

Novel vaginal danazol ring therapy for pelvic endometriosis, in particular deeply infiltrating endometriosis

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Danazol is routinely administered orally to inhibit ovulation and to treat pelvic endometriosis. However, recent evidence suggests that danazol can act directly on endometriotic tissue *in vitro* to inhibit DNA synthesis and induce apoptosis. Danazol was administered via the vagina in this study, using a vaginal ring drug delivery system containing 1500 mg of danazol. This therapy was effective for treatment of pelvic endometriosis, especially for deeply infiltrating endometriosis, resulting in a cure of dysmenorrhoea and tenderness in the cul-de-sac within 3 months, and of induration or nodularity in the cul-de-sac within 7 months. Moreover, conception was possible during insertion of the vaginal ring in 17 out of 31 infertile women with deeply infiltrating endometriosis, and in two out of eight infertile women with ovarian endometriotic cysts not adhering to the cul-de-sac and without deeply infiltrating endometriosis. Serum danazol concentrations, high during oral daily 400 mg danazol therapy, but undetectable during vaginal danazol ring therapy, explain why ovulation and conception could occur during insertion of the vaginal danazol ring, and why general side-effects, which are often observed during oral danazol therapy, were not observed during vaginal danazol ring therapy. Danazol seems to be absorbed through the vaginal mucosa and reaches the deeply infiltrating endometriosis via diffusion.

Key words: deeply infiltrating endometriosis/drug delivery system/local danazol therapy/pelvic endometriosis/vaginal danazol ring

Introduction

In oral danazol therapy and luteinizing hormone-releasing hormone (LHRH) analogue therapy for the treatment of pelvic endometriosis, the absorbed danazol and LHRH analogues stimulate the hypothalamo-pituitary system to inhibit luteinizing hormone (LH), follicle stimulating hormone (FSH) secretion and ovulation through the general circulation. Consequently, danazol, a weak androgen derivative, often induces side-effects such as liver dysfunction, increases in body weight and acne, whilst LHRH analogues induce side-effects such as post-menopausal-like symptoms and osteoporosis due to

inhibition of oestrogen secretion. Moreover, although a reduction of pelvic endometriosis is observed during both oral danazol and LHRH analogue therapies, recurrence of endometriosis often occurs after cessation of therapy due to resumption of oestrogen secretion. Recently accumulated evidence has suggested a direct inhibitory action of danazol on endometriotic tissue (Chamness *et al.*, 1980; Taketani and Mizuno, 1985; Surrey and Halme, 1992; Braun *et al.*, 1994; Chin *et al.*, 1997). Our preliminary report (Igarashi, 1990) confirmed the effect of clinical in-vivo local danazol therapy on pelvic endometriosis and uterine adenomyosis. In this study, serum danazol concentrations during oral danazol and vaginal danazol ring therapy were monitored and further detailed clinical results were presented.

Materials and methods

Diagnosis of pelvic endometriosis

Among infertile women complaining of severe dysmenorrhoea, the diagnosis of pelvic endometriosis was confirmed by manual examination, transvaginal ultrasonography, and laparoscopy. Laparoscopic surgery except adhesiolysis was not performed, because the effect of medical treatment without surgery should be strictly evaluated. Serum CA 125 concentrations were also assayed. Laparoscopy does not always detect the extraperitoneal, sub-cul-de-sac endometriosis, 'deeply infiltrating endometriosis' or 'recto-vaginal endometriosis'.

Deeply infiltrating endometriosis has been defined as pelvic endometriosis infiltrating deeper than 5 mm (Koninckx *et al.*, 1991; Koninckx and Martin, 1992). However, in this study, histological examination was not performed in order to investigate the effect of vaginal danazol ring on the conserved deeply infiltrating endometriosis. Therefore deeply infiltrating endometriosis was arbitrarily diagnosed on the basis of the following points: (i) severe dysmenorrhoea and/or extramenstrual disabling pelvic pain; (ii) laparoscopically subtle or small gun-shot lesions on the cul-de-sac which might correspond to type II or type III of the Koninckx' (1992) classification, or combined with ovarian endometriotic cysts adhering to the cul-de-sac which might correspond to type I of the Koninckx' classification; and (iii) tender nodules in the pouch of the cul-de-sac.

Out of 42 cases diagnosed as deeply infiltrating endometriosis according to the above-mentioned criteria, 24 cases were thought to correspond to type I and 18 to type II or III of Koninckx' classification. As a control, 14 cases of ovarian endometriotic cysts which did not satisfy criteria (ii) and (iii) were treated with vaginal danazol ring in the same way as the 42 cases with deeply infiltrating endometriosis.

Patients showing hyperprolactinaemia were excluded from this study. Ages of the patients were 29.1 ± 2.3 years (mean \pm SD) in the control group and 29.8 ± 3.4 years in the deeply infiltrating endometriosis group. Duration of infertility was 3.8 ± 1.4 years in the control group and 4.3 ± 1.5 years in the deeply infiltrating endometriosis group. Laparoscopic findings were 38.5 ± 9.3 according

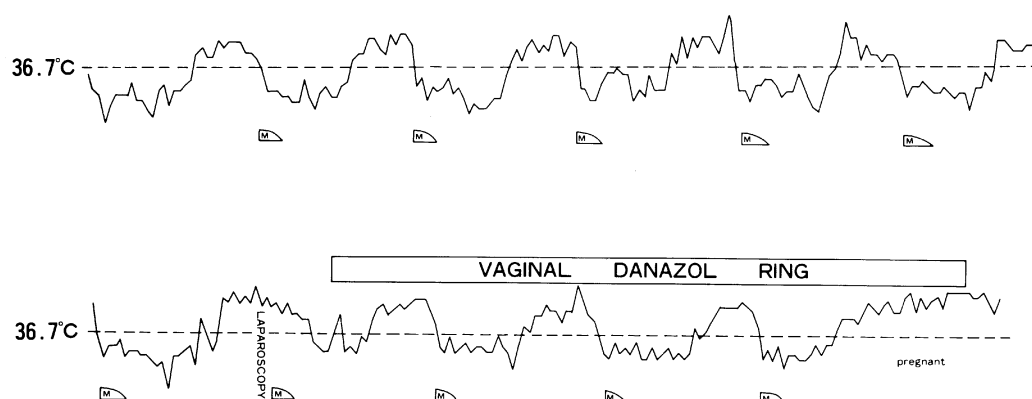


Figure 1. Basal body temperature (BBT) curves showing that ovulation, menstruation (M), and even conception could occur during insertion of the vaginal danazol ring. This BBT curve is from one patient, typical of all the patients who showed biphasic BBT curves during insertion of the vaginal ring containing 1500 mg danazol.

to the revised American Fertility Society score (American Fertility Society, 1985) in the control group and 42.4 ± 8.6 in the deeply infiltrating endometriosis group. Dyspareunia was checked but was not essential for diagnosis of deeply infiltrating endometriosis.

Occurrence of ovulation

All the patients were requested to record basal body temperature (BBT) in order to check the occurrence of ovulation during treatment.

Vaginal danazol ring

Doughnut-shaped rings made of silicon rubber, Silastic MDX 4-4210 (Dow-Corning Co., Midland, MI, USA), containing 1500 mg danazol were manufactured. This drug delivery system ring, sterilized by ethylene oxide gas, was inserted into the vagina in both the control group and the deeply infiltrating endometriosis group. The patients were not randomized. The vaginal danazol ring was replaced by a new ring every 1 or 2 months until pregnancy was successfully established or endometriosis was completely cured. The vaginal danazol ring was removed as soon as the establishment of conception was confirmed. Approval of this clinical research was obtained from the Medical Ethical Committee of the Gunma Central General Hospital. Informed consent was obtained from all patients, after the purpose and possible side-effects were explained. The patients were requested to record BBT every morning and to visit the hospital with BBT results every 2 or 4 weeks to record results and side-effects. For the statistical evaluation, Fisher's exact probability test was used.

Assay of serum danazol concentrations

Danazol was extracted from sera with a solution of n-hexane/chloroform (7/3) and then applied to a Sep-Pack Silicon cartridge absorption column (Millipore Co., Bedford, MA, USA). The danazol absorbed to the Sep-Pack column was eluted with chloroform. After evaporation of chloroform, the danazol was resolved in 200 μ l of n-hexane/chloroform solution and measured by high-pressure liquid chromatography (HPLC) with a Lichrosorb Si 605 column (250 \times 4.6 mm, Merck & Co., Rahway, NJ, USA). As a control, serum danazol concentrations in 15 patients taking danazol orally, 400 mg daily, for treatment of pelvic endometriosis were also assayed, after informed consent was obtained.

Results

All the 56 patients treated with the vaginal danazol ring showed biphasic BBT curves and normal menstruation, as in the pretreatment menstrual cycles (Figure 1). Moreover, 39

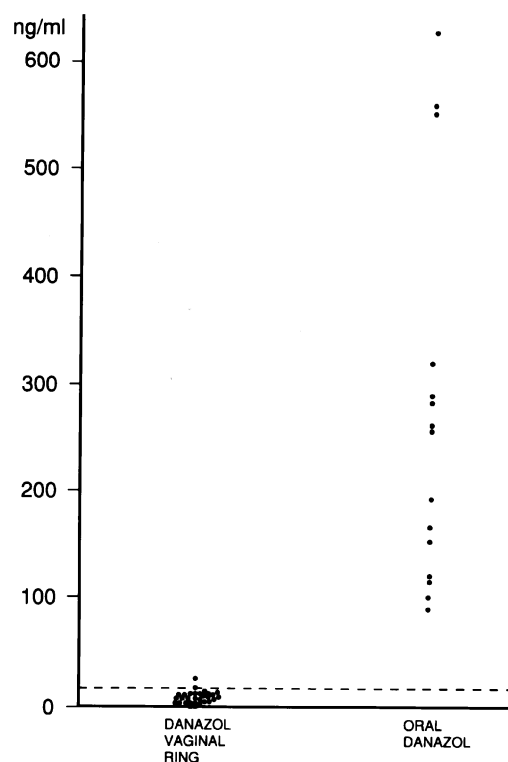


Figure 2. Serum danazol concentrations of the 15 patients during oral daily 400 mg danazol therapy (control) and of 30 patients during vaginal danazol (1500 mg) ring therapy assayed with HPLC. Serum danazol concentrations were always over 70 ng/ml during oral administration of daily 400 mg danazol, while they were undetectable during vaginal insertion of ring containing 1500 mg danazol.

infertile patients successfully conceived with the vaginal danazol ring *in situ* as shown in Figure 1. Serum danazol concentrations were moderately high, over 100 ng/ml in the women taking 400 mg danazol daily (200 mg twice) (Figure 2). On the other hand, danazol always remained undetectable in serum of 30 patients treated with the vaginal danazol ring.

As shown in Figure 3, dysmenorrhoea began to decrease within 2 months, and disappeared in 88.2% (15/17) of the group within 3 months. Tenderness on the cul-de-sac began to

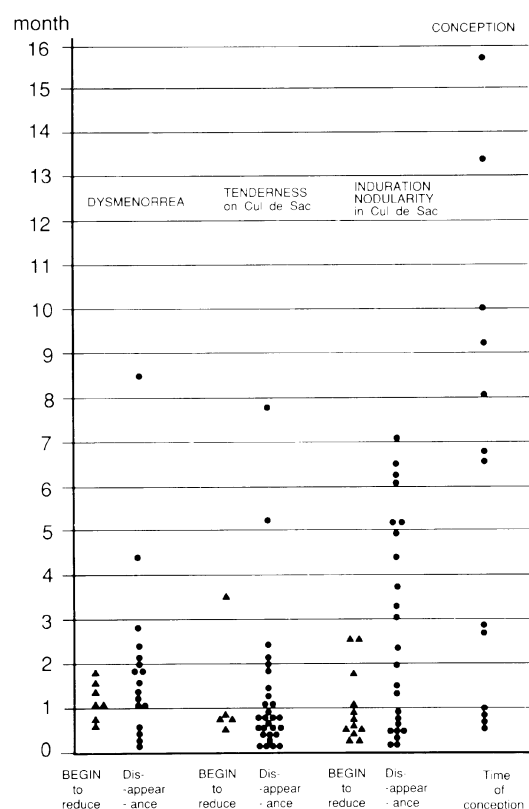


Figure 3. Time interval (months) until the initial manifestation of the clinical effects of vaginal danazol ring therapy on dysmenorrhoea, tenderness and induration or nodularity in the cul-de-sac, and conception in 17 patients. Dysmenorrhoea began to reduce within 2 months, and disappeared in 88.2% (15 cases out of 17) within 3 months. Tenderness on cul-de-sac began to reduce in four out of five cases within 1 month, and disappeared in 92.9% (26 cases out of 28) within 3 months. Induration or nodularities began to reduce within 3 months and disappeared within 7 months. Conception occurred in four cases within 1 month, in two further cases within 3 months, and in 11 cases (84.6%) in all within 10 months.

decrease within 1 month, and disappeared in 92.9% (26/28) of the group within 3 months. Induration or nodularities in the cul-de-sac began to decrease within 3 months, and disappeared after ~7 months in all of the 24 patients.

Successful conception in the infertile patients was observed in four cases within 1 month, in two patients within 3 months, and in another five patients within 10 months. The other two cases conceived in 13 months and 16 months (Figure 3). Eight male and 11 female babies were born during this vaginal danazol therapy, with no malformations including female clitoromegaly.

The effects of the vaginal danazol ring were compared between the types of pelvic endometriosis. As shown in Table I, endometriosis mass, measured by transvaginal ultrasonography, disappeared in 36 out of 42 patients, and was reduced in six out of 42 patients with deeply infiltrating endometriosis, but was unchanged in 11 out of 14 patients and reduced in three out of 14 patients with ovarian endometriotic cysts not adhering on the cul-de-sac and without deeply infiltrating endometriosis. The latter group acted as a control. The difference between the two groups was significant ($P < 0.001$).

Table I. Effects of vaginal danazol ring on endometriosis mass

	Disappeared	Reduced	Unchanged	Total
Deeply infiltrating endometriosis group	36	6	0	42
Ovarian endometriotic ^a cysts group without deeply infiltrating endometriosis (control)	0	3	11	14

^aThese ovarian endometriotic cysts did not adhere on the cul-de-sac. The groups were significantly different ($P < 0.0001$) by Fisher's exact test.

Table II. Effects of vaginal danazol ring on dysmenorrhoea

	Disappeared	Reduced	Unchanged	Total
Deeply infiltrating endometriosis group	32	9	1	42
Ovarian endometriotic ^a cysts group without deeply infiltrating endometriosis (control)	5	3	2	10 ^b

^aThese ovarian endometriotic cysts did not adhere on the cul-de-sac.

^bExcluding four patients for whom changes in dysmenorrhoea were not recorded.

The groups were not significantly different (Fisher's exact test).

Table III. Effects of vaginal danazol ring on establishment of pregnancy

	Got pregnant	Not pregnant	Total
Deeply infiltrating endometriosis group	17	14	31 ^b
Ovarian endometriotic ^a cysts group without deeply infiltrating endometriosis (control)	2	6	8 ^b

^aThese ovarian endometriotic cysts did not adhere on the cul-de-sac.

^bExcluding patients for whom an alternative cause for infertility apart from endometriosis had been recorded, e.g. severe oligozoospermia or bilateral tubal exclusion.

The groups were not significantly different (Fisher's exact test).

Dysmenorrhoea disappeared in 32 out of 42 vaginal danazol ring patients with deeply infiltrating endometriosis, but in only five out of 10 patients with the ovarian endometriotic cysts not adhering on the cul-de-sac and without deeply infiltrating endometriosis, as shown in Table II. Changes of dysmenorrhoea after treatment in four patients who belonged to the ovarian endometriotic cyst group were not described on the chart, so statistical analysis between these two groups was not performed. In Table III, the patients who had a cause for infertility in addition to endometriosis, such as severe oligozoospermia or bilateral tubal occlusion, were omitted, so that the total number of patients in both groups was different from that shown in Table I.

The pregnancy rate was 54.5% (17/31) in the deeply infiltrating endometriosis group and 25.0% (2/8) in the group with ovarian endometriotic cysts not adhering on the cul-de-sac and without deeply infiltrating endometriosis, as shown in Table III. However, the difference between the two groups was statistically not significant, probably because the number

of patients in the ovarian endometriotic cyst group was too small.

Discussion

Serum danazol concentrations were very high during 400 mg daily danazol therapy, but were undetectable during vaginal 1500 mg danazol ring therapy (Figure 2). It is also reported that serum danazol concentrations are undetectable or very low when a 100 mg or 200 mg danazol suppository is inserted into the vagina (Mizutani *et al.*, 1995; Tanaka *et al.*, 1996, 1997). These results clearly explain why inhibition of ovulation, which was always observed in oral danazol therapy, was not observed during vaginal danazol ring therapy, and also why the general side-effects often observed during oral danazol therapy (e.g. liver dysfunction, increases in body weight and acne) were not observed at all during vaginal danazol ring therapy.

It is interesting to speculate on the mechanism by which vaginally administered danazol effectively cured pelvic endometriosis without increasing serum danazol concentrations. We (M. Igarashi, unpublished data) have previously detected high danazol concentrations in the cervical mucus during vaginal danazol ring treatment, so at first it was presumed that danazol was transported via the transcervical–tubal route. However, Tanaka *et al.* (1997) demonstrated that danazol concentration in the peritoneal fluid and the pelvic organs, including the ovary and the tubes, was not less at vaginal administration of 200 mg danazol than at oral administration of 200 mg danazol. Moreover, they ruled out the above-mentioned transcervical–tubal route because there was no difference in danazol concentrations in peritoneal fluid between women with and without tubal patency, when 200 mg danazol was administered vaginally. So we presume the exact mechanism of transport is by direct absorption of danazol through the vaginal mucosa and subsequent transportation to the cul-de-sac and the pelvic organs via diffusion.

Recently accumulated evidence suggests direct action of danazol on endometrial or endometriotic cells *in vitro*. Danazol binds rat androgen and progesterone receptors (Chamness *et al.*, 1980). Uptake of [³H]-thymidine into cultured endometrial cells is inhibited by danazol (Taketani and Mizuno, 1985). Danazol, but not leuprolide acetate (an LHRH analogue), exerts direct effects to suppress growth of endometriotic implants (Surrey and Halme, 1992). Danazol reduced monocyte-enhanced endometrial proliferation in peripheral blood *in vitro* (Braun *et al.*, 1994). Danazol stimulated *in-vitro* apoptosis of the cultured cells derived from endometrial adenocarcinoma (Chin *et al.*, 1997). These direct *in-vitro* actions of danazol on endometriotic tissue have been confirmed *in vivo* by our present study. It is reported that LHRH analogues may act directly on uterine leiomyomata (Wiznitzer *et al.*, 1988), but there is no evidence that LHRH analogues act directly on endometriotic cells (Surrey and Halme, 1992). The mode and site of action of local danazol therapy seem to be quite different from those of both oral danazol therapy and LHRH analogue therapy. Local danazol therapy reduces endometriosis because danazol can act directly on endometriotic cells to inhibit DNA synthesis

and to induce apoptosis *in vitro*. On the other hand, LHRH analogue therapy and oral danazol therapy seem to act by preventing the progression of endometriosis because they act on the hypothalamo–pituitary system to inhibit LH-FSH secretion and subsequently to inhibit oestrogen secretion.

Acien *et al.* (1989) reported that oral danazol treatment reduced significantly the prolactin response to LHRH/thyrotrophin-releasing hormone test. In our research, the patients showing hyperprolactinaemia were excluded in advance and, moreover, danazol excreted from the vaginal ring did not appear in peripheral blood, so the influence of danazol on prolactin response to LHRH/ thyrotrophin-releasing hormone test is not relevant.

Deeply infiltrating endometriosis and extraperitoneal infiltration of endometriosis sometimes cannot be found through laparoscopy (Koninckx and Cornillie, 1993; Koninckx and Martin, 1994), but induce more severe pelvic pain than the various other types of endometriosis (Koninckx *et al.*, 1991). Moreover, deeply infiltrating endometriosis is often resistant and does not respond well to medical therapy such as oral danazol or LHRH analogues (Shaw, 1993). The best and only treatment is said to be surgical treatment which requires skill and is often accompanied by the risk of perforation of the rectum (Nezhat *et al.*, 1991; Donnez *et al.*, 1995). As shown in Tables I–III, vaginal danazol therapy had excellent effects on deeply infiltrating endometriosis, although its effect on the ovarian endometriotic cysts not adhering to the cul-de-sac was limited. Since danazol released from the vaginal ring invades through the vaginal wall into the nearest tissue via diffusion as mentioned above, it is conceivable that high concentrations of danazol may reach the cul-de-sac and invade the deeply infiltrating endometriosis, because the distance between the vagina and the cul-de-sac is only 3–5 mm.

The advantages of vaginal danazol ring therapy compared with the oral danazol and LHRH analogue therapies are as follows: (i) it is the best medical treatment for deeply infiltrating endometriosis, which is usually resistant to the oral danazol and LHRH analogue therapies; (ii) it has no general associated side-effects, which are often observed in the other two therapies; (iii) conception can occur even during therapy, while the patients remain infertile for 4 to 6 months during the other two therapies; and (iv) dysmenorrhoea and severe pelvic pain, induced by deeply infiltrating endometriosis, are cured more rapidly by this vaginal ring therapy than by the other two therapies. This vaginal danazol therapy has one disadvantage. The effects on uterine adenomyosis (Igarashi *et al.*, 1997) and ovarian endometriotic cysts not adhering to the cul-de-sac are insufficient and unsatisfactory. However, local danazol therapy using a danazol intrauterine device for uterine adenomyosis (Igarashi, 1990; Tamaoka *et al.*, 1997) and local danazol injection therapy for ovarian endometriotic cysts (Adachi *et al.*, 1994) are both useful and effective. Oral administration of danazol induces endometrial atrophy (Hickey *et al.*, 1996), but no endometrial atrophy was observed during vaginal danazol ring therapy (M. Igarashi, unpublished data) and conception could occur during insertion of vaginal danazol ring (Figures 1, 3 and Table III).

There were no general side-effects, such as are often

observed in oral danazol therapy, with the vaginal ring therapy. The only side-effect of this vaginal danazol ring therapy was colpitis. However, colpitis can be prevented by either special design of the ring, or by exchange of the ring every month, and can be treated with vaginal anti-trichomoniasis or anti-candidiasis drugs. Since danazol is a weak androgen and is transported by diffusion through the vaginal mucosa, there is a risk that the female fetus might be masculinized if the vaginal danazol ring is inserted in the vagina during the pregnancy. There are a few reports (Castro-Magana *et al.*, 1981; Duck and Katayama, 1981; Peress *et al.*, 1982) on the virilization of female fetuses in patients who received oral danazol therapy during pregnancy. In the 11 fetal virilization cases, the mother received oral danazol therapy during the first 12 to 18 gestational weeks or from the eighth to 12th weeks, or during the last 12 or 19 gestational weeks. However, none of the female infants born to our patients showed any masculinization symptoms such as clitoromegaly. All our patients were required to check BBT every morning and to come to visit the doctor when the high phase of BBT continued for over 21 days. During visits, if the urinary human chorionic gonadotrophin pregnancy test was positive, the vaginal danazol ring was removed. Since the critical period of development of androgen receptor sensitivity in genital ducts starts from the eighth week of embryogenesis, the virilization of the female fetus can be prevented if the vaginal danazol ring is removed before the eighth week of gestation. However, further studies are required before the effect on the fetus can be proven to be negligible.

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