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PLASMINOGEN ACTIVATORS AND PLASMINOGEN ACTIVATOR INHIBITORS IN ENDOMETRIOSIS

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1. ABSTRACT

Endometriosis is one of the most frequent benign gynecological diseases that affect women. Little is known about the pathogenesis and etiology of endometriosis, despite the numerous studies performed in this field. Although endometriosis is a benign disease, the endometrial tissue, after attachment to the peritoneum, has the ability to grow and invade the surrounding tissues. Similar to neoplastic growth, local extracellular proteolysis might take place, and therefore, the fibrinolytic system may be involved. An altered expression of several components of the fibrinolytic system in the endometrium and peritoneal fluid of women with the disease has been suggested as a key factor in the establishment of the endometriotic lesions. There is evidence of increased fibrinolytic activity in the eutopic endometrium of these women, resulting in endometrial fragments with a high potential to degrade the extracellular matrix and facilitate implantation. The peritoneum possesses an inherent fibrinolytic activity that is responsible for the degradation of the fibrin deposits originated after an injury. This physiological function allows a correct repair of the mesothelium, and therefore, prevents the formation of adhesions. Peritoneal fluid of women with endometriosis and pelvic adhesions has shown to have an increased fibrinolytic activity that may be implicated in reducing the formation of new adhesions. Endometriotic tissue has abnormal proteolytic capacity, which is determined by modifications of the fibrinolytic parameters in this tissue. Proteolytic status is determined by the imbalance between plasminogen activators and plasminogen activator inhibitors, which are expressed differently depending on the type of lesion considered and the stage of the disease. The aim of the present study is to review the role of the plasminogen activator system in endometriosis, consider the clinical implications and focus on possible further research efforts and therapeutic applications in this disease.

2. INTRODUCTION

Endometriosis is defined by the presence of endometrial glands and stroma outside the uterus. It is one of the most frequent benign gynecological diseases that affect women with pelvic pain or infertility during their reproductive age (1, 2). The etiology and pathogenesis of endometriosis are far from clear, despite several decades of research in this field. Little is known about the pathogenesis of endometriosis. However, it is thought that retrograde menstruation may transport endometrial tissue to ectopic locations. Several factors have been implicated as causes of endometriosis including immune system

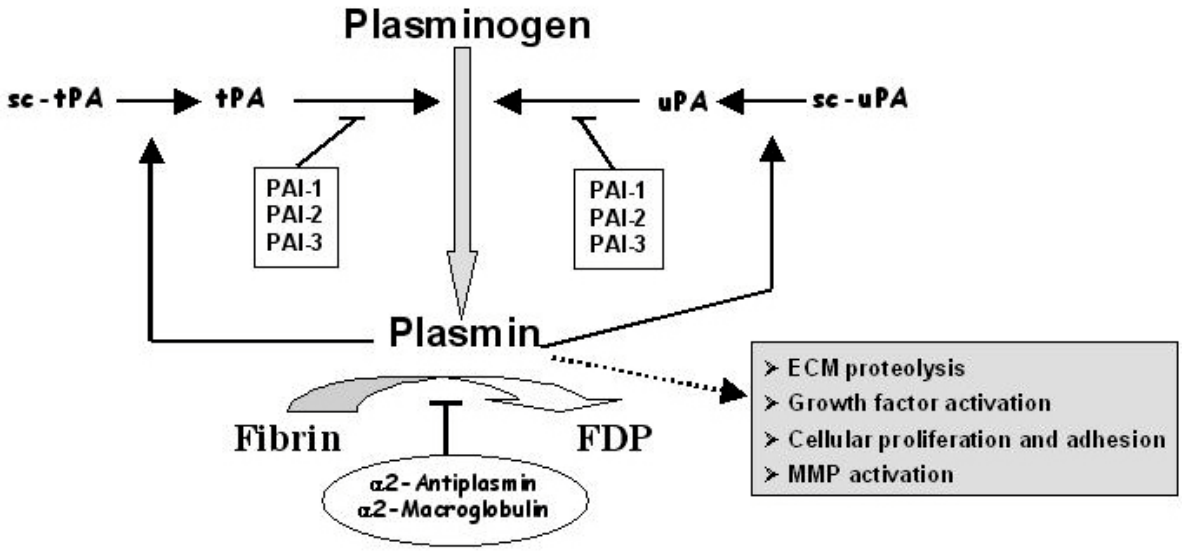

Figure 1. Fibrinolytic system. The plasminogen activator system is based on the conversion of an inactive proenzyme, plasminogen, to the active enzyme, plasmin. The activation of plasminogen to plasmin is mediated by two types of activators, urokinase-type plasminogen activator and tissue-type plasminogen activator. Plasmin is an active enzyme that degrades fibrin into soluble degradation products. The activity of the plasminogen activators is regulated by specific plasminogen activator inhibitors (PAIs). The principal PAIs are PAI-1, initially termed the endothelial cell PAI; PAI-2, historically known as placental-type PAI and PAI-3 that is identical to protein C inhibitor. Plasmin can be inhibited by specific plasmin inhibitors (mainly alpha$_2$-antiplasmin and also alpha$_2$-macroglobulin).

disorders, genetic predisposition, altered peritoneal environment and endometrial alterations (3, 4, 5). Although endometriosis is a benign disease, the endometrial tissue acquires the ability to attach and invade the peritoneum through local extracellular proteolysis (6, 7). The plasminogen activator system may be involved in this process.

The mechanisms by which the plasminogen activator system is implicated in the pathogenesis of endometriosis are not well defined. Besides their fibrinolytic function, plasmin and plasminogen activators are implicated in tissue proliferation, cellular adhesion and remodeling of extracellular matrix (8). Human endometrium is a dynamic tissue that exhibits cyclic changes during the menstrual cycle. The most widely accepted theory for the pathogenesis of endometriosis is the implantation theory, which suggests that retrograde menstruation may enable the transport of endometrial tissue to ectopic locations. Although retrograde menstruation is a frequent finding in menstruating women (9), endometriosis occurs only in some cases. Altered expression of several components of the fibrinolytic system in both eutopic and ectopic endometrium and peritoneal fluid of women with the disease has been implicated in the onset and progression of the endometriotic lesions.

Conflicting results have been published concerning gene expression, protein production and precise localization of the components of the fibrinolytic system analysed in endometriosis. However, the studies are not homogeneous with respect to the variety of tissues, models, conditions and techniques. There is a promising scope for defining the exact role of the plasminogen activator system in endometriosis and its clinical implications. Hopefully, further research lines focusing on the prevention of adherences and the development of endometriotic lesions will improve the current therapeutic management of women with endometriosis.

3. GENERAL ASPECTS OF THE FIBRINOLYTIC SYSTEM

The fibrinolytic system includes a broad spectrum of proteolytic enzymes with physiological and pathophysiological functions in several processes such as fibrinolysis, tissue remodeling, tumor invasion, angiogenesis and reproduction (8, 10, 11, 12, 13, 14, 15).

The main enzyme of the plasminogen activator system is plasmin, which is responsible of the degradation of fibrin into soluble degradation products. Plasminogen is an inactive proenzyme that can be converted to plasmin by proteolytic cleavage of a single peptide bond. The activation of plasminogen to plasmin is mediated by two types of activators, urokinase-type plasminogen activator (uPA) and tissue-type plasminogen activator (tPA) (figure 1). Additionally, plasmin also degrades a considerable variety of extracellular matrix proteins and activates matrix metalloproteinases (MMPs) and growth factors (8, 12). The activity of plasminogen activators (PAs) is regulated by specific PA inhibitors (PAIs). The principal PAIs are PAI type 1 (PAI-1), initially called the endothelial cell PAI (10, 16), PAI type 2 (PAI-2), also known as placental-type PAI (17, 18, 19) and PAI type 3 (PAI-3), which is identical to protein C inhibitor (20, 21). Plasmin can be inhibited by specific plasmin inhibitors (mainly alpha$_2$-antiplasmin and also alpha$_2$-macroglobulin) (figure 1).

Besides their fibrinolytic function, plasmin and plasminogen activators are implicated in tissue proliferation and cellular adhesion, since they can proteolytically degrade the extracellular matrix and regulate the activation of both growth factors and MMPs. By all these means, the fibrinolytic system is also involved in physiological processes, such as menstruation, ovulation and the implantation of the embryo (22, 23), and in pathological situations such as endometriosis (15, 24) or cancer (25, 26).

3.1. Plasminogen and plasmin

Plasminogen is a glycoprotein mostly synthesized in the liver, although other synthesis sites have also been described. Plasmin is the active enzyme of plasminogen. Two of its most specific substrates are fibrin and fibrinogen. It is known that plasmin binds to α_2-antiplasmin through the lysine binding sites (LBS) and the active center. Through the LBS, plasminogen and plasmin bind to cell surface plasminogen receptors (27). These receptors modulate the fibrinolysis process and, in particular, the proteolysis related to the cellular migration that take place on the cellular surface. The binding of plasmin or plasminogen to cell surfaces is the main mechanism that mediates its participation in pericellular proteolysis (27, 28). Once bound to cell surface, plasminogen is more efficiently activated to plasmin, and bound plasmin is partially protected from inactivation by its physiological inhibitors.

Plasmin has broad spectrum of proteolytic functions. It can directly degrade multiple matrix proteins (29), including fibronectin, laminin, and thrombospondin, as well as the major provisional matrix constituent, fibrin. Moreover, plasmin is an activator of several MMPs and it also possesses a proteolytic action on the tissue inhibitors of the metalloproteinases (TIMPs) (30).

3.2. Plasminogen activators

3.2.1.Tissue-type plasminogen activator (tPA)

tPA is a serine protease that is synthesized by endothelial cells and released into the circulating blood as a single chain precursor (sc-tPA). Plasmin converts sctPA to active two-chain form (tc-tPA or tPA) by cleaving a peptide bond (figure 1). The activation of plasminogen by tPA occurs on the fibrin surface and on the endothelial cell surface. This process allows efficient and localized plasminogen activation since tPA has a high affinity for fibrin and its enzymatic activity is enhanced by fibrin binding (31). tPA-mediated plasminogen activation is mainly involved in the lysis of fibrin at the site of vascular injury.

3.2.2. Urokinase-type plasminogen activator (uPA)

uPA is a serine protease that is mainly implicated in cellular proteolysis. uPA binds to a specific cellular receptor (u-PAR) resulting in enhanced activation of cell-bound plasminogen. uPA was initially isolated of the human urine as a two-chain form (tc-uPA). However, uPA is initially synthesized as a single chain polypeptide molecule (sc-uPA) (32). Following partial digestion by plasmin, sc-uPA is converted into a two-chain form denominated urokinase (uPA). sc-uPA acts directly on plasminogen generating plasmin but it is much less active than uPA. It has been described that uPA activates plasminogen after binding to its receptor (uPAR).

Besides its fibrinolytic activity, uPA also regulates the cellular migration process under physiologic and pathological conditions, such as angiogenesis, embryo implantation, inflammation and tumor metastasis (12, 33, 34, 35). uPA was obtained from ovarian carcinoma cell cultures (36) but its production has also been observed in the majority of malignant tumors (25, 26, 37, 38).

uPA plays an important role in the endometrial physiology (39) and the mechanisms underlying menstruation (40). uPA expression in endometrium is regulated by paracrine mechanisms and steroid environment (41). Progesterone stimulates the degradation of uPA in endometrial stromal cells by increasing the production of its inhibitor (PAI-1) and the surface expression of uPAR (42, 43).

3.3. Plasminogen activator inhibitors

3.3.1. Plasminogen activator inhibitor type 1 (PAI-1)

PAI-1 is a multifaceted proteolytic inhibitor (44). It does not function only as a fibrinolytic inhibitor, but also plays an important role in cellular signal transduction, cell adherence and migration (44, 45). It is a glycoprotein synthesized by a great variety of tissues and cells including endothelium, megakariocytes, human endometrium, peritoneal macrophages and mesothelial cells (16, 46, 47, 48, 49). PAI-1 belongs to the superfamily of the serine proteases inhibitors also called serpines. The PAI-1 is a relatively termostable protein when deposited on the subendothelial matrix (49). This stability is mainly due to the presence of a glycoprotein, vitronectin, which is able to bind and stabilize PAI-1 (51).

PAI-1 is one of the primary regulators of the fibrinolytic system *in vivo* (10). Overexpression of this inhibitor may compromise normal fibrin clearance mechanisms and thus promote pathological fibrin deposition and thrombotic events (52). Impaired fibrinolysis due to increased PAI-1 levels in plasma is a common finding in patients with deep vein thrombosis (53, 54, 55). A fibrinolytic hypofunction due to an increase in PAI-1 concentration has been detected in patients with coronary artery disease (56, 57, 58, 59). Moreover, some authors have pointed out that an increase in plasma levels of tPA:PAI-1 complex may constitute a risk factor for recurrent myocardial infarction (60). In addition, PAI-1 is increased in situations with high thrombotic risk, such as pregnancy (61, 62), and in certain obstetric complications, such as preeclampsia (63, 64, 65).

The fibrinolytic activity of human endometrium is essential in the process of tissue remodeling during the menstrual cycle. Plasmin is a proteolytic enzyme that is capable to activate MMPs and to degrade extracellular matrix proteins including diverse components of the basal membrane.

There is compelling clinical evidence which considers PAI-1 as a key factor for tumor invasion and

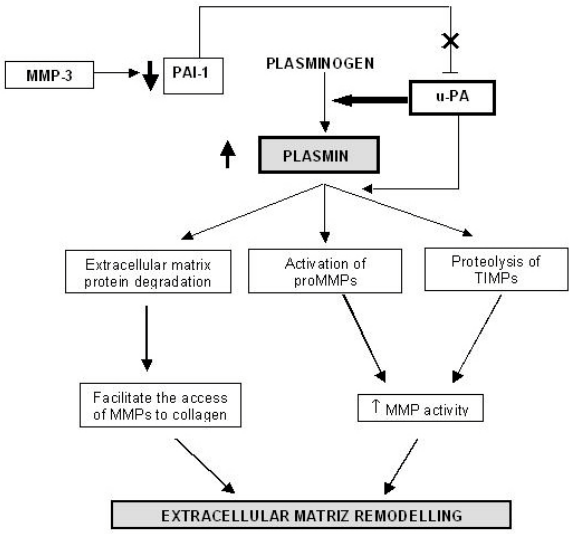

Figure 2. Relationship between fibrinolytic and metalloproteinase (MMP) systems. Plasmin is an active enzyme that degrades a variety of extracellular matrix proteins and activates MMPs. On the other hand, MMP- 3 specifically hydrolyses and inactivates human PAI-1 and may regulate cell-associated plasmin activity.

metastasis. Both uPA and PAI-1 have been considered poor prognostic factors in patients with several carcinomas, such as breast cancer (66, 67). Furthermore, high level of PAI-1 protein correlated with cancer invasion potential despite its well-known ability to inhibit uPA activity (67). No explanation has been found for this apparent paradox. It has been demonstrated that inhibition of proteolytic activity by PAI-1 is essential for tumor angiogenesis, suggesting that excessive plasmin proteolysis prevents the assembly of tumor vessels (68). A critical balance between proteases and PAI-1 levels would be necessary for optimal invasion.

3.3.2. Plasminogen activator inhibitor type 2 (PAI-2)

PAI-2 activity was first described in placental extracts (69). Initially, it was believed that PAI-2 expression was limited to placenta and monocytes/macrophages, however there is a broad distribution of PAI-2 in different tissues (17, 70).

In general, PAI-2 levels are not detectable in plasma from men and from non-pregnant women. However, plasma levels from both PAI-1 and PAI-2 increase during the course of pregnancy, suggesting that both PAIs may play a role in the maintenance of haemostasis during pregnancy and delivery. Moreover, it has been reported that PAI-2 can also be involved in fetal growth regulation or be a marker of placental function during pregnancy (62, 65, 71). In addition, PAI-2 may also exert many other functions. PAI-2 has been identified either as an intracellular nonglycosylated form or as an extracellular glycosylated protein. The major proportion of PAI-2 remains in the intracellular compartment but the function of this form has not been clearly elucidated (72). It may have cytoprotective functions and appears to play a role in apoptosis. Overexpression of PAI-2 protected from cytolysis induced by tumor necrosis factor (73). It has also been suggested that intracellular PAI-2 could protect placental cells from apoptosis in normal pregnancy and therefore, a reduction of its local concentration may decrease its cytoprotective effect, impair placental nutrient transport and finally result in intrauterine growth restriction (19).

3.3.3. Plasminogen activator inhibitor type 3 (PAI-3)

PAI-3, also known as protein C inhibitor and synthesized in the liver and in steroid-responsive organs, is present in plasma, urine, seminal and follicular fluid (74, 75, 76, 77, 78). PAI-3 was identified in human plasma and urine as a glycoprotein that inhibits uPA (79). Later, it was demonstrated that PAI-3 was identical to protein C inhibitor (PCI) (20), initially characterized by Suzuki *et al* (78). Previous studies have suggested that this inhibitor may be involved in human reproduction (74, 75, 76, 77). PAI-3 is a heparin-dependent serpine that inhibits several proteases, including uPA, tPA, activated protein C, thrombin, kallikrein and prostate specific antigen (74, 75, 77, 78, 80, 81).

In vivo studies have found uPA:PAI-3 and tPA-PAI-3 complexes in urine and semen (75), and during thrombolytic therapy with uPA or tPA in patients with myocardial infarction (81, 82). A decrease in plasma PAI-3 levels was observed in liver diseases, disseminated intravascular coagulation and in patients under heparin therapy (83). Although plasma levels of PAI-3 are higher than PAI-1, its plasma fibrinolytic activity is lower than PAI-1 (75, 79). Previous studies have suggested that PAI-3 may be linked to carcinogenesis in hormone-regulated tissues (77, 84). However, its biological role in remodeling of extracellular matrix, cell migration and tumor invasion is still unknown.

3.4. Relationship between plasminogen activator and metalloproteinase systems

Besides plasmin and plasminogen activators, MMPs are also enzymes involved in extracellular matrix remodeling (85) (figure 2). These proteases have been implicated in the endometrial remodeling during the menstrual cycle (86) and also in the growth of endometriotic tissue outside the uterus in patients with endometriosis (87). MMP activities are regulated by tissue inhibitors (TIMPs) which can inhibit in 1:1 molar ratio different classes of MMPs (88, 89).

Fibrinolytic and MMP systems are involved in both normal and pathological processes in which degradation of the extracellular matrix is a key event. Plasmin is an active enzyme that degrades a variety of extracellular matrix proteins and activates MMPs and growth factors (8). On the other hand, MMP- 3 specifically hydrolyses and inactivates human PAI-1 (30) and may regulate cell-associated plasmin activity (90). Although it is far from clear, the mechanism that regulates and determines the activity of the MMPs in the endometrium, peritoneal fluid and endometriotic tissue of women with endometriosis may also be implicated in the regulation of fibrinolytic system in this disease. Therefore, more studies are required in order to elucidate the ultimate connections between both systems.

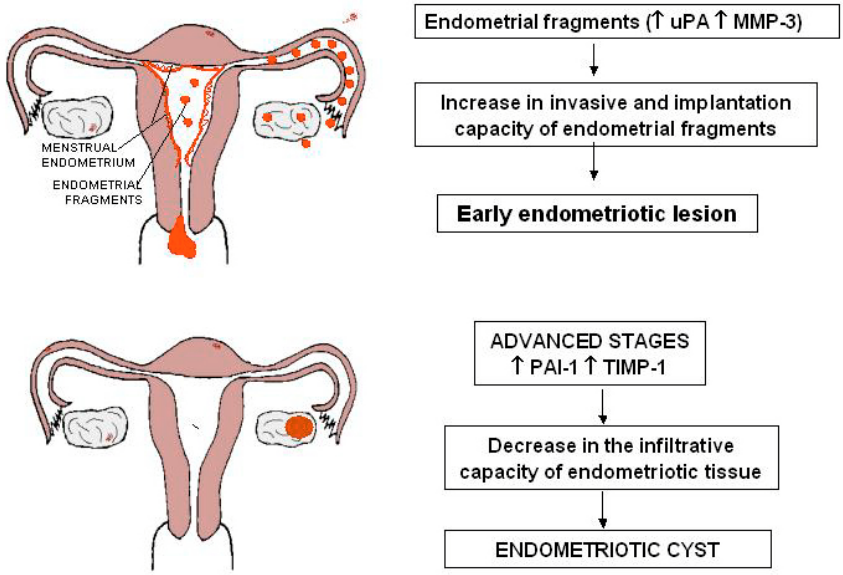

Figure 3. Fibrinolysis in endometriosis. The proteolytic capacity of endometrium from women with endometriosis and the peritoneal environment may have an important role in the establishment of the endometriotic lesion. The up-regulation of uPA and MMP observed in endometrium from women with endometriosis might be an important clue in the invasive potential and in the growth of endometrial tissue outside the uterus. This process would lead to the formation of early endometriotic lesions. Once the ovarian endometriotic cyst is developed, PAI-1 and TIMP-1 would increase and the proteolytic activity would, therefore, decrease. This observation would explain the frequent clinical finding of an isolated endometriotic cyst without invasion of the surrounding ovarian tissue.

4. THE ROLE OF FIBRINOLYTIC SYSTEM IN ENDOMETRIOSIS

Endometriosis is a frequent benign gynecological disease that usually affects women with pelvic pain or infertility during their reproductive age (1, 2). Little is known about the pathogenesis of this disease. Several theories have been proposed including immune system disbalances, genetic predisposition, altered peritoneal environment and endometrial disorders (3, 4, 5). However, the most widely accepted theory is the implantation theory, which suggests that retrograde menstruation may transport endometrial fragments to ectopic locations (91) (figure 3). By degrading extracellular matrix, the ectopic endometrium may be able to implant and invade the peritoneum and the surrounding structures (6, 7). However, menstrual reflux of endometrial tissue also occurs in healthy cycling women who do not develop endometriosis (9). A possible explanation for this finding is that the peculiar characteristics of the endometrium and the peritoneal environment of women with endometriosis may facilitate the ectopic implantation of this tissue. The following sections summarize the feasible mechanisms that support the observation that the plasminogen activator system might be involved in the pathogenesis of endometriosis.

4.1. Plasminogen activator system in normal endometrium

Human endometrium is a dynamic tissue that exhibits cyclic changes during the menstrual cycle. This tissue proliferates in the follicular phase under the stimulation of estrogens and differentiates by the action of progesterone in the secretory phase of the cycle. At the end of the secretory phase the decrease of progesterone levels initiates the menstrual shedding of the superficial layer of the endometrium. After menstruation the endometrium rapidly regenerates in the proliferative phase, once again under the influence of estradiol. *In vitro* studies with endometrial stromal cells have demonstrated that the presence of progesterone increases the synthesis of extracellular matrix components, such as collagen, laminin and fibronectin (92, 93), which may contribute to the differentiation of endometrium.

The plasminogen activator system is influenced by steroid levels in the human endometrium. Although it has been reported that the plasmin activity of the endometrium is mainly due to tPA, endometrial cells can also express uPA and minimal amounts of tPA *in vitro* (94). This can be explained by the storage of uPA as sc-uPA in endometrial cells, an inactive precursor that cannot be detected by all the assays (95). The expression of uPA throughout the menstrual cycle has been studied and a higher proteolytic activity due to high uPA levels has been reported during menstruation (41, 94). Studies with human endometrial cells have detected a higher production of uPA in the proliferative phase than in the secretory phase (41, 96). In agreement with this point, other authors have observed that the addition of progesterone to endometrial tissue cultures reduced the expression of uPA (41, 97).

Human endometrial stromal cells supplemented with progesterone express high levels of PAI-1 that can neutralize uPA and thereby prevent the degradation of the extracellular matrix (46). It has been pointed out that the reduced uPA activity observed in these cells is also

determined by an increased degradation of uPA (43). Moreover, steroid treatment increases the number of available cell surface binding sites for uPA and facilitates complexed uPA degradation and clearance from the cellular membranes. Therefore, the reduction of extracellular uPA may be secondary to increased internalization and degradation of uPA, phenomenon that is more efficient for uPA:PAI-1 complexes. The increase in uPARs combined with the reduction of extracellular uPA may explain the low fibrinolytic activity detected in the presence of high progesterone concentrations. The direct consequence of low uPA activity in the secretory endometrium is a significant reduction of the degradation of the extracellular matrix. Thus, endometrial bleeding is avoided and optimal conditions for the implantation of the embryo are assured. Although PAI-1 also inhibits tPA, non significant variations in tPA concentrations have been observed in the presence of progesterone. Moreover, several studies have detected an increase in tPA levels in healthy endometrium before menstruation, which is probably related to the degradation of fibrin clots (98, 99). The low fibrinolytic activity during the secretory phase may be altered by the withdrawal of progesterone at the end of this phase resulting in higher levels of uPA and tPA. This activation of the fibrinolytic system may enhance the proteolytic mechanisms that are implicated in menstruation.

Growth factors and cytokines may modulate the concentration of plasminogen activators and inhibitors (37). Epidermic growth factor increases the concentration of uPA (100), transforming growth factor-β (TGF-β) and PAI-1 and inhibits the growth of epithelial cells (37). Although there are multiple paracrine mechanisms that determine the fibrinolytic activity, the final regulation of this system is performed by the plasminogen activators and plasminogen activator inhibitors.

4.2. Plasminogen activator system in endometriosis

The mechanisms by which the plasminogen activator system is implicated in the pathogenesis of endometriosis are not well defined. Controversial results have been reported in this disease concerning gene expression, protein production and precise localization of different components of these systems. However, these studies are not homogeneous with respect to the variety of tissues, models, conditions and techniques.

Ectopic endometriotic lesions are histologically similar to eutopic endometrium. However, biochemical differences exist between these two tissues. Although there is little information regarding the differences between endometrium from women with and without endometriosis, evidences suggest that the eutopic endometrium from diseased women shows an abnormal expression of several components of the plasminogen activator system. This finding would tightly link the plasminogen activator system to the pathogenesis of the endometriosis.

Several reports have detected an increase in uPA local concentration in the endometrium from women with endometriosis (24, 15). *In situ* hybridization studies performed by Bruse *et al* (101) showed that uPA mRNA seems to be up-regulated in both endometriotic and endometrial tissue. The higher concentration of uPA in endometrium of women with endometriosis might result in tissue fragments with a higher potential to degrade the extracellular matrix and thus, in facilitated implantation processes (6, 7, 24, 102, 103). However, studies with isolated and cultured stromal cells from endometriotic tissue and endometrium from women with endometriosis showed lower uPA levels in all these cells when compared to endometrium of healthy controls (104). Obviously, *in vitro* models lack the environmental modulations of hormones, growth factors, or cytokines, which are usually present *in vivo* and would explain the observed differences.

In relation to endometriotic lesions, Lembessis *et al* (105) reported an increase in uPA mRNA expression in endometriotic lesions compared to eutopic endometrium. However, we did not notice any increase in uPA mRNA levels in ovarian endometriomas (15, 106). The characteristics of the endometriotic samples might be the reason for the disagreement of these results. As previously suggested, endometriosis seems to be a progressive disease and a reduction in the activity of endometriotic lesions has been observed in advanced stages (107). Our results show that uPA levels are higher in endometriotic implants than in ovarian endometriomas (106). Furthermore, ovarian endometriomas have higher PAI-1 antigenic levels than peritoneal implants. Therefore, the ratio uPA/PAI-1 (antigenic and mRNA levels) is higher in the peritoneal implants than in the ovarian endometriomas. These results suggest that peritoneal implants correspond to proteolytic active endometriotic lesions, while those advanced stages of endometriosis (ovarian endometriotic cysts) show less proteolytic activity.

Concerning the fibrinolytic inhibitors, previous studies have shown that antigenic concentrations of PAI-1 are higher in the eutopic endometrium from women with endometriosis than in endometrium from healthy controls. PAI-1 antigen levels are even higher in ovarian endometriomas than in endometrial tissue (24, 15). PAI-1 mRNA is also overexpressed in endometriotic tissue (106) and it seems to be located mainly in both glands and stroma (102). However, the increase in PAI-1 antigenic levels is higher than the increase in PAI-1 mRNA levels (107). A probable explanation of these findings could be that the low levels of activators in ovarian endometriomas may lead to less degradation of PAI-1 protein, and thus result in higher PAI-1 antigenic levels. The increase in PAI-1 protein expression in ovarian endometriomas might contribute to limit the invasive potential of the endometriotic tissue in advanced stages of this disease.

A decreased PAI-3 antigenic and mRNA levels in endometriotic cysts compared to endometrium has been reported (15, 106). PAI-3 is a protease inhibitor that may be involved in human reproduction (74, 75, 76). The precise role of this inhibitor in extracellular proteolysis has not been fully elucidated, but it has been suggested that PAI-3 protects uPA from the inactivation by PAI-1 (108). The reduced PAI-3 expression observed in ovarian endometriomas might enhance inhibition of uPA by PAI-1,

and therefore contribute to the reduced proteolytic activity of this tissue.

In relation to fibrinolytic receptors, soluble uPAR is overexpressed in endometrial cells of women with endometriosis (109). The soluble form of uPA receptor can increase the local availability of uPA by delaying its inhibition by PAI-1 (110). In addition, uPAR inhibits cell adhesion to vitronectin, which would facilitate cell migration (111) and PAI-1 stabilization (50). These findings reinforce the previously commented theory of retrograde menstruation of endometrial fragments with high proteolytic potential. *In vitro* studies, found that basal release of soluble uPAR in cultured endometriotic cells obtained from ovarian endometriomas of women with endometriosis was lower than in endometrium from women with and without the disease (104). This report would indicate a low proteolytic potential of the cells from ovarian endometrioma.

It is known that ovarian steroids have an important role in the progression of endometriosis. This disease is restricted to the reproductive age of the women and changes in the steroid status, such as menopause or pregnancy, induce a regression of the endometriotic lesions. On the other hand, several authors have suggested that endometriotic tissue has a different hormonal regulation than endometrium. In the endometriotic tissue the estrogen receptor levels remain low throughout the menstrual cycle and the progesterone receptors synthesized are not all biologically active (112). It has been shown that aromatase is overexpressed in endometriosis resulting in high levels of estradiol that promote endometrial gland growth. Additionally, endometriotic glandular cells are deficient in 17beta-hydroxisteroid dehydrogenase type 2, which impairs the inactivation of estradiol due to insensibility to progesterone (113, 114). *In vivo* studies have shown that estradiol would not be necessary for the implantation process (115, 116) but it would be determinant for the implantation and growth of endometriotic tissue (117).

There is compelling evidence that endometriosis is influenced by the estrogenic levels, but conflicting evidence exits about the effect of estrogens in the production of uPA, uPAR and PAI-1. The invasive ability of ectopic endometrial tissue has been studied in patients with adenomyosis, where endometriotic tissue is located in the uterine myometrium. An increase in uPA and its receptor (uPAR) has been reported in both endometrial and ectopic tissue of women with the disease and this may contribute to the invasive phenotype of heterotopic endometrium (7). In addition, uPA expression is dependent on the menstrual cycle with a significant increase in both eutopic and ectopic endometrial tissues during menstruation. uPAR is overexpressed in ectopic tissue in comparison to eutopic endometrium with an increase in the proliferative phase. Meanwhile uPAR expression is not influenced by steroid treatment in cultured endometrial cells, it is significantly higher in cells from patients with endometriosis (109). The different expression pattern of the fibrinolytic system in the endometriotic tissue may be implicated in the different biological behavior related to its invasion potential.

As previously indicated, studies with human endometrial cells have detected higher production of uPA in the proliferative phase than in the secretory phase (94, 96). It has been reported that secretory endometrium from healthy controls has lower uPA and MMP-3 mRNA levels than proliferative endometrium. However, no differences are observed between proliferative and secretory phase in the endometrium from patients with endometriosis (106). On the other hand, secretory endometrium from women with endometriosis has higher mRNA and protein levels of MMP-3 and uPA than secretory endometrium from controls. These findings may indicate a failure of progesterone or locally produced factors to suppress these enzymes in women with endometriosis and might facilitate the implantation of endometrial fragments after retrograde pass through the fallopian tubes.

There is evidence in the literature of the implication of the fibrinolytic system in the pathogenesis of endometriosis. The proteolytic capacity of endometrium from women with this disease and the peritoneal environment may have an important role in the establishment of the endometriotic lesion (figure 3). The up-regulation of uPA observed in endometrium from women with endometriosis might be an important clue in the invasive potential and in the growth of endometrial tissue outside the uterus. This process would lead to the formation of early endometriotic lesions. Once the ovarian endometriotic cyst is developed, PAI-1 would increase and the proteolytic activity would, therefore, decrease. This observation would explain the frequent clinical finding of an isolated endometriotic cyst without invasion of the surrounding ovarian tissue. On the other hand, peritoneal fluid of women with endometriosis has shown to have an increased fibrinolytic activity in the presence of pelvic adhesions, which may be implicated in reducing the formation of new adhesions.

4.3 Peritoneal fluid and endometriosis

The peritoneal fluid has been a target of research on endometriosis due to its proximity to the endometriotic lesions. This milieu might play an important role in the implantation of ectopic endometrium in the peritoneal and ovarian surface. The physiological balance of the different components of this fluid has been suggested to have a protective role in preventing the development of endometriosis through mechanisms such as macrophage digestion or secretion of inhibitory factors (118). Peritoneal fluid is mainly originated from plasma transudate and ovarian exudate (119). It is also composed of secretions from the mesothelial surface and tubal and uterine luminal fluid. The cellular components are formed by macrophages, red blood cells, neutrophils and endometrial cells. Macrophages secrete cytokines and growth factors creating an immunological environment where reproductive processes such as ovulation take place. There is evidence that alterations in the characteristics of the peritoneal fluid may be implicated in the pathogenesis of endometriosis.

When the peritoneum is injured an inflammatory reaction is initiated that leads to the formation of fibrin deposits, white cell exudates and mesothelial tissue necrosis. The generation of fibrin is a result of the

haemostatic process and has an important role in tissue repair by providing a matrix for invading fibroblasts and new vessels. Peritoneum possesses an inherent fibrinolytic activity, which is responsible of the degradation of the fibrin deposits in the cell surface allowing a correct repair of the mesothelium and thereby preventing the formation of adhesions. Fibrinolytic activity of the mesothelial cells has been studied in rodent models and a reduction in the fibrinolytic capacity has been observed after trauma (120, 121). The area of the peritoneal defect has a progressive recovery of its fibrinolytic activity when the healing process is initiated. Long-term analysis of a peritoneal adhesion model in the rat showed increased fibrinolytic activity, which was found to be associated with the persistence of adhesions (122).

There are a variety of cells in the peritoneal cavity capable of expressing different components of the fibrinolytic system (123). Macrophages produce tPA and PAI-1 but the main source of fibrinolytic factors is the mesothelial cells, which are fundamental for the peritoneal integrity (47, 48). There is a remodeling process in the peritoneum that is very similar to wound healing. Previous studies have shown that combined deficiencies of uPAR and tPA do not exhibit the widespread fibrin deposition, extensive multi-organ tissue damage and severe alteration in the wound healing that is observed in combined uPA and tPA deficient mice (124). This would suggest that uPA could be the main PA responsible of removing fibrin deposits in the mesothelium surface allowing a correct process of peritoneal repair. In addition, ischaemic and tissue trauma lead to a high incidence of adherence formation that is a consequence of a reduction in the peritoneal fibrinolytic activity. The peritoneal fibrinolytic capacity of the peritoneum decreases during a surgical procedure as a local response to trauma and consequently facilitates adhesion formation (125). Intraabdominal adhesions have been shown to result from the impairment of peritoneal fibrinolysis by inhibitors present in the ischaemic tissue.

Conflicting evidence exists in relation to the peritoneal fibrinolytic activity in patients with endometriosis and pelvic adhesions. Previous reports have suggested that there is no difference in fibrinolytic parameters in peritoneal fluid in patients with or without endometriosis (126) and/or pelvic adhesive disease. (127). Meanwhile, Astedt and Nordenskjold (128) have referred an increase in plasminogen activators in the peritoneal fluid of women with endometriosis. Dörr *et al* have stated that fibrinolysis occurs at a high rate, as measured in the peritoneal fluid and plasma (129). An increased uPA level has been reported in the peritoneal fluid of women with pelvic adhesions but not in initial endometriosis without signs of adhesion formation (130). It may indicate an increased fibrinolysis parallel to the finding of pelvic adhesions. In addition uPA is increased in peritoneal fluid of women with advanced stages of the disease where adhesions are often present and may suggest an activation of the fibrinolytic system to inhibit further formation of adhesions in these patients (15).

In relation to the role of plasminogen inhibitors in women with endometriosis several studies have shown changes in the peritoneal fluid. Bruse *et al* (24) reported higher PAI-2 levels in the peritoneal fluid of women with endometriosis than in controls. This could be due to an increased activity of macrophages in peritoneal fluid, which would lead to an inflammatory reaction that may contribute to the high concentration of PAI-2. On the other hand, when studying initial stages of the disease without peritoneal adhesions, a reduction in PAI-2 levels in peritoneal fluid is observed. PAI-2 increases significantly after adhesiolysis, reflecting the need for fibrin deposition in the initial reaction of the peritoneum after trauma (130). Gilabert-Estelles *et al*, studied PAI-1, PAI-2 and PAI-3 antigen levels in peritoneal fluid of women with advanced endometriosis (stages III-IV), and no significant difference was found in comparison to women without the disease (15).

The peritoneal fluid seems to play an important role in the implantation process and the development of endometriotic lesions of patients with endometriosis. The differences observed between studies may be related to the different characteristics of the samples and the different stages of the disease. An increase in fibrinolytic activity is observed in the presence of pelvic adhesions, which are more frequent in the advanced stages of the disease. In the initial stages an impaired peritoneal fibrinolysis may facilitate adhesion formation because of reduced fibrin clearance. The increase in plasminogen activators observed in the peritoneal fluid of women with advanced stages of the disease may be due to a peritoneal response in order to limit the inflammatory process and the formation of new adhesions.

5. CLINICAL IMPLICATIONS AND FUTURE PERSPECTIVES

There is an increasing number of studies regarding the pathogenesis and pathophysiology of endometriosis and, consequently, more targeted therapies have been proposed. Current research has focused upon drugs that have the capacity to modify the development and maintenance of the disease. These include progesterone receptor modulators, gonadotropin releasing hormone antagonists, aromatase inhibitors, tumor necrosis factor alpha inhibitors, angiogenesis inhibitors, MMP inhibitors, general immune modulators and estrogen receptor beta agonists (131-136). Meanwhile, alterations observed on fibrinolytic activity of eutopic endometrium and peritoneal fluid of women with endometriosis have opened new therapy options and experimental approaches for the management of endometriosis and pelvic adhesions.

The fibrinolytic activity of the peritoneal cavity has an important role in peritoneal healing after trauma and in the process of limitation of adhesion formation. The plasmin activity of peritoneal mesothelium determines whether fibrin, which is formed after peritoneal injury, is either lysed or organized into fibrous peritoneal adhesions (137). Endometriotic implants in the peritoneum alter the mesothelial integrity resulting in frequent adhesion formation during the progression of the disease. Impaired peritoneal fibrinolysis may determine adhesion formation

and may result from altered proportion of plasminogen activator and plasminogen activator inhibitors.

It is believed that reduction of fibrinolytic activity may be related to adhesion formation after peritoneal trauma. However, Bakkum *et al* found an increase in fibrinolysis associated with long-term persistence of adhesions (122). These findings suggest that the balance between plasminogen activators and plasminogen activator inhibitors may be critical in the induction of adhesion formation. Therefore several studies have been developed in order to test the usefulness of different molecules as targets to develop novel therapeutic strategies to reduce adhesion formation. Intraperitoneal administration of anti-PAI-1 antibodies has shown to be effective in reducing adhesion formation after a surgical trauma of the peritoneum by limiting the availability of PAI-1 for the inhibition of tPA (138). Several authors have administered intraperitoneally recombinant tissue plasminogen activator (rtPA) and have demonstrated *in vivo* a reduction in postoperative intraperitoneal adhesion (139, 140).

Sharpe-Timms (141) evaluated the effects of gonadotropin-releasing hormone (GnRHa) agonist on plasminogen activator and plasminogen activator inhibitor in peritoneal fluid of female rats relative to adhesion formation. GnRHa decreased the PA activity and increased the PAI activity resulting in an effective prevention of adhesion formation.

Confusion exists about the optimal therapeutic agent that should be tested in order to prevent the establishment of the endometriotic lesion and the formation of new adhesions. The broad variety of models and methods used may be a confounding factor that would make difficult interpretation of the results and the establishment of clinical basis for human application. In addition, other factors independent of the fibrinolytic system such as steroid environment or MMP activity may be implicated in the pathogenesis of endometriotic implants and pelvic adhesions. Since endometriosis is diagnosed during surgical procedures, somehow peritoneal trauma cannot be avoided. Hopefully, further research will be able to develop new molecules which will prevent adherences when administered intraperitoneally.

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Abbreviations: tPA: tissue type plasminogen activator, sc-tPA: single chain tPA, uPA: urokinase type plasminogen activator, sc-uPA: single chain uPA, PAI: plasminogen activator inhibitor, FDP: fibrin degradation products, ECM: extracellular matrix, MMP: metalloproteinase, TIMPs: tissue inhibitors of MMPs, PAI-1: plasminogen activator inhibitor type 1

Key Words: endometriosis, fibrinolysis, tPA, tissue type plasminogen activator, sc-tPA, single chain tPA, uPA, urokinase type plasminogen activator, sc-uPA, single chain uPA, PAI, plasminogen activator inhibitor, FDP, fibrin degradation products, ECM, extracellular matrix, MMP, metalloproteinase, TIMPs, tissue inhibitors of MMPs, PAI-1, plasminogen activator inhibitor type 1, Review

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