Endometrial vasculature in Norplant® users

P.A.W. Rogers

Department of Obstetrics and Gynaecology, Monash University, Monash Medical Centre, 246 Clayton Road, Clayton, 3168 Victoria, Australia

Disrupted, prolonged and irregular endometrial bleeding are major unwanted side-effects of progestin-only contraceptives. The aim of this paper is to review current information on steroid control of the microvasculature, microvascular heterogeneity and microvascular fragility, with emphasis on the relevance of these issues to the endometrial microvasculature in women receiving Norplant® implant contraception. Subjects were either Indonesian women with between 3 and 12 months exposure to Norplant (n = 191) or Caucasian controls recruited in Melbourne, Australia. Norplant endometrium was always thinner than control endometrium, with a varied histology that usually included a basalis-type appearance, signs of haemorrhage and some dilated and congested subepithelial vessels. Thin-walled vessels were seen which could have been either blood vascular or lymphatics. Steroid control of the vasculature can operate through numerous direct and indirect mechanisms, with up to 30 genes relevant to vascular function having consensus oestrogen response elements in their promoter regions. The vasoactive effects of progesterone are less well documented. However, experimental data for direct effects on the endometrial vasculature are mounting. Progestin-induced endometrial breakthrough bleeding is often focal, suggesting that microvascular heterogeneity may be an important factor in understanding this phenomenon. Increased susceptibility to bleeding may result from increased microvascular fragility, possibly as a consequence of progestins altering the balance of angiogenic promoters and inhibitors in the endometrium, thus leaving the vessels in a permanently weakened state.

Key words: breakthrough bleeding/endometrium/local mechanisms/microvasculature/Norplant®

Introduction

Disrupted endometrial bleeding, and in particular prolonged and irregular bleeding, is one of the major unwanted side-effects of progestin-only contraceptives. As progestin-only contraception now represents one of the major methods of choice of contraception for many couples worldwide, there is a steadily increasing focus on identifying the causes of breakthrough bleeding. A large part of this focus now rests on the endometrial vasculature (Alexander and d'Arcangues, 1992). Because major differences in bleeding patterns occur between women on identical contraceptive regimes, it is increasingly felt that local differences at the level of the endometrium are probably responsible for the bleeding. The aim of this paper is to review current information and concepts on three different aspects of the endometrial microvasculature in women receiving Norplant implant contraception. The three main areas that will be covered are steroid control of the microvasculature, microvascular heterogeneity and microvascular fragility.

Materials and methods

Data discussed here were obtained primarily from subjects recruited for World Health Organization (WHO) study 90901. Ethical approval for these
studies was obtained both at a local institutional level and from WHO. All subjects gave informed consent before participating. Patients were either Indonesian women who had received between 3 and 12 months exposure to Norplant, or Caucasian controls recruited in Melbourne, Australia. More detailed information about select subgroups of these subjects has been published in a number of previous reports (Critchley et al., 1993; Rogers et al., 1993; Goodger et al., 1994). The 191 women receiving Norplant in this study had a mean ± SEM exposure of 219 ± 7.2 days, a mean ± SEM weight of 51.0 ± 0.7 kg and a mean ± SEM age of 30.0 ± 0.3 years. On average (mean ± SEM), each woman had had 2.6 ± 0.1 pregnancies.

Norplant users recruited to the study kept a daily menstrual diary for a minimum of 90 days prior to endometrial biopsy. Starting 2 weeks prior to biopsy, peripheral blood samples were taken every 2 or 3 days from Norplant users (but not controls), resulting in six samples in the 2 weeks prior to the day of biopsy. These samples were assayed for oestrogen and progesterone for all Norplant subjects. The mean ± SEM peripheral oestrogen concentration for the whole Norplant user population was 369.8 ± 24.7 pmol/l, although concentrations were highly variable between women (Figure 1). In contrast, mean ± SEM progesterone concentrations were basal for these same women (0.9 ± 1.0 nmol/l). On average (mean ± SEM), each Norplant user had 21.9 ± 1.4 days of bleeding or spotting in the 90 day reference period prior to endometrial biopsy. However, the distribution for bleeding/spotting days was highly varied, as shown in Figure 2 where the number of bleeding/spotting days is plotted against the number of days of Norplant exposure. When analysing Norplant user group data, we were unable to find any correlation between oestrogen concentrations, bleeding and spotting days or the number of days Norplant had been inserted. Women using Norplant in our study had on average (mean ± SEM) 2.48 ± 0.14 bleeding episodes in the 90 days prior to biopsy. The mean ± SEM duration of each bleeding episode was 8.76 ± 0.86 days, while the mean ± SEM minimum length for a bleeding episode was 5.10 ± 0.71 days and the mean ± SEM maximum length for a bleeding episode was 12.00 ± 1.04 days.

Results and discussion

Endometrial histology

Endometrial biopsies containing sufficient tissue for histopathological diagnosis were obtained from 96 of the 191 biopsy procedures (Hadisaputra et al., 1996). The histological appearance of haematoxylin- and eosin-stained Norplant endometrial sections was quite varied, although the endometrium was always significantly thinner than that from the control cycle (e.g. 0.4 mm for Norplant versus 5.8 mm for control mid-secretory). A number of histological features can be considered
Endometrial musculature in Norplant® users

The endometrial musculature in Norplant® users was assessed in a study that recorded bleeding or spotting in each woman's menstrual diary over a 90-day time-span prior to endometrial biopsy. The results showed no particular pattern of blood loss during the first 12 months of Norplant® use, which is reasonably typical for Norplant endometrium from this group. These endometria often showed signs of haemorrhage, with numerous red blood cells in the tissues. However, because this can also be a common artefact caused by biopsy, especially suction curette, it needs to be interpreted with care. Subepithelial vessels were often dilated and congested, although there was considerable regional variability in this appearance. Some biopsies also had large very thin-walled vessels, often without red blood cells in them. It is possible that these may connect to either the blood vascular or the lymph system. Glands tended to be much reduced in size, with cuboidal epithelial cells. Glandular appearance was quite varied, ranging from proliferative with mitoses through to late secretory with apoptotic bodies. The surface epithelium was also quite varied, although in places it appeared to have breaks and signs of re-epithelialization. In biopsies taken by microhysteroscope, the junction between the endometrium and the myometrium could be evaluated. In many cases the junction appeared normal, although occasionally it was possible to find glandular tissue appearing relatively deep in the myometrium, suggestive of adenomyosis. In some endometrial biopsies there was a significant amount of oedema, although once again this may be an artefact caused by suction curette. It was also not uncommon to see spindle-shaped periglandular cells, typically not found around glands in control endometrium.

Steroid control of the endometrial microvasculature

While oestrogen and progesterone play a central role in controlling the endometrial vasculature, it is important to understand that these regulatory effects are superimposed on top of a myriad of mechanisms by which all blood vessels in the body are controlled. Our understanding of how oestrogen and progesterone regulate endometrial vascular function during complex events, such as implantation, menstruation and angiogenesis, is poor. A better understanding of how steroid control of the endometrial vasculature occurs is central to discovering new ways of controlling progestin-induced breakthrough bleeding. At present, the only effective treatment for progestin-induced breakthrough bleeding appears to be with oestrogen (Diaz et al., 1990; Witjaksono et al., 1996). There is now considerable immunohistochemical evidence suggesting that endometrial endothelial cells do not contain oestrogen or progesterone receptors (e.g. Sheridan and Weaker, 1992; Critchley et al., 1993; Perrot-Applanat et al., 1994). However, this issue may not be finally resolved because Wang et al. (1992) have reported progesterone receptors by immunohistochemistry in human decidual endothelial cells during pregnancy. In contrast to the endothelium, other cells that make up the vessel wall, such as pericytes and smooth muscle cells, have been shown to contain oestrogen and progesterone receptors by immunohistochemistry (Sheridan and Weaker, 1992; Critchley et al., 1993; Perrot-Applanat et al., 1994). Thus, there is a clear
avenue for oestrone and progesterone to have direct effects on much of the endometrial vasculature.

There is currently major interest in the atheroprotective effects of oestrone seen in premenopausal women. While the exact mechanisms responsible for this protective effect are unclear, experimental and theoretical molecular data exist to show that up to 30 genes with products that affect vascular structure or function have consensus oestrone response elements in their promoter regions (Mendelsohn and Karas, 1994). In other words, oestrone can have a wide range of effects on the vasculature by acting as a transcription factor, once it has bound to its receptor, to up-regulate the production of numerous vasoactive compounds. In addition to these wide-ranging genomic effects, oestrone has also documented rapid non-genomic effects on the vasculature, which usually result in vasodilatation (Mendelsohn and Karas, 1994). In contrast, there is very little information available on the vasoactive effects of progesterone outside the uterus, other than the general observation that it can act as an antagonist to oestrone and thus negate many of its beneficial atheroprotective effects.

In the one study to date on sex steroid receptor distribution in endometrium from long-term subdermal levonorgestrel users (Critchley et al., 1993), a major finding using immunohistochemistry was elevated stromal progesterone receptor in Norplant users. This study did not specifically investigate perivascular cells for steroid receptor content. In unpublished studies, we have found a significant number of vessel profiles in Norplant endometrium surrounded by cells which stained positive with an antibody to α-smooth muscle actin. It is possible that these cells are either pericytes or smooth muscle cells. It also seems likely that these same cells contain progesterone receptors based on the immunostaining of adjacent sections. Because these cells are clearly prime candidates for a role in mediating the effects of contraceptive progestins on the endometrial vasculature, they represent an important target for future research.

Recent data have provided evidence for a relatively direct effect of progestins on the endometrial vasculature. It has been shown that Norplant (0.05–0.08 mg/day levonorgestrel for 3–12 months) causes a significant increase in endometrial microvascular density (Rogers et al., 1993). In contrast, high-dose progestin exposure (5–30 mg/day norethisterone for 2 weeks–6 months or 15–80 mg/day medroxyprogesterone acetate for 8 days–5 months) results in a significant reduction in endometrial vascular density (Song et al., 1995). Both studies noted that vascular density did not correlate in any way with the histological appearance of the stroma or glands. In other words, the effects of progestins on the vasculature appear to be substantially independent of their effects on the other tissue compartments. The apparent conflict in the results of these two studies (an increase versus a decrease in vascular density) may not be that surprising, because it is not at all uncommon for bioactive substances to have both agonist- and antagonist-type activities when administered at significantly different concentrations.

**Microvascular heterogeneity in Norplant endometrium**

When reviewing normal histology slides of Norplant endometrium, it is rapidly apparent that the vasculature varies considerably from vessel to vessel. As discussed earlier, it is possible to find normal-looking capillaries alongside highly congested vessels, with large thin-walled vascular spaces nearby. It has been reported that bleeding sites in Norplant endometrium are often focal, suggesting that the localized rupture of vessels, rather than ubiquitous rupture throughout the whole endometrium, is responsible for breakthrough bleeding. Thus it is important as a concept to focus on the possibility that different subpopulations of endometrial vessels exist, and that perhaps only some particular vessel types are susceptible to bleeding. The concept of microvascular heterogeneity is now well established for many other tissues (Auerbach, 1992). Similarly, endothelial cells, both within the same vessel segment and between different vessels, can show a high degree of heterogeneity, particularly in tissues undergoing growth and remodelling, such as tumours (Kruzu et al., 1992). In endometrium, we have shown that antibodies to the endothelial cell marker CD34 will stain many more vessels than, for example,
antibody to Factor VIII (Au and Rogers, 1993; Rogers et al., 1993). In other immunohistochemical studies of endometrium, we have shown that the basement membrane of nearly all endometrial vessels contains collagen IV and laminin (Kelly et al., 1995). However, in the same study, we found that heparan sulphate proteoglycan stains only ~55% of vessels. This suggests that a percentage of vessels in the endometrium have different structural properties, which provides further evidence to support the hypothesis that some vessels may be more susceptible to bleeding than others.

**Microvascular fragility in Norplant endometrium**

It has been hypothesized that increased microvascular fragility may play an important role in the aetiology of progestin-induced breakthrough bleeding (Fraser and Peek, 1992). Theoretically there are a number of physical factors that can influence the fragility of a blood vessel. These include the state of the endothelial cells, including their thickness and cytoskeletal arrangement, the strength of the intercellular junctions between the endothelial cells, and the type and number of adhesion molecules anchoring the endothelial cells to the underlying basement membrane. It also includes the structure and composition of the basement membrane, as well as the structure and strength of the perivascular cells, such as pericytes and smooth muscle cells. Another factor that can have an important role in influencing vessel fragility is the blood pressure inside the vessel, because elevated blood pressure in a weakened capillary can rapidly lead to rupture. To date, there has been little or no work systematically investigating how any of the above factors may play a role in contributing to increased vascular fragility in the endometrium of Norplant users.

Many of the factors which can influence vessel fragility (outlined above) are parameters that alter during the process of new blood vessel formation or angiogenesis (Klagsbrun and D'Amore, 1991). Thus, during angiogenesis, endothelial cells activate and alter their complement of surface adhesion molecules. Cell junctions are reduced and proteolytic enzymes released to break down the basement membrane. To migrate towards an angiogenic stimulus, endothelial cells need to break away from the existing vessels and thus considerably weaken their links with adjacent endothelial cells. During this process, the blood vessel will be considerably weakened and more susceptible to rupture.

Based on the very low levels of endothelial cell proliferation seen in the endometrium of Norplant users (Goodger et al., 1994), it seems unlikely that significant angiogenesis is occurring. However, as discussed above, angiogenesis comprises a series of steps, each of which can undoubtedly be regulated independently (Klagsbrun and D'Amore, 1991). Thus, for example, it is possible to have endothelial cell migration without endothelial cell proliferation (Sholley et al., 1984). Furthermore, the regulation of each of these steps can be quite complex, with the final outcome being a balance between promoting and inhibiting factors (Goodger and Rogers, 1995). With evidence now available that progestins have significant effects on endometrial vascular density (Rogers et al., 1993; Song et al., 1995), which is in turn a consequence of angiogenic activity, and that oestrogen can have a wide range of effects on the vasculature, it seems highly probable that one or more steps of the normal angiogenic process are being perturbed by the administration of exogenous progestins. This could lead to a situation where any of the parameters mentioned above, e.g. basement membrane breakdown or endothelial cell adhesion molecule expression, are altered, resulting in the vessels becoming more fragile. Clearly further work on these issues is required before such a hypothesis can be substantiated.

**Acknowledgements**

Thanks are due to Dr Biran Affandi, Julianto Witjaksono and Wachyu Hadisaputra for help with biopsy collection, Dr Beatrice Susil for the histopathological assessment of endometrial sections, Sr Rosminah Hubardina Animo for patient coordination in Jakarta, Indonesia, Sr Nancy Taylor for patient coordination in Melbourne, Australia, Ida Iriani and Yudiati for expert technical assistance, and Kathy Craven for typing the manuscript. This work was supported by WHO project grant no. 90901 through the WHO Special Programme of Research, Development and Research Training in Human Reproduction.
References


