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Research Article

Effect of vaginal gestrinone in Pentravan® on endometriosis patients using Mirena®: A preliminary report

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Abstract

Objective: To investigate the effect of low doses of vaginal gestrinone with oral Pinus pinaster extract and resveratrol on endometriosis-related pain in patients with deep endometriosis using Mirena® and still experiencing symptoms during use of this levonorgestrel-releasing intrauterine system.

Patient and methods: This was an open observational study conducted with 20 patients with deep endometriosis and severe pain to investigate the clinical effectiveness of low doses of vaginal gestrinone to improve pain and induce amenorrhea in patients not responding adequately to Mirena®. In Group A (n=14), treatment with vaginal gestrinone, oral Pinus pinaster extract and resveratrol began 3-6 months after Mirena® insertion when it was found that these patients were not responding satisfactorily to treatment. In Group B (n=6), patients initiated treatment with vaginal gestrinone, oral Pinus pinaster extract and resveratrol at the time of Mirena® insertion. The effect of gestrinone on aromatase expression in the eutopic endometrium was also investigated by immunohistochemistry in all patients.

Results: In Group A, the use of Mirena® alone resulted in a small but significant decrease in pain scores. However, these patients were still experiencing breakthrough bleeding and pelvic pain. The introduction of low doses of vaginal gestrinone in Pentravan® together with oral Pinus pinaster extract and resveratrol led to a further decrease in pain score, rendering these patients pain-free by the end of the second month of this combination treatment. In Group B, pain scores similar to those found in Group A after introduction of the combination treatment were achieved after the first treatment month.

Conclusion: Concomitant use of Mirena® with low doses of vaginal gestrinone in association with oral antioxidants is an effective treatment for deep endometriosis-related pain.

Introduction

Initial trials using a levonorgestrel intrauterine system (LNG-IUS) have shown the effectiveness of this device for the treatment of pelvic pain and to reduce the size of lesions in deep infiltrating endometriosis [1,2]. However, despite these encouraging initial results, pain may persist in some patients [3]. This lack of response may be a consequence of the development of some form of progesterone resistance caused by a reduction in progesterone receptor isoform B in both the endometriosis lesions and the eutopic endometrium, triggered by exposure to inflammatory mediators [4,5]. One medical solution to this problem is to use the vaginal route to administer hormones that interact with the androgen receptor instead of the progesterone receptor, combined with natural NF-Kappa.b inhibitors to curb down inflammation. Absorption of the hormone through the vagina offers a unique advantage not offered by other routes of administration because of the first uterine pass effect, an effective mechanism through which to concentrate pharmacologically active agents such as gestrinone in the pelvic organs before they are diluted into the systemic circulation [6,7].

In the present report, the effect of lower doses of vaginal gestrinone in Pentravan® together with a combination of natural polyphenols with antioxidant and antiinflammatory properties was investigated in patients with deep endometriosis who continued to have breakthrough bleeding and pain despite being in use of Mirena®. Two polyphenols, Pinus pinaster extract and resveratrol, were used concomitantly [8]. These natural polyphenols are a complex mixture of flavonoids extracted from the bark of a pine tree (Pinus pinaster) or present in red wine (resveratrol), and are able to block both NF-Kappa.b and aromatase activity [9,10]. The effect of vaginal gestrinone associated with these natural polyphenols on aromatase expression in the endometrium of these patients was also investigated.

Patient and methods

This was an open clinical study to investigate the effect of

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an association of natural polyphenols and low doses of vaginal gestrinone on patients with endometriosis who were using Mirena® but who continued to experience pain and breakthrough bleeding. The combined effects of vaginal gestrinone together with the natural polyphenols on pain scores and bleeding patterns were also evaluated in endometriosis patients in whom treatment was initiated at the time of Mirena® insertion.

The primary endpoint of this study was to investigate the combined use of an intrauterine levonorgestrel system (IUS) (Mirena®) with low doses of vaginal gestrinone and natural polyphenols on pain scores and bleeding patterns in patients with deep endometriosis. Other associated medical conditions such as ovarian endometriotic cysts (n=2), adenomyosis and uterine fibroids (n=12) were also present.

The patients formed two separate groups. Patients in Group A (n=14) had already been using Mirena® for 3-6 months prior to commencing use of vaginal gestrinone (Fagron, the Netherlands) together with oral Pinus pinaster extract (Fagron, the Netherlands) and resveratrol (Fagron, the Netherlands). In Group B (n=6), patients with deep endometriosis initiated treatment with gestrinone, oral Pinus pinaster extract and resveratrol at the time of Mirena® insertion. Prior to initiating vaginal gestrinone treatment, all patients were evaluated clinically. Global pain scores, applied to both dysmenorrhea and dyspareunia, were rated by the patient at the time of the first interview prior to gestrinone use and 2 months after treatment using a visual analogic scale in which 0 was indicative of no pain and 10 reflected the worst pain imaginable. Pain scores prior to Mirena® insertion in Group A were obtained from the patients' medical records and confirmed retrospectively at the time of the first medical interview to initiate treatment with gestrinone, Pinus pinaster extract and resveratrol. In Group A, the patients using Mirena® were treated with a combination of 2.5 mg of vaginal gestrinone in Pentravan® (Fagron, the Netherlands) twice a week, together with 100 mg of oral Pinus pinaster extract (Fagron, the Netherlands) and 30 mg of resveratrol (Fagron, the Netherlands) daily. In Group B, these medications were initiated at the time of Mirena® insertion. In all cases, they were prepared by a licensed compounding pharmacy. The Pinus pinaster extract (100 mg) and resveratrol (30 mg) were prepared and placed in the same capsule at a local compounding pharmacy supervised by the same pharmacists (MCH, WSDS). The Mirena® devices were manufactured by Bayer and were inserted by the same doctors (HM, CH) in all cases, under intracervical block achieved with 10 ml of lidocaine 1%. Gestrinone is licensed by the Brazilian drug regulatory authorities (ANVISA) for use as an oral medication for the treatment of endometriosis. It was prepared for vaginal use at the concentration of 2.5 mg in Pentravan® (Fagron, the Netherlands), always by same pharmacist (WSDS).

The patients were instructed to insert the gestrinone/Pentravan® preparation into the vagina at bedtime, twice a week, on Mondays and Fridays, using a disposable plastic applicator. Resveratrol and Pinus pinaster extract were dispensed together in the same V caps (Fagron, the Netherlands) and patients were instructed to take one capsule orally every day at bedtime.

This study was conducted at the *Instituto da Mulher*, Itaigara Memorial Hospital. The institution's internal review board approved the study protocol. The *Instituto da Mulher* is a private medical facility specializing in women's health and the treatment of endometriosis. All patients were counseled with respect to this treatment regimen and gave their informed consent to participate in the study.

Hysteroscopy with endometrial biopsy was performed in the 14 patients with endometriosis in Group A prior to and after two months of gestrinone use to evaluate aromatase expression by immunohistochemistry. During hysteroscopy, the uterine cavity was evaluated and an endometrial biopsy was taken, with samples being sent to pathology for routine histology and immunohistochemistry. The same two surgeons (HM and CH) performed all the hysteroscopic procedures. Anesthesia was achieved with the use of a paracervical block and light intravenous sedation with propofol. When evaluation of the uterine cavity was complete, the hysteroscope was removed and a 4 mm Karman curette attached to a 10 ml disposable plastic syringe was introduced and the endometrium was aspirated. The samples were immediately fixed in 4% formalin and sent to pathology. Routine histology using hematoxylin & eosin (HE) staining was performed on all samples. The same pathologist (NP) performed both the routine pathology and immunohistochemical evaluation of the endometrium.

The presence of aromatase expression in the endometrium was determined by immunohistochemistry following antigen retrieval. Aromatase expression was investigated using a commercially available monoclonal antibody supplied by Serotech, Raleigh, NC, USA. Antigen retrieval was performed using the Tris-EDTA buffer at pH 8.0. The reaction was revealed using the DAKO EnVision Flex detection system + Linker followed by DAB + substrate chromogen mix (DAKO). The presence of aromatase expression was rated either as positive if there was any detectable staining reaction in the endometrium or negative when no reaction was observed.

Statistical analysis was performed using the chi-square test to detect differences in the percentages of endometria testing positive for aromatase expression before and after gestrinone use. Student's t-test was used to detect differences in mean pain scores before and after gestrinone treatment. Significance was established at $p<0.05$.

Results

The effect of Gestrinone and Mirena® on pain scores

In Group A (n=14), use of Mirena® alone resulted in a modest but significant reduction in pain score from a mean of 9 to a mean of 6 ($p=0.01$) after 3-6 months of use; however, the patients were still reporting pain at this time and none were in amenorrhea. In all cases, the presence of breakthrough bleeding coincided with flare-ups of dysmenorrhea-like pain. Nevertheless, when gestrinone (2.5 mg) twice weekly + daily oral Pinus pinaster extract and resveratrol were introduced, dysmenorrhea pain scores further decreased after one month of treatment to a mean of 1 ($p<0.0001$). After the second month of this combination treatment, all patients in this group became pain-free and amenorrheic. With respect to other forms of pelvic pain such as dyspareunia and dyschesia, pain scores decreased significantly from a mean of 8 to a mean of 1 after the first month of gestrinone treatment.

In Group B, insertion of Mirena® with immediate implementation of a daily regimen of gestrinone + Pinus pinaster extract and resveratrol led to a significant reduction in dysmenorrhea scores from a mean of 9 to a mean of 2 ($p=0.004$) after the first month of treatment. By the end of the second month of treatment, all patients were completely pain-free and remained so throughout treatment. There were no statistically significant differences in pain scores between Groups A and B at any time period following implementation of gestrinone with Pinus pinaster extract and resveratrol. No untoward systemic effects were found with these low doses of gestrinone except for acne in 20% of patients.

Aromatase expression in the endometrium

In endometriosis patients using Mirena® and still experiencing pain and breakthrough bleeding, aromatase expression was detected in the endometrium of patients in 88% of the cases. Aromatase expression was detected by immunohistochemistry solely in the stroma, while no staining reaction was found in the glandular epithelium. In these cases, histology revealed the presence of a strong decidual reaction in the endometrium. After the introduction of vaginal gestrinone with oral Pinus pinaster extract and resveratrol, the percentage of aromatase-positive endometria decreased to 20% by the end of the second month of combination treatment. Aromatase expression remained positive in only three patients and these patients were still reporting pain, albeit much less. The endometrium was labeled as basal/inactive and the decidual reaction in the stroma disappeared. At hysteroscopy, the mucosa was thin and avascular.

Discussion

The present report showed that in patients with deep endometriosis experiencing pain and breakthrough bleeding while in use of Mirena® the introduction of low doses of vaginal gestrinone in Pentravan® with oral Pinus pinaster extract and resveratrol was highly effective in improving pain and inducing amenorrhea.

In patients with endometriosis, the use of Pinus pinaster extract with resveratrol successfully potentiated the inhibitory effect of oral contraceptives in extended regimens on aromatase expression in the eutopic endometrium [8]. Since aromatase gene transcriptions are directly or indirectly increased by NF-kappa.b activation, the reduction in their expression in the eutopic endometrium of endometriosis patients following the use of gestrinone with Pinus pinaster extract + resveratrol may be a consequence of the more effective inhibition of this transcription factor [11]. The unabated aromatase expression in the eutopic endometrium of symptomatic endometriosis patients using Mirena® suggests that the persistence of pain may be a consequence of the continued expression of this enzyme in the endometrium, probably due to the development of progesterone resistance caused by increased inflammation [4,5,11]. This is the most likely explanation for the lack of effect of the LNG-IUS on aromatase expression in the endometrium of these patients, since inhibition of this enzyme correlates positively with higher rates of amenorrhea [8]. The persistence of pain in endometriosis patients using Mirena® may be caused by the presence of inflammatory cytokines and prostaglandins in the endometrium, since these may reduce the expression of progesterone B receptors, rendering both the endometrium and endometriosis lesions resistant to the effect of levonorgestrel [5,11,12]. In patients who experience breakthrough bleeding during continuous use of oral contraceptives containing gestodene/ethinylestradiol, NF-Kappa.b remains actively bound to cell nuclei where it will increase the transcription of several genes directly or indirectly related to inflammation [13]. This will eventually maintain the transcription of the aromatase gene active and the ensuing local estrogen production will further augment inflammation [11]. Estrogens can increase Cox-2 activity, thus increasing prostaglandin production, which in turn can stimulate the expression of the aromatase gene, thus establishing a link between estrogens and increased inflammation [11,14,15]. The more effective suppression of aromatase expression following vaginal gestrinone treatment with oral Pinus pinaster + resveratrol in endometriosis patients using Mirena® is a consequence of the reduction in inflammation prompted by activation of the androgen receptor by gestrinone and the concomitant blockade of NF-Kappa.b activity by the oral flavonoids. This results in higher rates of amenorrhea and pain-free intervals after just one month of treatment. Gestrinone acts in the endometrium by stimulating the androgen receptors, and the ensuing inhibition of aromatase expression may be a consequence of the reduction in inflammation, since gestrinone has been shown to have no direct effect on aromatase expression in cultured cells from endometriotic cysts [16]. Inflammation plays a pivotal role in the progression of endometriosis [11] and activation of the androgen receptors may represent an alternative for those patients in whom progestin therapy is unsuccessful. The combination of Mirena® with low doses of gestrinone and oral antioxidants effectively diminished aromatase expression in the endometrium of patients in use of Mirena® who had dysmenorrhea and breakthrough bleeding, thus rendering them pain-free and amenorrheic. Because of the strong anti-progesterone and anti-estrogenic effect of gestrinone, the decidual reaction in the stroma disappeared completely, rendering the endometrium thin and avascular. As shown in the present paper, the combination of Mirena® with low doses of vaginal gestrinone was as effective as gestrinone used alone in higher doses, as shown previously by our group [7].

The association of Mirena® with vaginal gestrinone and oral antioxidants, as reported in the present paper, induces high rates of amenorrhea, with a considerable improvement in pain after just one month of treatment, irrespective of whether this treatment begins at the time of Mirena® insertion or afterwards in the case of patients who remain symptomatic after 3 to 6 months' use of the device. The decrease in pain score was similar in the two groups. The use of low doses of gestrinone together with Mirena® may increase patient compliance by reducing the side effects and the cost of treatment, while maintaining the same effectiveness as that achieved with higher doses. Since endometriosis is an inflammatory, estrogen-producing pathology, the combination of the anti-estrogenic effects of gestrinone with the antioxidant properties of resveratrol and Pinus pinaster extract may improve control of the disease, thus explaining the rapid relief of pelvic pain experienced by these patients.

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