Levonorgestrel-releasing intrauterine device-wearing women express contraceptive glycodelin A in endometrium during midcycle: another contraceptive mechanism?

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Intrauterine devices (IUDs) exert contraceptive action by interfering with sperm transport, ovum development, fertilization and implantation. Glycodelin A (GdA) is a uterine glycoprotein that has local contraceptive activity by inhibiting sperm–egg binding. GdA is normally absent from endometrium during the fertile midcycle and it is not expressed until the fifth postovulatory day. The phase of menstrual cycle addressed in this study covers the phase when conception is most likely to follow an unprotected intercourse and when GdA is normally absent. We present here evidence that levonorgestrel-releasing IUD (LNg-IUD) is accompanied by ‘inappropriate’ expression of GdA in endometrium between days 7 and 16 of the menstrual cycle (six out of six cases). The same was also found in copper-releasing IUD (Cu-IUD)-wearing women, but less frequently (four out of 11 cases, P < 0.0345, Fisher’s exact test). In-situ hybridization localized GdA mRNA into endometrial glands in the midcycle endometrium, confirming the cellular site of synthesis. Based on the potent inhibitory activity of GdA on sperm–egg binding, the presence of GdA in uterine glands of IUD wearers may lead to prior exposure of sperm to contraceptive GdA, thus contributing to the contraceptive activity of the IUD.

Key words: contraception/glycodelin/gynaecology/IUD/reproduction

Introduction

Intrauterine devices (IUD) exert contraceptive action by interfering with sperm transport, ovum development, fertilization and implantation (Aref et al., 1984; Ortiz and Croxatto, 1987). Levonorgestrel-releasing IUD (LNg-IUD) has additional effects which include reduction of menstrual blood loss and a high incidence of oligo-amenorrhea due to glandular atrophy and decidualization of endometrial stroma (Silverberg et al., 1986; Luukkainen and Toivonen, 1995). These changes are believed to be related to the contraceptive effects of LNg-IUD.

The uterus exhibits cyclical contraceptive activity by secreting a glycoprotein named glycodelin A (GdA) which potently and dose-dependently inhibits binding of sperm to the zona pellucida (Oehninger et al., 1995). The contraceptive activity of GdA is based on its unique carbohydrate structure (Dell et al., 1995), because a differentially glycosylated GdS from seminal plasma has no contraceptive activity in spite of the fact that both the primary structure and immunological reactivity of GdA and GdS are identical (Morris et al., 1996; Koistinen et al., 1996).

Secretion of GdA is cyclical. Thus, GdA is normally absent from endometrium during the proliferative and immediate postovulatory phases of the cycle (Julkunen et al., 1986a; Waites et al., 1988), it appears in endometrial glands on days 5–6 after ovulation (Seppälä et al., 1988), and increases toward the end of ovulatory cycles (Julkunen et al., 1986a, 1988; Waites et al., 1988). A similar menstrual cycle-related increase in GdA concentration has been reported for uterine fluid (Li et al., 1993) in which the spermatozoa migrate through the uterus to fertilize in the fallopian tube where the GdA concentration is low (Julkunen et al., 1986b).

Endometrial GdA secretion appears to be related to the action of progesterone. This is suggested by expression of GdA in secretory endometrium only, elevation of serum glycodelin concentration in response to cyclical progesterone treatment of women with unexplained infertility (Seppälä et al., 1987b), and the finding of three putative corticosteroid/progesterone responsive elements in the glycodelin gene (Vaisse et al., 1990).

A recent study indicates that, in a normal menstrual cycle, the fertile phase for intercourse to result in conception is a 6 day period ending in ovulation (Wilcox et al., 1995). This coincides with absence of contraceptive GdA in the uterus, necessary for a ‘fertilization window’ to be open (Seppälä et al., 1997). We postulated that LNg-IUD may cause ‘inappropriate’ GdA secretion during this potentially fertile period and studied the GdA expression in endometrium specimens taken at this narrow phase of the cycle from IUD-wearing women.

Materials and methods

The use of human tissues for research was based on informed consent and approved by the Ethical Committee of the Department of Obstetrics and Gynaecology, Helsinki University Central Hospital. Fresh normal endometrium specimens were taken by biopsy or after hysterectomy from six women wearing LNg-IUD (Levonova®; Leiras/ Schering, Turku, Finland), with menstrual cycles in which the specimens were taken between days 7 and 16 of the cycle, dated according to the first day of the last menstrual period. Another eleven specimens came from copper-IUD (Cu-IUD)-wearing women, also taken between days 7 and 16 of the cycle.
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**Figure 1.** A representative example demonstrating glycodelin mRNA by in-situ hybridization in endometrium taken on day 10 of the cycle from a woman. (A) Antisense strand of glycodelin mRNA showing positive signal in endometrial glands. (B) Control experiment on adjacent section using sense strand of glycodelin mRNA (negative control). Magnification ×400.

**Immunohistochemistry**

Sections (5 μm) of formalin-fixed, paraffin-embedded normal human endometrial tissues were examined using affinity-purified polyclonal anti-glycodelin IgG for staining by the immunoperoxidase method essentially as described by Kämäräinen et al. (1996). Briefly, the sections were cleared and subjected to microwave heat treatment to enhance immunoreactivity. Rabbit anti-glycodelin IgG was used as the first antibody (Koistinen et al., 1996), and normal rabbit IgG (Vector Laboratories, Burlingame, CA, USA) was used as a control. The second antibody was biotinylated porcine anti-rabbit immunoglobulin (Dako A/S, Glostrup, Denmark). Endogenous peroxidase activity was blocked by treating the samples with 0.6% perhydrol in methanol. Immunostaining was carried out using Vectastain ABC kit (Vector) and 3-amino-9-ethylcarbazole as a substrate. The sections were counterstained with haematoxylin.

**In-situ hybridization**

Six endometrium specimens were also studied by in-situ hybridization to demonstrate cellular localization of glycodelin mRNA as described elsewhere (Koistinen et al., 1997). Briefly, proteinase K-treated sections were fixed in 4% paraformaldehyde. In-vitro transcriptions of sense and antisense probes were prepared using 413 bp long cloned glycodelin DNA as a template. Hybridization was carried out using a digoxigenin-labelled RNA probe after acetic anhydride treatment. After washing, immunological detection of digoxigenin-labelled RNA probe using anti-digoxigenin phosphatase Fab fragments (Boehringer Mannheim, Germany) was done according to the manufacturer’s instructions using Fast Red tablets (Boehringer Mannheim) as a substrate.

**Statistical analysis**

Staining was regarded as positive when a distinct red colour was seen in the luminal epithelium of endometrial glands (the most sensitive area where GdA appeared first), distinct from the blue background staining of the same specimen, and when there was clear distinction from the staining of the adjacent control specimen which was blue throughout the sample. The result was negative when only the blue background staining was present, and there was no distinction between the test and the control stainings. Fisher’s exact test (Instat®, San Diego, CA, USA) was used to compare the number of GdA-positive and GdA-negative endometrium specimens in the two sets of women: between six LNG-IUD wearers and eleven Cu-IUD wearers.

**Results**

In keeping with earlier reports (Silverberg et al., 1986), irrespective of the duration of LNG-IUD use, the major morphological changes in the endometrium samples from LNG-IUD wearers were epithelial atrophy and stromal decidualization. We found that endometrium from all women wearing LNG-IUD contained both GdA mRNA and protein at midcycle (Figures 1 and 2, Table I), irrespective of the duration of IUD use. GdA was localized to endometrial glands, with only sporadic faint patches in the stroma. There was some variation in staining intensity from one specimen to another. GdA-positive endometria were less frequent among Cu-IUD wearing women (P = 0.0345) (Table I).

**Discussion**

Previous studies have clearly shown that GdA is not expressed in endometrium during the midcycle (Julkunen et al., 1986a, 1990; Waites et al., 1988; Seppälä et al., 1988). Therefore we addressed this phase in particular by taking the endometrial specimens on days 7–16 after the last menstrual period, which also is the most fertile phase of the cycle. This phase is not used for routine endometrial biopsies to study endometrial responses to ovarian stimuli, but there was no reason to repeat the previous studies on the usual premenstrual phase, where GdA expression is well established (Julkunen et al., 1986a, 1990; Waites et al., 1988; Seppälä et al., 1988). Because GdA was found in all the women wearing LNG-IUD in the midcycle when GdA is not normally present, the result indicated...
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**Figure 2.** Immunohistochemical localization of glycodelin A (GdA) in endometrium from women wearing a levonorgestrel (LNg)- or copper (Cu)-releasing intrauterine device (IUD). (A) A typical staining with anti-GdA IgG showing localization of GdA protein in endometrial glands on day 10 of the cycle of an LNg-IUD-wearing woman. Note that the surface epithelium is very thin, the glands show secretory changes and there is stromal decidualization (original magnification $\times$100). (B) An example of staining with anti-GdA IgG, showing absence of GdA from proliferative endometrium on day 10 of the cycle of a Cu-IUD-wearing woman. All the glands were GdA-negative (original magnification $\times$400). (C) Endometrium from an LNg-IUD wearer on day 10 of the cycle shows localization of GdA in endometrial gland (original magnification $\times$400). (D) Control staining for the former (adjacent tissue slice) using rabbit IgG instead of rabbit anti-glycodelin IgG (original magnification $\times$400). Table I shows positive stainings for GdA on the days of menstrual cycle from which the LNg-IUD specimens were obtained.

That GdA was induced by the locally administered LNg, IUD, or both. Of great interest is our finding that all the LNg-IUD wearers had GdA in endometrium throughout the otherwise ‘fertile’ phase of the cycle, i.e. the 6 day period ending on the day of ovulation (Wilcox et al., 1995). Indeed, this study demonstrates for the first time by in-situ hybridization and immunohistochemical staining that both GdA mRNA and protein are expressed in the endometrium taken before and at midcycle in women wearing LNg-IUD.

A recent study indicates that the circulating levels of PP14/GdA are lower in Cu-IUD users compared to controls, and the authors interpreted this as signifying defective endometrial function (Seleem et al., 1996). Their material included no case of LNg-IUD, so it remains to be studied whether the induction of GdA in endometrium of LNg-IUD wearers is accompanied by any changes in the circulating GdA concentration at the midcycle.

It was also of interest to learn that some of the Cu-IUD wearers had ‘inappropriate’ GdA expression in the endometrium. However, this was less frequent than among LNg-IUD wearers, suggesting that local progestogen can induce or enhance endometrial GdA synthesis.
In view of the potent contraceptive effect of GdA, our results are significant because the uterine cavity is expected to be free of contraceptive activity at the time when spermatozoa pass through the uterus to the Fallopian tubes. Indeed, previous studies have shown that prior exposure of spermatozoa to GdA renders them incapable of binding to the zona pellucida, whereas prior exposure of the zonae to GdA has no effect (Oehninger et al., 1995). Thus, prior exposure to endometrial GdA may provide one mechanism by which the fertilizing capacity of spermatozoa may be reduced in LNG-IUD-wearing women, and also in some Cu-IUD-wearing women. Our observation was not totally unexpected, because cyclical oral oestrogen/LNg replacement treatment for postmenopausal women has been found to increase the serum GdA concentration in those women whose uterus is intact compared to those women who have undergone hysterectomy (Seppälä et al., 1987a).

With regard to progestogen-releasing IUDs, they obviously prevent fertilization and/or implantation by interference with sperm migration and disruption of the cyclicity of the endometrium. Our finding of contraceptive GdA in the endometrium over the fertile midcycle should provide a possible molecular explanation for this, and it could also be of considerable interest for contraceptive development, by search of local and systemic compounds that can induce GdA synthesis (Stewart et al., 1997). It has been implied that IUDs prevent implantation of a fertilized ovum, i.e. that they act after fertilization but before nidation is completed. Quite clearly none of the currently existing IUDs are infallible, as pregnancies and abortions do occasionally occur (Luukkainen and Toivonen, 1995). By definition, abortion means detachment of an implanted embryo, demonstrated by transient occurrence of trophoblastic markers in blood or urine. Like overt pregnancies, biochemical pregnancies are uncommon among IUD-wearing women, as documented by rare occurrence of chorionic gonadotrophin or other trophoblastic markers in the serum of IUD-wearing women (Klein and Mishell, 1977; Seppälä et al., 1978; Segal et al., 1985; Wilcox et al., 1985). So far, failure to implant has been difficult to detect after conception in vivo, but theoretical calculations have indicated that this may occur in as many as 30% of the cycles of couples having regular intercourse without contraception (Whittaker et al., 1983; Chard, 1991). Whether IUD make any difference to this figure has not been estimated.

However, as pointed out recently (Sivin et al., 1992), the consensus of informed opinion is that Cu-IUD greatly reduce the likelihood of fertilization. Perhaps one of the mechanisms involved here is enhancement of GdA synthesis in the uterus. Although the number of women was small in the present study which focused on the critical ‘fertile’ phase, the unequivocal documentation of both GdA mRNA and protein during the first half and peri-ovulatory period of the menstrual cycle provides a basis for larger scale clinical studies on the consistency of GdA expression outside its normal cyclical expression in women wearing LNG-IUD and other types of IUD. Our study also raises the possibility of a new contraceptive principle, based on induction of a contraceptive uterine substance that has the potential of inhibiting fertilization.

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