The role of prostaglandin E$_2$ in endometriosis

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Endometriosis is a leading cause of infertility in women of reproductive age. It involves the occurrence of endometrial tissue outside the uterine endometrium, mainly in the peritoneal cavity. Prostaglandin E$_2$ is up regulated in the peritoneal cavity in endometriosis and is produced by macrophages and ectopic endometrial cells. This prostaglandin is involved in the pathophysiology of the disease and elicits cell signals via four receptor types.

Prostaglandin E$_2$ increases estrogen synthesis by up regulating disease and elicits cell signals via four receptor types. This the peritoneal cavity in endometriosis and is produced from arachidonic acid metabolism. Their effects are ubiquitous and up regulate fibroblast growth factor-9 (FGF-9) promoting cell proliferation. Prostaglandin E$_2$ affects leukocyte populations and promotes angiogenesis through its effect on estrogen and up regulation of vascular endothelial growth factor (VEGF). Dienogest is a synthetic progestin targeting expression of genes involved in prostaglandin synthesis.

**Keywords:** Endometriosis, infertility, estrogens, prostaglandins

**Abbreviations:** cAMP, Cyclic adenosine monophosphate; CD36, Cluster of Differentiation 36; COUP-TF, Chicken ovalbumin upstream-transcription factor; COX, Cyclooxygenase enzyme; CREB, cAMP response element-binding; ELK-1, Ets Like gene1; EP1/2/3/4, Prostaglandin receptor subtypes 1, 2, 3 and 4; FGF-2, Fibroblast Growth Factor-2; FGF-9, Fibroblast Growth Factor-9; FGRF1, Fibroblast growth factor receptor-1; GnRH, Gonadotropin-releasing hormone; IL-1, Interleukin-1; IL-1β, Interleukin-1β; IL-6, Interleukin-6; MIF, Macrophage migration inhibitory factor; MMP-9, Matrix metalloproteinase-9; mPGES-1, Microsomal prostaglandin E synthase-1; PGE, synthase Prostaglandin E synthase; PGE$_2$, Prostaglandin E$_2$; PGH$_2$, Prostaglandin H$_2$; PKA, Protein kinase A; PKCδ, Protein kinase Cδ; SF1, Steroidogenic factor-1; Star, Steroidogenic acute regulatory protein; TNF-α, Tumour necrosis factor-alpha; VEGF, Vascular endothelial growth factor

**Introduction**

Prostaglandins are a group of bioactive lipid products derived from arachidonic acid metabolism. Their effects are ubiquitous both in physiological and pathophysiological mechanisms. Free arachidonic acid is the prostaglandin precursor. The synthesis of the various prostaglandin subtypes is governed by a committed step, catalysed by cyclooxygenase (COX) enzyme. At present, three isoforms are known to catalyse this step (COX-1, COX-2 and COX-3 [1]).

Various studies have shown that the COX-2 isoform is over expressed in ectopic endometrial cells and that there is an elevated level of a specific prostaglandin, prostaglandin E$_2$ (PGE$_2$) in uterine tissues of women with menorrhagia, dysmenorrhoea and endometriosis [2–4]. Such evidence has lead to questioning the role of this prostaglandin in the pathogenesis of endometriosis. This review aims to highlight the various underlying mechanisms elucidated so far, by which prostaglandin E$_2$ exerts its effect on ectopic endometrial cells. This provides a clearer understanding of the pathophysiology of the disease to elucidate the potential for novel drugs that target such mechanisms.

**Endometriosis: an overview**

Endometriosis is a female pelvic disorder whereby endometrial tissue is found outside the uterine cavity, mainly within the pelvic cavity. With a prevalence of 6–10% in the general female population, this condition is a major cause of infertility [5,6]. Endometriotic foci may settle and proliferate in the fallopian tubes, the ovaries or enter the peritoneal cavity and deposit in sites such as the recto-uterine pouch [7].

Laparoscopy is the key tool for diagnosing this condition and treating visible endometrial foci. As yet, medical therapy aims to minimise pain as a result of endometriosis [8]. The various drugs available are either oral contraceptives, aromatase inhibitors, gonadotropin-releasing hormone (GnRH) receptor agonists and androgenic agents. These aim to reduce ovarian estrogen production or inhibit ovarian activity [9]. Surgery through operative laparoscopy is used to excise visible foci. However such treatments are far from ideal as the recurrence rate following surgery or termination of therapy is 50–60% [10]. Precise aetiology and pathogenesis is primarily required to enable formation of drugs that target the pathophysiological mechanisms of endometriosis.

At present, there are various theories on the pathogenesis of endometriosis. The retrograde menstruation hypothesis suggests that endometriosis develops as a result of endometrial cells being swept towards the infundibulum of the fallopian tube and into the pelvic cavity [11,12]. Other theories are those of metaplasia [13] and the development of endometrial tissue from induced mesenchyme [14,15]. Estrogens seem to play a critical role in the development of the disease [16–19].
Production of PGE$_2$ and its receptors

The key cell types responsible for prostaglandin E$_2$ synthesis within the peritoneal cavity are ectopic endometrial cells [20]. Moreover, peritoneal macrophages are also involved in local prostaglandin production [2]. COX converts arachidonic acid to prostaglandin H$_2$ (PGH$_2$) in the rate-limiting stage of prostaglandin synthesis. The latter then acts as the substrate of the enzyme prostaglandin E (PGE) synthase which converts it to PGE$_2$ [21]. COX-2 is the key isoform which is over expressed in endometriosis. Such over expression is triggered by factors such as cytokines, tumour promoters, hormones and pro-inflammatory agents [22,23]. In fact, interleukin-1β (IL-1β), tumour necrosis factor-alpha (TNF-α) and PGE$_2$ were found to induce over expression of COX-2 gene in endometriosis. Hence, COX-2 is mainly over expressed in macrophages while COX-1 is rarely over expressed except in the severe stages of the disease [21,22].

There are four main subtypes of PGE$_2$ receptors-EPI, EP2, EP3 and EP4. Signal transduction is modulated depending on which receptor is bound by prostaglandin E$_2$ [24]. Interaction with the EP1 receptor elicits the intracellular calcium-inositol triphosphate pathway while the secondary messenger with EP2 and EP4 interaction is cyclic adenosine monophosphate (cAMP) [25]). Interaction with EP3 may elicit both pathways, depending on cell type and receptor splice variety [26]. Following the release of intracellular calcium via EP1 or EP3, PGE$_2$ can also act directly on target genes hence regulating their activity [27,28].

Effect on estrogen levels

Persistent endometriosis is dependent upon estrogen production, and ectopic endometrial cells in themselves are capable of estrogen synthesis [21]. PGE$_2$ is able to stimulate estrogen synthesis in ectopic endometrial cells by acting on major regulatory pathway [29]. There are two main regulatory steps in estrogen synthesis. These involve steroidogenic acute regulatory protein (StAR) and aromatase. The former acts as a cholesterol transporter across the inner mitochondrial membrane [30]. Aromatase is required for converting androstenedione to estrone.

PGE$_2$ mediates STAR expression via the EP2 receptor. This form of signal transduction occurs solely in ectopic endometrial cells [31]. The cell signalling pathway is via Gs protein that interacts with adenyl cyclase producing cAMP. A G protein (guanine nucleotide-binding protein) transmits extracellular signals to bring about an intracellular change, while a Gs protein is a G protein which leads to cAMP production. The resultant secondary messenger cAMP activates protein kinase A (PKA) which phosphorylates the nuclear protein, cAMP response element-binding (CREB). The activated CREB is then involved in histone acetylation which is key in DNA unwinding and facilitating transcription of the StAR gene [32].

PGE$_2$ mediates transcription of the aromatase gene, P450arom, in a similar fashion to that of StAR. However, apart from binding to the EP2 receptor, it may also bind the EP4 receptor. PGE$_2$-enhanced aromatase expression also occurs through steroidogenic factor-1 (SF1) which binds to the promoter of the aromatase gene-P450arom. This factor is abnormally over expressed in ectopic endometrial cells and competes with chicken ovalbumin upstream transcription factor (COUP-TF) and Wilm's tumor-1, both of which acts as inhibitors of the P450arom promoter [29].

The resultant increase in transcription of these two genes leads to an increase in estrogen levels. Estrogen is in turn involved in a positive-feedback loop leading to increased PGE$_2$ synthesis since it is an activator of COX-2 [33].

Apoptosis inhibition and cell proliferation

A high level of COX-2 and hence PGE$_2$ is associated with a decreased tendency for apoptosis. This has been observed both in endometrial cells and carcinomas of the reproductive tract [34]. It is also known that cells having over expressed COX-2 are capable of increased proliferation and escaping apoptosis. This is achieved through cyclin D which prolongs the G1 phase [35].

Estrogen is crucial in cell signalling leading to cell proliferation. This is achieved through up regulated transcription of peptide growth factors [36,37]. One crucial growth factor regulated by PGE 2 in ectopic endometrial cells, is fibroblast growth factor 9 [38]. PGE$_2$ induces FGF-9 transcription through two mechanisms. A direct mechanism involves the EP3 receptor whereby signal transduction proceeds via protein kinase C-δ (PKCδ). This mechanism leads to the phosphorylation of Ets LiK gene1 (ELK-1) transcription factor which binds the promoter region of FGF-9, enhancing its transcription [39]. Since estrogen also induces the expression of the FGF-9 gene [40], then PGE$_2$, indirectly contributes to FGF-9 expression through an indirect pathway—that of stimulating key enzymes involved in estrogen synthesis. FGF-9 is only one amongst various growth factors; hence the direct impact of PGE$_2$ on ectopic endometrial cell proliferation cannot as yet be quantified.

Apart from cell proliferation, PGE$_2$ could facilitate ectopic cell invasion. Cells over expressing COX-2 exhibit increased production of matrix metalloproteinase-2 enzyme (MMP-2). The contribution of MMPs is significant in erosion of extracellular matrix and hence enabling tissue invasion. In fact, administration of COX-2 inhibitors during in vitro studies prevented cells from invading extracellular matrix [41].

Immunosuppression

It was observed that B-cell and T-cell proliferation may be inhibited with high concentrations of PGE$_2$ [42]. Moreover, it has been shown that phagocytic activity is decreased in endometriosis [43]. Macrophages are the main immune cells present in the peritoneal cavity following retrograde menstruation. These act via two pathways, the first involving release of MMPs [44,45] while the other involves increased expression of scavenger receptor on their cell surface [46]. In patients with endometriosis, macrophages in the peritoneal cavity have down regulated MMP-9 as well as scavenger receptor CD36 [47,48]. PGE$_2$ is the main molecule responsible for this down regulation [21]. This inhibitory effect is exerted by PGE$_2$ via the EP2 and EP4 receptors, both of which involve the PKA cell signal [21].

PGE$_2$ in angiogenesis

For endometrial cells to thrive they require a blood supply, hence angiogenesis is key to their survival [49]. Various angiogenic factors play a role in the formation of new blood vessels. These include cytokines, endostatin, angiostatin, vascular endothelial growth factor (VEGF) and hormones such as estrogen [50]. Of these, VEGF and estrogen are well-linked to the influence exerted by PGE$_2$. It has been shown that estrogen and COX-2 can enhance VEGF expression [51,52]. The effect of COX-2 is mediated by the production of PGE$_2$ [52,53]. In particular, estrogen promotes both the expression of VEGF and that of MMPs, both being associated with angiogenesis [54,55].

Most of the angiogenic factors involved in endometriosis are also present in tumour endothelial cells [56]. VEGF is considered to be the key angiogenic factor involved in endometriosis with a key role in increasing vascular permeability [57].
lesions, neutrophils and macrophages in the peritoneal cavity express VEGF [58]. Compared to eutopic endometrium, the levels of VEGF are much higher in ectopic tissue and within the peritoneal cavity [59,60]. Apart from PGE2 and estrogen, factors such as hypoxia, progesterone and interleukins 1 and 6 (IL-1 and IL-6) also enhance VEGF expression so the extent of PGE2 on VEGF expression remains to be seen [52,53].

A less familiar angiogenic pathway involves PGE2 stimulating fibroblast growth factor receptor-1 (FGFR1), a tyrosine kinase receptor of fibroblast growth factor-2 (FGF-2 [61]). FGF-2 is involved in tumor angiogenesis and mediates cell proliferation and cellular migration in angiogenic pathways [62,63]. However, it has not been established whether this mechanism takes place in endometriotic angiogenesis.

Both VEGF and FGF-2 stimulate PGE2 synthesis and contribute to a positive-feedback loop [61]. While further research is essential to uncover the molecular pathways involving PGE2 in angiogenesis, it is also suggested that PGE2 directly stimulates both endothelial cell proliferation and migration. Such processes are mediated via the EP2 and EP4 receptors [64,65]. Moreover, PGE2 also mediates in vitro tube formation via the EP4 receptor [65]. Further research on the molecular mechanism of angiogenesis could enable new regimens that inhibit the settlement of cells beyond their usual confines in endometriosis and metastasis.

**Current treatment in endometriosis**

At present, medical treatment aims to stop the proliferation of endometriotic lesions [66]. GnRH agonists are popular drugs of choice. They suppress the pituitary-ovary axis and cause hypoestrogenism when administered in a non-pulsatile fashion [67]. Danazol is widely used and promotes endometrial atrophy by increasing free testosterone levels leading to a hypoestrogenic state [68,69]. Since endometriosis is estrogen-dependant, such drugs will decrease the severity of the disease and help relieve the symptoms. Moreover, the levels of PGE2 should theoretically fall as estrogen operates in a positive feedback-loop promoting PGE2 synthesis by activating COX-2 [33]. Aromatase inhibitors elicit a similar effect as they irreversibly bind to aromatase hence decreasing estrogen synthesis [70].

Pharmaceuticals have been developed, which target the prostaglandin E2 synthetic pathway. Dienogest is a synthetic progestin that inhibits the expression of key genes involved in PGE2 synthesis. These include the inhibition of PGE2 synthase,
COX-2 and microsomal prostaglandin E synthase (mPGES)-1. Unlike COX-2 inhibitors, the inhibitory effect does not take place at the enzyme level but rather by inhibiting gene transcription of critical enzymes involved in PGE$_2$ synthesis. This inhibitory effect persists for 24 hours following drug administration [71].

Moreover, it has been shown that dienogest directly suppresses aromatase mRNA expression. Dienogest also inhibits cellular proliferation by suppressing the expression of cyclin D, the cyclin that is responsible for the transition from the G1 phase to the S phase of the cell cycle [72]. On the contrary, PGE$_2$ maintains cyclin D which is one of the causes for increased cell proliferation.

While dienogest promises to be a key inhibitor that may target the roots of PGE$_2$ synthesis, it exerts its main effect by binding to the progesterone receptor. Dienogest selectively binds to the progesterone receptor and promotes progestational effects [73]. Thus it inhibits both ovulatory activity and endometrial cell proliferation [74,75]. Moreover, it also lowers cytokine levels produced by endometriotic cells [76].

Compared to conventional endometriotic drugs, dienogest lacks androgenic effects and can be regarded as a safe progestin through its high receptor selectivity [77]. GnRH agonists side-effects include hot flushes and decreased libido [78] while Danazol may lead to androgenic effects [78,79]. Furthermore, COX-2 inhibitors are not the current solution as they either exert a weak inhibitory effect or worse still can lead to cardiovascular complications [80,81]. Thus, dienogest seems to be a promising future by targeting the causative roots of this disease. Despite this, dienogest administration also has typical progestogen side-effects, namely headaches, weight gain and elevated blood pressure. Moreover, cases of depression were also reported [77,82].

Conclusions

While prostaglandin E$_2$ and COX-2 have a key role in the pathogenesis of endometriosis, other factors play a significant role in this disease. Endometriosis is a complex disease and as yet remains a psychosocial burden for women of reproductive age. The way forward is the development of novel pharmaceuticals that are more specific inhibitors of prostaglandin E$_2$ in the peritoneal cavity and target the mechanism by which it is exerts its effect. EP receptor antagonists could also prove to be a potential site for blocking the effects of PGE$_2$.

Declaration of Interest: The authors declare no conflict of interest.

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