Future directions for research on endometrial bleeding

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Disturbances in endometrial bleeding remain a major problem, and the primary reason for discontinuation of progestin-only methods of contraception. However, some interesting leads were uncovered in the course of this meeting on 'Current research on progestin-only contraceptives and endometrial bleeding', and a number of suggestions made about techniques and research topics. This paper attempts to summarize these suggestions, with a view to stimulating further research. Topics identified included the development of objective tests for vessel fragility, identification and properties of vessels likely to bleed, the location of bleeding vessels, the basis of the increased microvascular density, the integrity of the surface epithelium, the state of the stroma, the function of steroid receptors, and the properties of progestins and oestrogens. Several animal models are discussed, including monkeys, SCID mice and steroid-treated ovariectomized mice, and potential therapeutic agents suitable for human use are identified.

Key words: animal models/clinical treatments/endometrial bleeding/research suggestions

Introduction

A clear outcome from the meeting and the information in this Supplement is that disturbances in endometrial bleeding remain a major problem and the primary reason for discontinuation of progestin-only methods of contraception. However, some interesting leads were uncovered and a number of suggestions made about techniques and research topics during the course of the meeting and in the discussions which followed. This paper attempts to summarize those suggestions, with a view to stimulating further research and providing guidance about priority areas. It should be read in conjunction with the background information and research suggestions found in the papers published in this Supplement, as well as in monographs edited by Diczfalusy et al. (1980), Baird and Michie (1985), d'Arcangues et al. (1990) and Alexander and d'Arcangues (1992).

Bleeding versus non-bleeding sites

Progestin-induced bleeding occurs at focal points and not simultaneously across the whole endometrial surface. It has been contended that a comparison of the function of tissue collected from bleeding sites and non-bleeding sites could provide a clue to the underlying differences between bleeding in normal endometrium and irregular bleeding in 'progestin-only' endometrium. Several issues arise here. Firstly, it can be difficult, ethically and technically, to identify and obtain tissue in sufficient amounts from bleeding and non-bleeding sites using the currently available sampling devices, particularly from subjects using progestin-only contraceptives where the endometrium is very thin. It was suggested that a better sampling device is needed, such as one based on core sampling.

Secondly, it is not possible at present to predict which area of the endometrium will bleed next, and the dynamic changes in blood vessels and blood flow cannot be readily assessed with the currently available methods. What is really needed is a method to assess the function and fragility of endometrial vessels in vivo which can be correlated.
with histopathological analyses. The hysteroscopic approach described by Martha Hickey (pp. 35–44) could be modified to measure vascular fragility and blood flow and so identify morphologically abnormal vessels with increased fragility which are likely to bleed. Until such a method is available, an analysis of tissue from non-bleeding sites may not differentiate between those events which initiate bleeding in normal versus 'progestin-only' endometrium.

Finally, a comparison between tissues collected from bleeding sites of normal women and women using progestin-only contraceptives may identify differences in the mechanisms which stop bleeding. Data presented by Julianto Witjaksono (pp. 109–114) showed that oestrogen treatment of women bleeding excessively in response to Norplant® reduced the length of each bleeding episode, suggesting an action on clotting and tissue repair rather than the initiation of bleeding. It was suggested that haemostasis and clotting should be reinvestigated in Norplant endometrium.

Robert Brenner (pp. 150–164) suggested that a comparison of the endometrium of monkeys which did or did not stop bleeding after the withdrawal of oestradiol and progesterone could be a useful animal model to address these issues.

A related issue concerns the previous menstrual history of those women who experience bleeding problems when using progestin-only methods. Do these women have more irregular cycles and heavier bleeding before beginning the method than women who do not experience bleeding problems?

Which vessels are bleeding?

Although it is thought that the bleeding which occurs from the endometrium of users of progestin-only contraceptives occurs principally from the superficial vessels in the subepithelial stroma rather than the spiral arterioles, several pieces of information presented at the meeting suggest that this may not always be the case. Martha Hickey (pp. 35–44) described bleeding from a ‘variety’ of vessels observed through the hysteroscope. Considering that 50% of Norplant users have little or no endometrium, it is possible that the vascular plexus between the myometrium and the endometrium could be a source of bleeding. It was suggested that a standardized objective test of vessel fragility developed for the hysteroscope could be used to compare vessels in the endometrium of normal subjects and users of progestin-only contraceptives. The question of a difference between the properties of endothelial cells in the endometrium and those in vessels in the rest of the body was also raised.

Four possible causes of disturbed bleeding were identified which could be used as the basis of future research strategies. They were (i) inappropriate angiogenesis and vasculogenesis, (ii) the fragility of existing vessels, (iii) damage to normal or fragile vessels, and (iv) inappropriate regression of vessels.

The integrity of the surface epithelium

A particularly interesting question concerned the role of the surface epithelium in determining the number and extent of focal bleeding points and whether or not the integrity of the surface epithelium was more compromised in the case of progestin-only users. It appears that very little attention has been paid to the role of the surface epithelium in normal endometrial bleeding. It was hypothesized that in progestin-only users, there may be more frequent and extensive breakdown of the surface epithelium, allowing more frequent bleeding into the uterine cavity. It was suggested that techniques should be developed to examine the integrity of the surface epithelium at normal menstruation and on the endometrium of users of progestin-only contraceptives, and that attempts should be made to relate this integrity to bleeding. The integrity of the basement membrane underlying the epithelial cells should also be examined.

It was mentioned by Ian Fraser that vessels in the endometrium of women receiving Danazol® are more fragile but they do not show breakthrough bleeding, although there is subepithelial haemorrhage. This suggests that the epithelium, basement membrane and/or extracellular matrix (ECM) is more intact than in subjects receiving Norplant and that electron microscopy studies would be appropriate.

Increased microvascular density

Peter Rogers (pp. 45–50) made the interesting observation that there was an increased microvas-
cular density in the endometrium of Norplant users. This could provide more opportunities for bleeding from this increased density of vessels. However, it raises a number of questions. Is the increased microvascular density occurring early after Norplant insertion and does it remain thereafter? Does it in fact represent the regression of surrounding tissue or is it an increased number of vessels or an increase in fractional volume? Is the increase in the number of vessels caused by a more rapid rate of their formation from existing vessels (angiogenesis) or, less likely, from angioblasts (vasculogenesis), or is it caused by a decrease in the rate of vessel regression? These questions indicate a need for more morphometric studies of the endometrium in women using progestin-only methods. Are these vessels more fragile than those in the endometrium of normal subjects because of either altered morphogenesis or the presence of cytokines, such as tumour necrosis factor α, associated with cellular regression? There may be some parallels with microvascular density in regressing tumours and in diabetic retinopathy which could be investigated.

The state of the stroma

The question of whether or not the stroma undergoes decidualization in users of progestin-only contraceptives does not appear to have been investigated. It was suggested that there may be a short phase of 'decidualization' immediately after beginning the method. These may not be true decidual cells and they may produce factors not normally associated with decidual cells which are detrimental to the vessels. There is a need to identify the nature of all perivascular cells around vessels in normal and 'progestin-only' endometrium, because these cells could be the source of vasoactive substances like endothelin or paracrine regulators which can directly or indirectly influence vessel function.

The extent of perivascular support in the endometrium was also raised. It was suggested that silver staining, as described by Pierre Courtoy (pp. 134–143), could be used to visualize the ECM surrounding the vessels as a means of assessing the perivascular support. Specific ECM components such as fibronectin should also be examined. In addition, it was suggested that the balance between matrix metalloproteinases (MMP) and their tissue inhibitors (TIMP), which are products of stromal cells, could be an important determinant of vessel fragility. The whole question of the role of MMP and TIMP in normal and abnormal endometrial bleeding was considered to be a high priority and one where there are appropriate techniques available to investigate the subject (Salamonsen, pp. 124–133; Courtoy, pp. 134–143).

Infiltrating cells are a major component of the stroma and could provide a source of cytokines and enzymes capable of compromising the integrity of the endometrial vasculature and the surrounding tissues. For example, mast cells are present in the endometrium throughout the cycle but only release their products at specific times, and particularly at around the onset of menstruation in normal subjects (Salamonsen, pp. 124–133). The role of macrophages as a source of vascular endothelial growth factor in the normal endometrium is being investigated (Smith, pp. 56–61). Although there is some information on the nature and extent of cellular infiltration in normal endometrium, little work has focused on the endometrium of users of progestin-only contraceptives.

The dilemma of the steroid receptors

Tseng Lau (pp. 90–94) reported that in the endometrium of Norplant users, there was an increase in the immunostaining for the progesterone receptor but a decrease in the progesterone receptor mRNA measured by in-situ hybridization. This suggests a change in the turnover rate of the progesterone receptor mRNA and protein, which needs to be investigated further. It also raises a question about the function of the progesterone receptor in the endometrium of Norplant users. Are these receptors coupled to progesterone-dependent processes which can be measured in the endometrial cells? Markers of oestradiol and progesterone action on the endometrium which could be examined include fatty acid synthase, DB 91, insulin-like growth factor binding protein/placental protein 14, 17α-hydroxysteroid dehydrogenase type II, angiotensin II, transforming growth factor (TGF)-β, enkephalinase and markers of decidualization such as prolactin. It should also be determined
whether there are focal differences in the functionality of the progesterone receptor in different cell types, e.g. between stromal cells, vascular smooth muscle cells and pericytes. There is also the possibility that the synthetic progestins are acting outside the classic steroid receptor system or via an orphan receptor. In that regard, it was suggested that the androgen receptor status should be examined in the endometrium of progestin-only users.

All progestins are not equal
It was reported by Biran Affandi that Uniplant®, which contains nomegestrol acetate, caused less amenorrhoea and less prolonged and frequent bleeding than Norplant, which contains levonorgestrel. In progestin-responsive cells, such as breast and endometrial cancer cells, different progestins can have different effects on TGF-β1 and -β2 mRNA, protein and activation. Different progestins can also have different interactions with the other steroid receptors, and can be metabolized to oestrogen and androgen-like compounds. This suggests that beyond the classic potency differences, all progestins are not equal. However, they all produce breakthrough bleeding. More clinical trials are needed to confirm that nomegestrol acetate induces less breakthrough bleeding than levonorgestrel. If this is confirmed, it opens the possibility of designing progestins which have fewer side-effects on bleeding but the same contraceptive efficacy.

Level and type of oestrogen
Oestrogens have been used to treat the bleeding disturbances in users of progestin-only contraceptives with mixed success, as described by Catherine d’Arcangues (pp. 1–13), Julianto Witjaksono (pp. 109–114) and Wisut Boonkasemsanti (pp. 115–123). This could be related to the metabolism of the synthetic oestrogen and the activities of the metabolized forms. The type II hydroxysteroid dehydrogenase may not metabolize some synthetic oestrogens. Oestrogens increase the concentrations of sex hormone-binding globulin, which can influence the pharmacokinetics of levonorgestrel. There have been many measurements made of circulating concentrations of oestradiol in users of progestin-only contraceptives, but there are no data on the local oestradiol concentrations in the endometrium where oestrogen receptors have been identified.

Animal models
There are very few animal models suitable to study endometrial bleeding. Only the cattarhine primates viz. women, the great apes and Old World monkeys have the spiral arterioles in the endometrium and menstruate at the end of the cycle. Even within the menstruating primates the endometrial structure and form of bleeding can be different to that in the human. Nevertheless, we still rely to this day on the primary observations made by Markee >50 years ago, who used endometrial autotransplants in the rhesus monkey to study the regulation of menstruation. In addition to the monkey model, described by Robert Brennner (pp. 150–164), two other potential in-vivo animal models were discussed, one of which might allow observations on human endometrium.

SCID mice with xenografts of human tissue implanted in the abdominal wall (Aoki et al., 1994) offer the possibility of observing the hormonal regulation of human endometrium, including endometrial bleeding. It may be possible to use this model to study the effects of natural and synthetic steroids on blood flow and vessel fragility.

A mouse model of ‘endometrial bleeding’ has been described (Finn and Pope, 1984) which should be tested for its usefulness to study the regulation of endometrial bleeding, particularly in the presence of progestins used as contraceptives.

The question of whether or not non-human primates receiving progestins experience breakthrough bleeding was discussed. Rhesus monkeys receiving progesterone develop breakthrough bleeding as diagnosed with serial vaginal swabs, but there does not appear to be any data on the effects of Norplant or other long-acting progestins. One limitation of such a study would be the number of serial biopsies that could be collected from the endometrium of an individual animal per cycle. Three to four serial biopsies were thought to be ethically acceptable and technically possible. Baboons also show breakthrough bleeding when treated with progestins. Experiments to test the effects of progestins on endometrial bleeding in
monkeys and baboons were considered to be an important step in developing an animal model for breakthrough bleeding.

The similarity between the perivascular stroma around the spiral arterioles in human and monkey endometrium suggests that it may be possible to use organ culture of monkey endometrium to examine the influence of synthetic steroids on the vessels and the perivascular cells. Finally, endometrial edema has been associated with the use of progestin-only contraceptives, although the extent and incidence have not received much attention. The sexual skin of monkeys can be very edematous with increased synthesis of glycosaminoglycans. Do endometrial stromal cells also manufacture similar compounds which attract fluid, particularly when they are under the influence of progestins?

**Clinical treatments**

The consensus of the group was that while treatment with oestrogens may reduce the bleeding temporarily, it is not a cure. More research is needed therefore on potential therapeutic agents which would result in fewer users discontinuing the progestin-only methods of contraception.

The following were suggested on the grounds that many of the substances were already approved for human use: low-dose oestrogens; steroidal and non-steroidal anti-oestrogens (more specific than tamoxifen); combined oral contraceptives; anti-progestins; anti-prostaglandins and non-steroidal anti-inflammatory agents; anti-histamines; anti-fibrinolytics; vitamin E; modulators of tissue remodelling; modulators of vasoactive compounds (e.g. Parathyroid Hormone related Peptide and endothelin antagonists); and tetracycline analogues (which can influence MMP).

It was also suggested that the development of a biodegradable ‘slimline’ system, suitable for 2–3 weeks of intrauterine administration, was worthy of consideration as a way of avoiding the peripheral effects of parenteral administration of the drugs to treat the bleeding problems and possibly as a means of administering progestin-only contraceptives.

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**References**


