Abnormal endometrial bleeding continues to be a significant problem for women using long-acting gestagens and not only reduces the use of these agents, thus restricting the options available to women, but also reduces the quality of their lives. This symposium addressed a range of basic and clinical studies that have tried over the past 3 years to define the problem. Two areas have been identified. The first is the structure of the blood vessels themselves and the second is the environment of the endometrium in relation to these vessels. This reflects a shift in research interest away from factors that simply control epithelial–mesenchyme interactions. The expression of angiogenic inhibitors and stimulators is being defined and the critical role of the matrix and the immunocompetent cells of the endometrium elucidated. The disappointing results of simple oestrogen supplementation were confirmed. Three broad areas of research were considered to be important for future studies. The first of these is not related to molecular and cellular function but rather to understanding better the attitudes of women to vaginal bleeding in the multicultural diverse situations in which this event occurs. It was recognized that a broader constituency was not interested in this problem with the increasing use of continuous combined regimes of hormone replacement therapy in the developed world. Studies that listen to and educate women about this problem are needed to ensure that these techniques are felt to be better owned by the women themselves. Molecular and cellular studies were identified that attempted to define the factors necessary for the development and maintenance of healthy, non-leaky vessels. These included studies of the pro- and anti-angiogenic agents expressed in endometrium and a definition of their interactions with matrix metalloproteinases in maintaining vessel integrity. Finally there was recognition of the need to understand how neutrophils traffic through the endometrium and to determine how they effect endometrial bleeding.

**Key words:** contraception/endometrium/gestagens/steroids

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**Introduction**

The meeting continued to recognize the importance of breakthrough bleeding (BTB) as the primary reason for women to discontinue long-acting gestagen contraception. Since the last meeting two further developments have emphasized the importance of this problem. The first is the increasing preference for continuous combined regimes of hormone replacement therapy (HRT) in the developed world (Keating et al., 1999) and secondly the introduction in Europe of the levonorgestrel intrauterine system (LNG-IUS) for the management of abnormal uterine bleeding (Kulatunga and Fraser, 1998). Whilst the primary interest of the World Health Organization (WHO) continues to be contraception, it was recognized...
that a broader constituency of agencies would now become concerned with the problem. As HRT and abnormal bleeding applies to women in the developed world and provides a large market opportunity, it was considered likely that pharmaceutical companies may be more willing to participate with governmental and international agencies in pursuing an effective resolution to the problem.

A critical issue at the last meeting held in Bali, Indonesia in 1995 was the completion of clinical studies that used oestrogens to reduce BTB in women receiving long-acting gestagens (Witjaksono et al., 1996). Completed clinical trials in which ethinyl oestradiol or combined oral contraceptives (COC) were administered were presented to the meeting. Witjaksono (not represented in this supplement) reported reduction in the duration of spotting with ethinyl oestradiol in a study undertaken in Indonesia but other reports contradict these findings and it was not possible to advocate the widespread use of these regimes (Boonkasemsanti et al., 1996). Results are awaited from clinical trials using vitamin E (Subakir et al., 2000), low-dose aspirin and anti-progestins but it was felt that there was little justification for further large clinical trials in the absence of developments in the understanding of how steroids influence endometrial bleeding.

Emphasis was placed at the last meeting on the need to identify bleeding from non-bleeding sites in the endometrial biopsy. In general the experience of the past 3 years has been that this is not possible to advocate the widespread use of these regimes (Boonkasemsanti et al., 1996). Results are awaited from clinical trials using vitamin E (Subakir et al., 2000), low-dose aspirin and anti-progestins but it was felt that there was little justification for further large clinical trials in the absence of developments in the understanding of how steroids influence endometrial bleeding.

Endometrial vasculature

Several studies have confirmed the presence of abnormal blood vessels in the endometrium of women with BTB on long-acting gestagens. The initial studies of Hickey have been confirmed (Hickey et al., 1996) and as reported by Thomas (Thomas et al., 2000) the presence of dilated venules on the surface was consistent with the histological findings of Rogers of dilated venules and reduced pericyte development in women taking Norplant (Rogers et al., 2000). In the latter study a loose association with excessive days of bleeding was also reported. Hickey described reduced collagen IV, laminin and heparin sulphate proteoglycans in the basement membranes of endometrial blood vessels obtained from women receiving Norplant (Hickey and Fraser, 2000).

Several participants described the possible role of a range of agents in this process. Professor Schatz described the increased expression of tissue factor at the site of bleeding in Norplant users (Krikun et al., 1998) and confirmed the presence of increased lumen size. Tissue factor is the key initiator of the coagulation cascade and these observations support the clinical trials of the anticoagulant, aspirin, in the management of abnormal bleeding.

Casey described the role of transforming growth factor (TGF)-β in angiogenesis and the complex regulation including stromal cell membrane-attached urokinase plasminogen activator; activating plasmin to convert latent TGFβ to bioactive TGFβ was considered an important mediator of endometrial angiogenesis. In view of its tight regulation by progesterone and its inhibitory action on enkephalinase, the importance of TGFβ as a mediator of steroid action was confirmed.

The possible role of the insulin-like growth factor (IGF) family of genes and its binding proteins was considered by Rutanen who showed increased concentrations of IGF-binding proteins and IGF-II but reduced concentrations of IGF-I in users of the LNG-IUS (Rutanen, 2000). However, focal changes were not shown in respect of bleeding sites or differences in bleeding duration.

Rogers (Rogers et al., 2000), Arispe (Rodriques-Manzaneque et al., 2000) and Charnock-Jones (Charnock-Jones et al., 2000) reported several key
developments in the understanding of angiogenesis. Firstly that three processes, sprouting, elongation and intussusception are all involved in the process of angiogenesis. Arispe, in a series of delicate experiments confirmed the presence of both the A and B progesterone receptor in human endometrial endothelial cells. This new observation, hotly disputed in the past, needs further studies to define the direct role of steroids on endothelial cell function.

Secondly it is now clear that simple measures of endothelial cell proliferation and cell count are difficult to interpret and that sprouting occurs in the absence of proliferation and that the importance of endothelial cell apoptosis needs to be studied more closely. This is particularly relevant because withdrawal of angiogenic growth factor support induces atrophy that is restricted to the stromal cells in close apposition to the apoptotic endothelial cells (Benjamin and Keshet, 1997). How is it that only certain areas of endometrium show endothelial cell destruction with bleeding whilst closely proximate regions show an integral endothelium?

**The endometrial environment**

**The action of steroids**

Several new aspects of steroidal action were considered at the meeting. Clarke (Mote et al., 2000) described the differential expression of PR-A and PR-B receptors in endometrial epithelial cells and in the stroma in the late luteal phase. As it is binding of anti-progestins to PR-A that determines progesterone antagonism, these findings have implications for the actions of anti-progestins which were found to be highly effective when given to women receiving long-acting gestagens. Although dilated venules were found in the atrophic endometrium, Chwalisz and Garfield (2000) found the bleeding profiles of the women to be significantly improved. Further studies using continuous low dose anti-progestins are underway on the basis of these initial observations.

The importance of considering the variable biological effects of different steroids was outlined by Charnock-Jones who found significant differences in immunoreactivity for VEGF induced by different gestagens. The importance of this point was reconfirmed from the last meeting.

Grow raised the prospect of using anti-oestrogens to counter the presumed influence of raised oestrogens on BTB as suggested by the higher incidence of BTB in women of perimenopausal age, and of elevated endogenous concentrations of oestradiol in women with prolonged BTB (Van de Weijer et al., 1999; Grow and Reece, 2000). Currently there is little information on the use of selective oestrogen response modulators (SERM) in this indication. It was considered an important goal to seek consultation with the companies developing these agents with a view to clinical trials in the future.

**Extracellular matrix**

Marbaix showed that gelatinases A and B (MMP-2 and -9) activity is increased at the bleeding sites of women experiencing BTB (Marbaix et al., 2000). Tissue inhibitor of matrix metalloproteinase (TIMP)-1 was reduced in the samples. He suggests that, in conjunction with reduced collagen fibres at the bleeding site, this represents enhanced breakdown of the matrix (Marbaix et al., 1996).

Salamonsen indicated that mast cells regulate stromal MMP expression and activation and that this is triggered before the onset of bleeding. Moreover, it is clear that a number of MMP are expressed in high amounts by macrophages, large granular lymphocytes, polymorphonuclear leukocytes and to a lesser degree by eosinophils whilst mast cells and neutrophils provide enzymes which can trigger latent MMP activity (Vincent et al., 1999; Salamonsen et al., 2000; Vincent and Salamonsen, 2000).

A novel feature of this process was described by Professor Murphy who demonstrated the need for a loss of epithelial basement membrane integrity for bleeding to be experienced (Murphy, 2000).

**Immunocompetent cells in endometrium**

There was a consensus of opinion that these cells are of critical importance in regulating endometrial events and that they are likely to be involved in tissue breakdown and vascular remodelling. Jones indicated that large numbers of large granular lymphocytes (LGL) are found in endometrium of women taking LNG-IUS and this complements the findings of Salamonsen of infiltration and/or proliferation of macrophages, eosinophils, neutro-
phils and activation of mast cells in endometrium primed to bleed (Jones and Critchley, 2000; Salamonsen et al., 2000; Salamonsen and Lathbury 2000). The finding that these cells express multiple forms of the VEGF and angiopoietin family of genes by Charnock-Jones and Smith provides an initial mechanism for their involvement in vascular remodelling. There was a suggestion that the numbers of these cells invading the endometrium is high after initiation of systemic or IUS-progestin treatment but that this alters over time. This provides the first indication of a possible mechanism to explain the clinical observations of increased bleeding at the start of treatment. Further studies are needed before this can be confirmed.

**Recommendations arising from the WHO/NIH Meeting on the Mechanisms of Endometrial Bleeding**

Three areas of research were identified that should be pursued in order to improve the social and individual impact of abnormal bleeding on women using long-acting gestagens. These areas may be broadly divided into attitudinal studies, basic molecular and cellular studies and clinical trials.

**The consumer view**

Great attention was placed at the conference on the views of researchers to unwanted endometrial bleeding, yet the views of consumers have not been given sufficient attention. Many women are unaware that irregular bleeding is not in itself a sign of ill health. A programme to determine these views needs to be expanded in the context of a woman-centred educational programme that targets both consumers and health care workers.

These views are likely to reflect diverse cultural, social, ethical and economic factors and studies in the future must not apply universal criteria to the assessment of endometrial bleeding in differing societies. This is particularly relevant in the context of increased interest in endometrial bleeding from postmenopausal women using HRT.

**The molecular and cellular basis of endometrial bleeding**

The complex interactions that arise from the multiplicity of molecules derived from the broad range of cells that constitute the endometrium needs to be addressed in understanding the multiple factors that result in endometrial bleeding.

**The endometrial environment**

(i) Studies are needed to determine the factors that regulate focal expression of MMP.

(ii) Detailed studies are needed to determine how endometrial angiogenic growth factor expression regulates endometrial blood vessel growth. These studies need to consider the different functional layers of the endometrium, and to determine how the agents most likely to be involved in regulating this structure interact to determine the three-dimensional structure of the blood vessels. Complex imaging studies are needed to define blood vessel structure.

(iii) The role that immunocompetent cells play in the mechanism of endometrial bleeding needs to be better understood, especially as the early bleeding events seem to be related to these cells. These studies need to identify the mechanisms whereby neutrophils, macrophages, natural killer cells, eosinophils and mast cells traffic from the vascular compartment into the endometrial stroma and how it is that these cells regulate angiogenesis.

**Endometrial vasculature**

(i) Studies need to be undertaken on endothelial cells isolated and prepared from human endometrium. Whilst there will always be a limitation on this tissue, newer techniques of separation and permissive culture provide a greater source of these cells than in the past.

(ii) The precise nature of the oestrogen and progesterone receptors expressed by these cells needs to be determined and the direct effects of steroids on endothelial cell function identified.

(iii) The cell–cell regulation of endothelial cells needs to be determined in the context of the endometrium, particularly the endothelial–pericyte and endothelial–macrophage–natural killer cell interactions.

**Clinical studies**

(i) The effect of matrix metalloproteinase inhib-
itors on endometrial bleeding needs to be determined. The results of studies undertaken in women of reproductive age need to be reported and new studies undertaken to determine the effect of the oral TIMP on menstrual bleeding in women receiving long-acting gestagens and HRT.

(ii) The effects of selective oestrogen receptor modulators on endometrial bleeding need to be determined. The use of these agents either alone as a contraceptive or in combination with long-acting gestagens needs to be considered.

(iii) Further studies are needed to determine the role of low-dose anti-progestin therapy in women using long-acting gestagens.

General

(i) Studies relating to the molecular and cellular regulation of the endometrial vasculature need to concentrate on mechanisms and should not be of a solely descriptive nature.

(ii) The importance of understanding the complex interplay of gene expression that regulates vascular structure and function in the endometrium needs to be addressed by in-vivo and in-vitro functional genomic and proteomic analysis of the endometrium.

Conclusion

The cellular and clinical studies identified at the first meeting have led to important insights into the underlying biology of this process. The next phase of research needs to bring these leads together and to apply the latest techniques of cellular and molecular biology to the problem. Whilst this approach is likely to lead to improved treatments, there is also a need for increased understanding by women of the consequences of bleeding and the social, religious and individual context of vaginal bleeding must reflect cultural diversity and better inform the research programmes. Research programmes are needed that provide a greater understanding of the mechanisms of bleeding and should concentrate on mechanistic studies and not be simply descriptive. There was weak support for large-scale clinical programmes; rather, these should focus on defined areas such as the use of matrix metalloproteinase inhibitors, selective oestrogen modulators and low-dose anti-progestins. The problem of BTB continues to be a significant factor in reducing the quality of life for women and this is only likely to be resolved by a greater understanding of the basic mechanisms of endometrial bleeding.

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References


S.K. Smith


