithstanding the various models of endometriosis-associated inflammation yet available, no data exist on the role of the endocannabinoid system in endometriosis-associated inflammation and on the possibility of targeting the endocannabinoid system to modulate the inflammatory process.

**Angiogenesis in endometriosis and the cannabinoid system**

A web of blood vessels often surrounds endometriotic implants and extrapelvic endometriosis and in line, angiogenic factors are increased in the peritoneal fluid of patients with endometriosis, in peritoneal implants and in ovarian endometriomas (Taylor et al., 2009).

The role of cannabinoids and derivatives in angiogenesis has been broadly studied. They may cause a lower vascular density of experimental tumors as assessed by the reduced presence of CD31-positive cells in experimental tumor xenografts from glioma, melanoma and non-melanoma skin cancer and lung tumor cells. Furthermore, experimental tumors from animals treated with cannabinoids were characterized by a small, undifferentiated and impermeable vascular network. In fact, numerous cannabinoids that bind to CB1 and/or CB2 receptors inhibit vascular endothelial cell survival and migration as part of their anti-angiogenic action (Kogan et al., 2006). Nonetheless, the pharmacologic effect of cannabinoid-based drugs, usually administrated at micromolar doses, does not necessarily reflect the action of endogenously produced endocannabinoids whose concentration is at most in the nanomolar range. As a matter of fact, the CB1 receptor expression is induced during angiogenesis in endothelial cells and anandamide can stimulate bFGF (fibroblast growth factor)-induced proliferation of endothelial cells in the nanomolar physiologic concentration range (Pisanti et al., 2011).

The mechanism and the extent of endocannabinoid-mediated anti-angiogenic effects have not been fully explored to date. Therefore, novel investigations need to be carried out to assess and define the possible role of the endocannabinoid system in the angiogenic process in endometriosis.

**The endocannabinoid system and endometriosis-related pain**

Endometriosis is usually associated with pelvic pain such as chronic dysmenorrhea, intermenstrual abdominal and pelvic pain, back pain, dysuria, dyschezia and dyspareunia.

The neural mechanisms of pain in endometriosis have been extensively described elsewhere (Stratton and Berkley, 2011). Three mechanisms, nociceptive, inflammatory and neuropathic, seem to be relevant to endometriosis-associated pelvic pain (Howard, 2009).

Cannabinoids have been used for thousands of years to provide relief from pain, but only recently have they been critically evaluated.
in clinical trials. Cannabinoid receptors, endocannabinoids and enzymes controlling their synthesis and degradation are localized at multiple levels of the neuraxis, from the periphery to the central nervous system. These components are present in central nervous system regions associated with pain, such as the amygdala, periaqueductal gray and the dorsal horn of the spinal cord, supporting the idea that they are positioned in the appropriate neuroanatomical locations to regulate pain (Schlosburg et al., 2009).

The effect of exogenous cannabinoid agents in endometriosis-associated pain in a rat model was recently evaluated by Dmitrieva et al. (2010). A double-labeling fluorescence immunohistochemistry revealed that >75% of sympathetic fibers positive for vesicular monoamine transporter 2 and many (50–75%) sensory fibers positive for calcitonin gene related peptide in the cysts co-labeled with an antibody for the CB1 receptor. Moreover, the CB1 receptor could be localized on the somata of sensory and sympathetic neurons that innervate the cysts, thus supporting the idea that endocannabinoids might be able to regulate lesion innervation. Interestingly, treatment of the rats with the CB1 receptor agonist WIN 55212-2 (from 1 to 3 mg/kg) decreased endometriosis-associated hyperalgesia as demonstrated by recording the electromyographic activity using a model of visceromotor reflex to vaginal distention. Conversely, the CB1 receptor antagonist AM251 (from 1 to 3 mg/kg) increased the symptomatology while the CB2 receptor antagonist AM630 (1 mg/kg) had no effect on referred nociception. These findings suggest that cannabinoids could suppress endometriosis-induced hyperalgesia via the CB1 receptor (Dmitrieva et al., 2010). A promising new direction for developing new treatments for pain suffered by women with endometriosis may arise from these data.

Conclusions
The potential role of cannabinoids in the pathogenesis of endometriosis is still to be clarified. While agonists appear to have a favorable effect in limiting cell proliferation and controlling disease-associated pain, they also tend to sustain cell migration that represents a critical process in disease maintenance.

Figure 2 shows some of the pathways and molecules involved in endometriosis establishment and development that might be affected upon pharmacological targeting of the endocannabinoid system. Since both endogenous and synthetic cannabinoids can: (i) bind to the different cannabinoid receptors with different affinities, (ii) act with receptor-independent mechanisms and (iii) have a dose-dependent effect, their biological outcomes cannot be accurately predicted. Thus, more careful and comprehensive studies are surely needed to critically evaluate the possibility of targeting the cannabinoid system in endometriosis.

Authors’ roles
A.S. and P.V. provided a substantial contribution to the review conception and drafted the article; A.M. contributed to the interpretation of the data. P.P. and M.C. critically revised the manuscript. All the authors approved the final version of the article.

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None declared.

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