A functional model for progestogen-induced breakthrough bleeding

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During the past 5 years, a number of important advances have been made in our understanding of the mechanisms of sex steroid-induced breakthrough bleeding (BTB). These observations suggest that superficial endometrial vascular fragility may be the mechanism underlying BTB, and molecular changes in the microvasculature as well as hysteroscopic observations have supported this hypothesis. This paper aims to present a unified picture of our current understanding of BTB, particularly that associated with progestogens, to indicate current gaps in our knowledge and possible directions for future research.

Key words: breakthrough bleeding/endometrium/microvasculature/progestogens

Introduction

Contraceptive steroid-induced breakthrough bleeding (BTB) is a major social and clinical problem for women world-wide (Odlind and Fraser, 1990). Irregular and prolonged bleeding is particularly common in users of progestogen-only contraceptives, but can occur with combined oral contraceptives and with hormone replacement therapy (HRT) use. There is no established long-term treatment for this BTB and it commonly leads to discontinuation when effective alternatives may not be suitable or utilized (Belsey, 1988).

This paper aims to introduce a functional model to explain the mechanisms of progestogen-induced BTB. In particular, to emphasize where recent progress has been made, and to identify areas needing further study.

Mechanisms of BTB

Endogenous and exogenous sex steroids are likely to exert overall control over the endometrium, but since receptors for oestrogen and progesterone have not previously been identified in small endometrial blood vessels (Perrot-Applanat et al., 1988), local mechanisms have been presumed to maintain direct control over endometrial microvessels. However, recent data (Rodriguez-Manzaneque et al., 2000, see pp. 39–47, this supplement) suggest that these progesterone receptors may be present in some endometrial vessels, and the possible implications of these observations have not yet been explored.

Disrupted bleeding patterns may reflect a change in the type of endometrial vessel from which the bleeding is arising (vessel source), vessel integrity, haemostasis or a combination of these factors.

The pattern, quantity and appearance of BTB suggests that it may arise from a different vascular source than normal menstrual bleeding (Odlind and Fraser, 1990). Normal menstrual bleeding is thought to arise primarily from the spiral arterioles, with endometrial capillaries making little contribution (Bartelmez, 1937; Markee, 1940). Breaks in capillaries and veins
adjacent to the uterine lumen (Johanisson, 1990) suggest that BTB arises primarily from these vessels and is related to increased capillary and venous fragility (Odlind and Fraser, 1990; Hickey et al., 1997). Hysteroscopic studies in women using the low-dose levonorgestrel (LNG) contraceptive implant system Norplant have suggested that the superficial endometrial vessels are abnormally fragile (Hickey et al., 1997, 1998, 2000a,b).

Mechanisms by which exogenous sex steroids may compromise the stability and integrity of the endometrium

Changes in endometrial vascular morphology

There is increasing evidence from in-vivo and in-vitro studies that the endometrial microvascular appearance is altered by progestogen exposure. Superficial vascular dilatation (Hickey et al., 1997; Runic et al., 1997) and neovascular formations (Hickey et al., 1997) suggest that the normal tight control over vascular development is altered in women exposed to low-dose progestogens. Ultrastructural studies of individual endometrial vessels have demonstrated dilated vessels of abnormal shape and structure (F.Manconi et al., unpublished). It is likely that the systemic dose and also the local endometrial concentration of progestogen will be of importance. Endometrial biopsies from women exposed to high-dose progestogens (as DMPA) have also shown dilated superficial vessels (Song et al., 1995; I.S.Fraser, unpublished observation). The mechanisms leading to dilatation are unclear, and altered morphology alone will not lead to bleeding. However, this changed vascular appearance may indicate altered vascular function.

In addition, low-dose progestogen exposure is associated with a change in the balance between vascular and non-vascular elements in the endometrium, with a relative increase in endometrial vascular density (Rogers et al., 1993; Hickey et al., 1999a). This lack of stromal support for endometrial microvessels may contribute to vascular fragility.

Vascular endothelial growth factor (VEGF) contributes to vascular permeability and provokes dilatation. Increased VEGF expression has been observed in Norplant users (Lau et al., 1999). The regulation of other angiogenic factors such as basic fibroblast growth factor (FGF)-α and β, VEGF, transforming growth factor (TGF)-β, angiogenin and cytokines, may also be disturbed and contribute to abnormal endometrial vascular morphology.

Changes in vascular structural integrity

Small endometrial vessels are composed only of endothelial cells, their surrounding basement membrane and pericytes. Breakthrough bleeding is most common during the initial months of exposure to Norplant. At this time, the number of endometrial vessels surrounded by the basement membrane components laminin, collagen IV and heparan sulphate proteoglycan are reduced (Hickey et al., 1999b). This is not seen in longer term Norplant users, when fewer bleeding problems occur (Palmer et al., 1996). Changes may also occur in pericyte support as demonstrated by a reduction in vascular smooth muscle α-actin expression in progestogen-exposed endometrium (Rogers et al., 2000).

Increased capacity for cell breakdown

It is thought that normal menstruation is associated with breakdown of the superficial endometrial vessels and shedding of the extravascular compartment above the regenerative basalis level (Markee, 1940). Little is known about tissue shed during BTB, and the extravascular component of this loss.

Normal menstruation is preceded by an influx of proteolytic substances able to rapidly breakdown the extracellular matrix, and contributing to endometrial vascular repair via the activation of pro-angiogenic factors. Increased migratory leukocytes precede menstruation (Bulmer et al., 1988, 1991), and are also seen in association with high- (Song et al., 1996) and low-dose (Clark et al., 1996) progestogen exposure. Similarly, menstruation is associated with increased matrix metalloproteinase (MMP) activity, and a change in the balance between MMP and their tissue inhibitors (TIMP; Hampton and Salamonsen, 1994). Exposure to low-dose progestogen contraceptives is associated with a change in the ratio of MMP to TIMP in favor of proteolytic MMP (Skinner et al., 1999; Vincent et al., 1999), factors which may lead to vascular breakdown and BTB. However, the mechanism leading to this observed increase in MMP-9 localization is unknown.
**Changes in endometrial perfusion and oxygenation**

Changes in exogenous oestrogens and progestogens are known to affect uterine perfusion, as assessed from uterine artery Doppler ultrasound (Exacoustos et al., 1999). Assessing endometrial perfusion using $^{133}$Xe clearance, it was found that altered endometrial perfusion accompanied spontaneous dysfunctional uterine bleeding (Fraser et al., 1987). Vasomotion describes the spontaneous and rhythmic dilation and constriction of microvessels. Pilot studies using laser-Doppler show reduced endometrial perfusion in early Norplant users and profound alteration of normal vasomotion patterns with an almost total loss of short-term vasomotion (Hickey et al., 2000a).

It is possible that reduced endometrial perfusion leads to relative endometrial hypoxia. Hypoxia is a potent vascular destabilizer; compromising endothelial integrity (Ali et al., 1998), inducing vascular breakdown and stimulating angiogenesis via VEGF release (Smith, 1997). Hypoxic changes have not been studied in human endometrial tissue.

**Changes in endometrial responsiveness to sex steroids**

Conflicting results have been obtained regarding endometrial sex steroid expression in Norplant users (Critchley et al., 1993; Lau et al., 1996). In women using the 20 μg levonorgestrel-releasing intrauterine system (Mirena®; Schering Health, W. Sussex, UK), progesterone receptor expression is reduced, rendering the endometrium functionally unresponsive to endogenous and exogenous progestogens (Critchley et al., 1988). A fall in progesterone is known to stimulate MMP activity, and consequent breakdown of the extracellular matrix (ECM; Marbaix et al., 1995). An endometrium unresponsive to progestogens because of profound receptor downregulation may thus stimulate endometrial vascular breakdown in progestogen users. A likely cause of this differential receptor expression may be the variations in endometrial concentration of LNG seen in the contraceptive systems studied. Intrauterine LNG produces LNG concentrations 1000-fold greater than serum concentrations (Pekonen et al., 1992).

**Changes in endometrial vascular haemostasis**

Normal menstrual bleeding is thought to arise predominantly from the spiral arterioles (Markee, 1940). Spiral arteriole development is markedly curtailed following progestogen exposure (Hourihan et al., 1986, 1991), and bleeding may arise from smaller superficial vessels in the endometrium (Hickey et al., 1996). Since spiral arteriole constriction is an essential early mechanism of menstrual haemostasis (Markee, 1940), bleeding arising from other vessels may result in compromised haemostasis and prolonged bleeding. In addition, the powerful vasoconstrictor molecules endothelins are reduced in Norplant users (Marsh et al., 1995), and the endothelial metabolite neural endopeptidase (NEP) is increased (Salamonsen et al., 1999). Reduced endothelial activity may compromise vascular repair as well as haemostasis.

Tissue factor (TF) is a primary inhibitor of endometrial haemostasis. mRNA for TF is reduced in Norplant users compared to secretory controls (Runic et al., 1997).

The overall effect of these vascular changes may be to increase endometrial vascular fragility. If the endometrium contains comparatively more microvessels with comparatively less structural support, and greater influx and activity of local molecules able to break down vessels, in conjunction with compromised haemostatic mechanisms, this may induce and perpetuate BTB.

**Possible interventions to prevent BTB**

**Increasing vascular stability**

In other organ systems, such as the human retina, loss of vascular stability is associated with oxidative stress and the release of free radicals (Kowluru et al., 1999). At a molecular level, this vascular fragility is associated with reduced integrity of endothelial cell tight junctions and vascular basement membrane competence and clinically leads to vascular breakdown and retinal bleeding (Martin et al., 1988). Flavonoids, part of the vitamin B complex, have been shown in controlled trials to increase peripheral capillary resistance and to improve the systemic symptoms of capillary fragility such as epistaxes, petechiae and conjunctival haemorrhages (Galley and Thiollet, 1993). Recent pilot data (Subakir et al., 2000) have
suggested that oral vitamin E (an antioxidant) given during bleeding episodes may reduce bleeding in users of low-dose progestogens.

**Reduce vessel destabilizers**
The introduction of agents specifically targeted to block molecules stimulating breakdown of endometrial vessels and extracellular matrix may help to reduce BTB. MMP activity could be antagonized by selective use of TIMPs.

**Improve epithelial integrity**
Hysteroscopic studies in Norplant users strongly suggest that subepithelial bleeds (seen as petechiae and ecchymoses) are common in these women when vaginal bleeding has not been observed by the patient (Hickey et al., 1997). Norplant use appears to reduce epithelial integrity by interfering with cytokeratin deposition (Wonodiresko et al., 1996). Since endometrial bleeding is not problematic unless it manifests as vaginal bleeding, agents which maintain epithelial integrity may also act to contain bleeding. Oestrogens induce endometrial epithelial proliferation and may thus effectively terminate prolonged bleeding episodes in progestogen-users. Oestrogens may also act to maintain endothelial cell junctional integrity, but there is currently little known about the regulation of these tight junctions in the endometrium. Since the addition of oestrogens to progestogen-only contraception essentially undermines many of the advantages of these preparations, non-oestrogenic agents to maintain epithelial integrity are needed. Current selective oestrogen receptor modifying preparations (SERM) aim to avoid endometrial receptor targets. There may be a role for SERM acting to selectively stimulate the endometrium but not other tissues.

**Unresolved issues in progestogen-induced BTB**
Despite these advances in understanding of the mechanisms of BTB, current information does not provide a comprehensive picture of this common clinical problem. There are a number of important unresolved issues: (i) What is the underlying process leading to increased endometrial vascular fragility? Is vascular basement membrane the whole story? What is the role of endothelial cell tight junctions in maintaining vascular integrity following progestogen exposure? Angiopoietins have recently been characterized in human endometrium (Charnock-Jones et al., 2000), and these molecules may play a vital role in the assembly and breakdown of microvessels. (ii) How are vascular haemostatic and repair mechanisms compromised by progestogens? (iii) What accounts for the apparent increase in endometrial microvascular density? There is currently no evidence for microvascular angiogenesis, using conventional markers of endothelial cell proliferation in Norplant users (Rogers et al., 1993; Goodger et al., 1994). In addition, superficial vessels with apparent neovascular formations have commonly been seen in these subjects (Hickey and Fraser, 2000). This suggests that increased vascular density may be due to differential regression of non-vascular elements. Alternatively, that vascular proliferation may be occurring by another mechanism. These hypotheses require further investigation. (iv) Despite changes in endometrial vasculature associated with progestogen use, very few single parameters have been directly associated with bleeding. Is there a delay between vascular breakdown and vaginal bleeding as observed by the woman? In Norplant users, subepithelial bleeding is common (Hickey et al., 1997). Alterations in epithelial integrity following sex steroid exposure require further exploration. (v) How can this process be interrupted? It seems unlikely that endometrial ‘normality’ can be restored, and methods involving systemic oestrogens are ultimately not going to be practical. There is an urgent need to identify and target specific points in the cascade of vascular breakdown that can be selectively inhibited, resulting in vaginal bleeding not occurring or being contained beneath the epithelium such that it is not seen by the contraceptive user. (vi) Since the uterus is a mobile organ within the normal pelvis, there may be a role for movement of uterine walls and shearing stresses in provoking superficial vascular breakdown.

**Conclusion**
Effective and acceptable management of contraceptive-induced breakthrough bleeding is unlikely to be achieved without a fuller understanding of the
factors provoking this bleeding, and of molecular changes in the endometrial vessels which undermine their integrity. Recent information has greatly advanced this understanding, but a number of areas require further study before the mechanisms of BTB can be defined. In addition, further information is required from women using these preparations in developing and developed countries regarding their contraceptive needs and the relative tolerability of varying bleeding patterns and of amenorrhoea.

References


