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Mechanisms of Normal and Abnormal Endometrial Bleeding

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Abstract

Expression of tissue factor (TF), the primary initiator of coagulation, is enhanced in decidualized human endometrial stromal cells (HESC) during the progesterone-dominated luteal phase. Progesterone also augments a second HESC hemostatic factor, plasminogen activator inhibitor-1 (PAI-1). In contrast, progestins inhibit HESC matrix metalloproteinase (MMP)-1, 3 and 9 expression to stabilize endometrial stromal and vascular extracellular matrix. Through these mechanisms decidualized endometrium is rendered both hemostatic and resistant to excess trophoblast invasion in the mid-luteal phase and throughout gestation to prevent hemorrhage and accreta. In non-fertile cycles, progesterone withdrawal results in decreased HESC TF and PAI-expression and increased MMP activity and inflammatory cytokine production promoting the controlled hemorrhage of menstruation and related tissue sloughing. In contrast to these well ordered biochemical processes, unpredictable endometrial bleeding associated with anovulation reflects absence of progestational effects on TF, PAI-1 and MMP activity as well as unrestrained angiogenesis rendering the endometrium non-hemostatic, proteolytic and highly vascular. Abnormal bleeding associated with long-term progestin-only contraceptives results not from impaired hemostasis but from unrestrained angiogenesis leading to large fragile endometrial vessels. This abnormal angiogenesis reflects progestational inhibition of endometrial blood flow promoting local hypoxia and generation of reactive oxygen species that increase production of angiogenic factors such as vascular endothelial growth factor (VEGF) in HESCs and Angiopoietin-2 (Ang-2) in endometrial endothelial cells while decreasing HESC expression of angiostatic, Ang-1. The resulting vessel fragility promotes bleeding. Aberrant angiogenesis also underlies abnormal bleeding associated with myomas and endometrial polyps however there are gaps in our understanding of this pathology.

Keywords

Tissue Factor; endometrium; hemostasis; angiogenesis; bleeding

Normal menstrual cycle hemostasis and menstruation

Following menstruation, follicular phase estradiol (E_2) induces proliferation of endometrial epithelial and stromal cells while E2 and/or hypoxia enhance angiogenesis by inducing

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human endometrial stromal cells (HESC) to express vascular endothelial growth factor (VEGF) and endometrial endothelial cells to express angiopoietin-2 (Ang-2) (1,2). In the early to mid luteal phase, progesterone induces the decidualization reaction initially around blood vessels. There progesterone augments expression of HESC Angiopoietin-1 (Ang-1), an angiostatic agent that stabilizes vessels and blocks further unrestrained angiogenesis.

Progesterone also induces tissue factor (TF) mRNA and protein in decidualized HESCs of luteal phase and gestational endometrium (3). Tissue factor is a 46 kDa cell membrane-bound glycoprotein consisting of a hydrophilic extracellular domain, which acts as a receptor for coagulation factor VII and its active form, factor VIIa. Tissue factor also has a membrane-spanning hydrophobic domain, and a cytoplasmic tail. Binding of circulating factor VII/VIIa to TF initiates the clotting cascade which promotes hemostasis by forming fibrin and platelet plugs. The absence of canonical progesterone response elements (PREs) in the TF gene promoter led us to a series of experiments that elucidated the underlying molecular mechanisms by which progesterone enhances TF expression in HESCs. In brief, epidermal growth factor receptor (EGFR) is induced by progesterone through a canonical PRE in HESCs, and ligand binding to EGFR is required for HESC TF expression (4). Since EGF is an inducer of the transcription factor Sp1, whose response elements festoon the TF gene promoter, we next ascertained that Sp1 augments while Sp3 inhibits HESC TF gene transcription (5). Subsequent studies indicated that decidualized HESCs continue to express TF throughout pregnancy (6).

Additional studies indicated that progesterone induces a second hemostatic protein, plasminogen activator inhibitor-1 (PAI-1), in HESCs via similar molecular mechanisms (7,8). In addition to its anti-fibrinolytic properties PAI-1 restrains trophoblast invasion mediated by urokinase –type plasminogen activator (9). Thus, the progesterone-dominated mid-luteal phase is associated with maximal hemostatic, anti-fibrinolytic and anti-proteolytic properties to mitigate the threat of potentially pregnancy-threatening hemorrhage during implantation and of unrestrained trophoblast invasion associated with placenta accrete, a condition marked by inadequate or absent decidua due to uterine scarring.

Conversely, following the failure of conception, progesterone withdrawal in the perimenstrual period, or progestin withdrawal and/or treatment with the antiprogestin RU486 *in vitro* elicits reductions in HESC TF and PAI-1 expression (10). Thus, failure of conception and progestational withdrawal creates a pro-hemorrhagic environment around endometrial blood vessels promoting menstrual bleeding.

In parallel with these changes in luteal phase hemostatic factor expression is a parallel progestational inhibition of HESC matrix metalloproteinase (MMP) expression. Thus, progestins inhibit expression of immunoreactive and functional active MMP-1, 3 and 9 expression while progesterone withdrawal or treatment with RU486 augments their expression in HESCs (11–14). In contrast, neither progestins nor progestational withdrawal consistently affect MMP-2 or tissue inhibitors of MMPs (11,12). Progesterone withdrawal is also associated with up-regulation of the neutrophil and macrophage chemoattractants, interleukin-8 (CXCL8) and macrophage chemoattractant protein-1 (CCL2), respectively (15). Thus, luteal phase and gestational endometrium is associated with reduced MMP activity, stabilizing stromal and underlying vascular extracellular matricies to impede endometrial hemorrhage during pregnancy. Conversely, perimenstrual progesterone withdrawal in non-fertile cycles is associated with increases in HESC MMP expression as well as chemokines promoting leukocyte infiltration which add to the proteolytic milieu, promoting menstrual bleeding and tissue sloughing.

Abnormal endometrial bleeding

In contrast to the well ordered and highly regulated processes underlying normal menstrual cycle hemostasis and menstruation, abnormal endometrial bleeding reflects derangements in these physiological processes. There are two principal causes of abnormal endometrial bleeding (Table 1) – impaired endometrial hemostasis and structural damage to endometrial vessels usually due to unrestrained aberrant angiogenesis.

Bleeding dyscrasias

Bleeding diatheses generally present as menorrhagia (heavy menstrual bleeding) commencing at menarche and are present in 10.7% of such patients compared to their occurrence in 3.2% of controls (16). Von Willebrand's disease is the most common defect associated with menorrhagia with a prevalence of 5–20% in five published studies (17). Screening includes activated partial thromboplastin time (aPTT) and ristocetin cofactor assay. Treatment consists of combined hormonal contraceptives which presumably induce HESC TF and PAI-1 levels to compensate for the hemostatic defect attendant such patients.

Abnormal uterine bleeding during long-term progestin-only contraception (LTPOC)

Long-term progestin-only contraceptives (LTPOCs) are efficient, safe and discrete forms of birth control. Formulations include Mirena, an intrauterine device that releases levonorgestrel for 5 years; Implanon, an implantable subdermal system that releases etonogestrel over a 3-year period; and Depo-Provera, an injectable form of medroxyprogesterone acetate which persists for 3 months. LTPOCs are safe to use during lactation and among patients in whom estrogen-containing contraceptives are contraindicated. Unfortunately, abnormal endometrial bleeding occurs in the majority of LTPOC users and is the leading cause for their discontinuation. In contrast to the organized and global response to circulating progesterone withdrawal triggering normal menstruation, LTPOC-associated bleeding occurs in a scattered and sporadic fashion from irregularly distributed, superficial, abnormally enlarged, fragile capillaries and venules embedded in a collapsed stromal extracellular matrix (18).

Hysteroscopic endometrial biopsies obtained from LTPOC users reveals the presence of enlarged, thin walled blood vessels at bleeding sites despite increased TF expression (19,20). Endometrial biopsies also reveal a dramatic increase in immature and partially mature vessel number, area and density (21). Immunostaining and Western blotting also revealed no significant differences at bleeding or non-bleeding sites in levels of the EGFR and progesterone receptor levels. In contrast, these blood vessels were associated with elevated expression of HESC VEGF and endothelial Ang-2 with reduced expression of HESC Ang-1, an angiostatic agent (22,23). Thus, aberrant, unrestrained angiogenesis producing fragile, leaky blood vessels rather than impaired hemostasis underlies LTPOC-induced abnormal endometrial bleeding. This begged the question of what initiates this aberrant angiogenesis.

Since hypoxia is a major stimulus for angiogenesis we measured endometrial blood flow following LTPOC administration using laser-Doppler fluxmetry and observed impaired endometrial blood flow in microvascular beds (24). Consistent with this reduced endometrial perfusion and resultant enhanced hypoxia/reperfusion injury, LTPOC use was accompanied by elevated endometrial lipid peroxidation and increased expression of reactive oxygen species (ROS)including 8-Hydroxy-2'-deoxyguanosine (8-OHdG), a marker of oxidative DNA damage, and nitrotyrosine, a marker of oxidative protein damage (24). We then showed that this oxidative stress induces synthesis of angiogenic factors such as HESC VEGF and endometrial endothelial cell Ang-2 expression while suppressing HESC Ang-1 expression (25). While physiological levels of VEGF promote angiogenesis, over-

expressed VEGF induces endothelial "leakiness" and perivascular ECM dissolution due to excess MMP-2 activity culminating in bleeding and collapsed stroma – precisely the characteristics of LTPOC treated endometria (26). Thus, LTPOC-induced reductions in endometrial blood flow cause hypoxia-reperfusion injury and free radical production that promotes aberrant angiogenesis via enhanced expression of VEGF and Ang-2 and reciprocal inhibition of Ang-1 as well as directly damaging blood vessels that bleed despite the surfeit of available perivascular TF.

Abnormal endometrial bleeding associated with myomas and endometrial polyps

While abnormal endometrial bleeding associated with myomas is a leading cause of hysterectomy the precise mechanisms for this bleeding are yet to be fully elucidated. Menorrhagia rather than metorrhagia (intracycle bleeding) is the most common presentation and is present in about a third of cases (27). The classical vascular lesion associated with myomas is venule ectasia. This is now thought to reflect the actions of tumor and/or hypoxia-derived angiogenic factors and increased estrogen receptor expression which presumably causes spiral artery vasodilation (28). Among the associated angiogenic factors putatively over-expressed by myomas and/or their adjacent endometrium are basic fibroblast growth factor, VEGF, heparin-binding epidermal growth factor (HBEGF), platelet-derived growth factor, transforming growth factor-beta, parathyroid hormone related protein and prolactin (28). The resultant large dilated vessels presumably account for the observed menorrhagia.

In contrast, endometrial polyps more often present as metorrhagia. Little is known about the mechanism for such bleeding but increased polyp MMP and cyclo-oxygenase production have been noted (29). Microvascular density also appears increased (30). These findings suggest that aberrant angiogenesis may also play a role in polyp-associated abnormal endometrial bleeding. Thus, polyp-associated dilated, fragile superficial endometrial vessels would be prone to intermittent bleeding as is seen in LTPOC treated endometria.

Despite these observations, the cause of abnormal uterine bleeding in patients with myomas and polyps remain a major gap in the care of reproductive age women. Additional, research is needed in this area.

Conclusion

The menstrual cycle is associated with maximal hemostasis and vascular stability in the mid-luteal phase followed by the controlled hemorrhage and tissue sloughing of menstruation in non-fertile cycles. This process is tightly regulated by progestational induction of TF and PAI-1 expression and inhibition of MMP activity along with regulation of angiogenesis. Conversely, progesterone withdrawal reduces hemostatic and increases MMP activity to provoke controlled hemorrhage. In contrast to this tightly regulated process, anovulatory bleeding is associated with both impaired hemostasis due to an absence of progestational induction of TF and PAI-1 and both increased MMP and angiogenesis due to unrestrained estrogenic effects. On the other hand, LTPOC-associated bleeding reflects sustained hemostasis with persistently elevated TF expression but vessel damage due to impaired endometrial blood flow with hypoxia-induced unrestrained angiogenesis. Similar defects appear to account for abnormal bleeding with myomas and endometrial polyps, though in the former condition macrovascular changes lead to menorrhagia while in the latter microvascular changes promote metorrhagia.

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References

1. Krikun G, Schatz F, Lockwood CJ. Endometrial angiogenesis: from physiology to pathology. Ann N Y Acad Sci. 2004; 1034:27–35. [PubMed: 15731297]
2. Schatz F, Krikun G, Caze R, Rahman M, Lockwood CJ. Progestin-regulated expression of tissue factor in decidual cells: implications in endometrial hemostasis, menstruation and angiogenesis. Steroids. 2003; 68:849–860. [PubMed: 14667977]
3. Lockwood CJ, Nemerson Y, Guller S, Krikun G, Alvarez M, Hausknecht V, Gurpide E, Schatz F. Progestational regulation of human endometrial stromal cell tissue factor expression during decidualization. J Clin Endocrinol Metab. 1993; 76:231–236. [PubMed: 8421090]
4. Lockwood CJ, Krikun G, Runic R, Schwartz LB, Mesia AF, Schatz F. Progestin-epidermal growth factor regulation of tissue factor expression during decidualization of human endometrial stromal cells. J Clin Endocrinol Metab. 2000; 85:297–301. [PubMed: 10634402]
5. Krikun G, Schatz F, Mackman N, Guller S, Demopoulos R, Lockwood CJ. Regulation of tissue factor gene expression in human endometrium by transcription factors Sp1 and Sp3. Mol Endocrinol. 2000; 14:393–400. [PubMed: 10707957]
6. Lockwood CJ, Murk W, Kayisli UA, Buchwalder LF, Huang ST, Funai EF, Krikun G, Schatz F. Progestin and thrombin regulate tissue factor expression in human term decidual cells. J Clin Endocrinol Metab. 2009; 94:2164–2170. [PubMed: 19276228]
7. Krikun G, Lockwood CJ. Steroid hormones, endometrial gene regulation and the Sp1 family of proteins. J Soc Gynecol Investig. 2002; 9:329–334.
8. Lockwood CJ. Regulation of plasminogen activator inhibitor 1 expression by interaction of epidermal growth factor with progestin during decidualization of human endometrial stromal cells. Am J Obstet Gynecol. 2001; 184:798–804. [PubMed: 11303186]
9. Zini JM, Murray SC, Graham CH, Lala PK, Karikó K, Barnathan ES, Mazar A, Henkin J, Cines DB, McCrae KR. Characterization of urokinase receptor expression by human placental trophoblasts. Blood. 1992; 79:2917–2929. [PubMed: 1316787]
10. Papp C, Schatz F, Krikun G, Hausknecht V, Lockwood CJ. Biological mechanisms underlying the clinical effects of mifepristone (RU 486) on the endometrium. Early Pregnancy. 2000; 4:230–239. [PubMed: 11742418]
11. Lockwood CJ, Krikun G, Hausknecht VA, Papp C, Schatz F. Matrix metalloproteinase and matrix metalloproteinase inhibitor expression in endometrial stromal cells during progestin-initiated decidualization and menstruation-related progestin withdrawal. Endocrinology. 1998; 139:4607–4613. [PubMed: 9794472]
12. Schatz F, Papp C, Aigner S, Krikun G, Hausknecht V, Lockwood CJ. Biological mechanisms underlying the clinical effects of RU 486: modulation of cultured endometrial stromal cell stromelysin-1 and prolactin expression. J Clin Endocrinol Metab. 1997; 82:188–193. [PubMed: 8989257]
13. Li L, Zhou Z, Huang L. Abnormal expression of MMP-9 and imbalance of MMP-9/TIMP-1 is associated with prolonged uterine bleeding after a medical abortion with mifepristone and misoprostol. Acta Obstet Gynecol Scand. 2009; 88(6):673–679. [PubMed: 19353331]
14. Chen JL, Lin QH, Fang XL, Tao GS. Huang FY Effect of progesterone on the secretion of matrix metalloproteinase-2 and matrix metalloproteinase-9 in human ectopic endometrial stromal cells. Zhong Nan Da Xue Xue Bao Yi Xue Ban. 2005; 30:307–311. [PubMed: 16045020]
15. Critchley HO, Jones RL, Lea RG, Drudy TA, Kelly RW, Williams AR, Baird DT. Role of inflammatory mediators in human endometrium during progesterone withdrawal and early pregnancy. J Clin Endocrinol Metab. 1999; 84:240–248. [PubMed: 9920091]
16. Dilley A, Drews C, Miller C, Lally C, Austin H, Ramaswamy D, Lurye D, Evatt B. von Willebrand disease and other inherited bleeding disorders in women with diagnosed menorrhagia. Obstet Gynecol. 2001; 97:630–636. [PubMed: 11275041]
17. James A, Matchar DB, Myers ER. Testing for von Willebrand disease in women with menorrhagia: a systematic review. Obstet Gynecol. 2004; 104:381–388. [PubMed: 15292016]
18. Rogers PA. Endometrial vasculature in Norplant users. Hum Reprod. 1996; 11 Suppl 2:45–50. [PubMed: 8982745]

19. Runic R, Schatz F, Wan L, Demopoulos R, Krikun G, Lockwood CJ. Effects of Norplant on endometrial tissue factor expression and blood vessel structure. J Clin Endocrinol Metab. 2000; 85:3853–3859. [PubMed: 11061549]

20. Lockwood CJ, Runic R, Wan L, Krikun G, Demopolous R, Schatz F. The role of tissue factor in regulating endometrial haemostasis: implications for progestin-only contraception. Hum Reprod. 2000; 15 Suppl 3:144–151. [PubMed: 11041230]

21. Stéphanie R, Labied S, Blacher S, Frankenne F, Munaut C, Fridman V, Beliard A, Foidart JM, Nisolle M. Endometrial vessel maturation in women exposed to levonorgestrel-releasing intrauterine system for a short or prolonged period of time. Hum Reprod. 2007; 22(12):3084–3091. [PubMed: 17921480]

22. Schatz F, Krikun G, Caze R, Rahman M, Lockwood CJ. Progestin-regulated expression of tissue factor in decidual cells: implications in endometrial hemostasis, menstruation and angiogenesis. Steroids. 2003; 68:849–860. [PubMed: 14667977]

23. Krikun G, Sakkas D, Schatz F, Buchwalder L, Hylton D, Tang C, Lockwood CJ. Endometrial angiopoietin expression and modulation by thrombin and steroid hormones: a mechanism for abnormal angiogenesis following long-term progestin-only contraception. Am J Pathol. 2004; 164:2101–2107. [PubMed: 15161644]

24. Hickey M, Krikun G, Kodaman P, et al. Long-term progestin-only contraceptives result in reduced endometrial blood flow and oxidative stress. J Clin Endocrinol Metab. 2006; 91:3633–3638. [PubMed: 16757524]

25. Krikun G, Critchley H, Schatz F, Wan L, Caze R, Baergen RN, Lockwood CJ. Abnormal uterine bleeding during progestin-only contraception may result from free radical-induced alterations in angiopoietin expression. Am J Pathol. 2002; 161:979–986. [PubMed: 12213726]

26. Dvorak HF, Brown LF, Detmar M, Dvorak AM. Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. Am J Pathol. 1995; 146:1029–1039. [PubMed: 7538264]

27. Fraser IS, Critchley HO, Munro MG, Broder M. A process designed to lead to international agreement on terminologies and definitions used to describe abnormalities of menstrual bleeding. Fertil Steril. 2007; 87:466–476. [PubMed: 17362717]

28. Stewart EA, Nowak RA. Leiomyoma-related bleeding: a classic hypothesis updated for the molecular era. Hum Reprod Update. 1996; 2:295–306. [PubMed: 9080227]

29. Erdemoglu E, Güney M, Karahan N, Mungan T. Expression of cyclooxygenase-2, matrix metalloproteinase-2 and matrix metalloproteinase-9 in premenopausal and postmenopausal endometrial polyps. Maturitas. 2008; 59:268–274. [PubMed: 18378414]

30. Tokyol C, Aktepe F, Dilek FH, Sahin O, Arioz DT. Expression of cyclooxygenase-2 and matrix metalloproteinase-2 in adenomyosis and endometrial polyps and its correlation with angiogenesis. Int J Gynecol Pathol. 2009; 28:148–156. [PubMed: 19188819]

Table 1

Causes of Abnormal Endometrial Bleeding

Primary Defect	Examples
Impaired hemostasis.	Von Willebrand's disease and other primary disorders of clotting exacerbate normal menstrual bleeding.
Impaired hemostasis with some derangement in blood vessels.	Anovulatory bleeding – absence of progesterone effects results in reduced endometrial stromal cell tissue factor and plasminogen activator inhibitor-1 production, increased matrix metalloproteinase activity and increased angiogenic factor expression. This creates vascular instability with greatly impaired hemostasis, each predisposing to break-through bleeding.
Aberrant blood vessels with normal hemostasis.	Long-term, progestin-only contraceptives reduce endometrial blood flow, and the resultant hypoxia and reactive oxygen species drive expression of angiogenic factors and perivascular MMP-2 production. This creates large, fragile, easily fractured superficial endometrial blood vessels held in place by a collapsing stromal extracellular matrix that promotes intermittent bleeding.
Aberrant blood vessels with normal hemostasis.	Myomas – are associated with long-standing increased angiogenesis and an increasingly estrogenic milieu which leads to dilated veins (venule ectasia) and increased arterial flow promoting menorrhagia.
Aberrant blood vessels with normal hemostasis.	Polyps – are associated with increased angiogenesis and focal vascular abnormalities leading to metorrhagia.