The effects of Ulipristal on surgically induced endometriosis in a rat model

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Abstract

Objective(s). *We conducted a prospective, randomized, controlled, experimental study to evaluate Ulipristal effects on surgically induced endometriosis in a rat model at Experimental Research Center of "Iuliu Hatieganu" University of Medicine and Pharmacy Cluj-Napoca, Romania. According to our knowledge at this moment no clinical or experimental study was published about the effect of Ulipristal on endometriosis.* ***Methods.*** *We used sixty female Wistar albino rats. Endometriosis was induced by transplanting two autologous fragments of uterine horn on bowel mesentery. After induction period we formed two groups: first treated with Ulipristal and second only with the vehicle used for Ulipristal. The volume and mass of the implants were measured, before and after treatment. A pathologist examined microscopically the sections for histological hallmarks of endometriosis.* ***Results.*** *For volumes and masses, Ulipristal reduced the average values meaning that is effective (P=0,01). The treatment induced a more than 50% reduction of the volume and masses of endometrial implants and the histological findings correspond to this.* ***Conclusion(s).*** *Ulipristal determined regression and atrophy of endometriotic lesions in rats.*

Keywords: *endometriosis, experimental endometriosis models, Selective Progesterone Receptor Modulator SPRM, Ulipristal*

Introduction

Endometriosis is an estrogen-dependent benign condition defined as the presence of endometrial-like tissue outside the uterine cavity, which induces a chronic, inflammatory reaction, predominantly in women of reproductive age, from all ethnic and social groups[(1)]. The disease affects an estimated 10% of women in the reproductive age group, rising up to 30-50% in women with infertility or pain[(2)].

Considering the side effects associated with the established medical treatment new approaches are being considered. This article focuses on the role of SPRMs in the treatment of endometriosis, evaluating the effects of a SPRM on surgically induced endometriosis in a rat model. Since the discovery of Mifepristone (RU 486) the first progesterone antagonist and glucocorticoid receptor antagonist, numerous related compounds were subsequently synthesized. These latter compounds are also known as selective progesterone receptor modulators and may function as progesterone agonists, progesterone antagonists or as mixed agonists-antagonists.

The beneficial effects of the treatment with SPRMs are probably related to their antiproliferative effects which have been well described in the primate endometrium. SPRMs are associated with an increase in estrogen receptors (ER), progesterone receptors (PR) and androgen receptor (AR). Androgens suppress estrogen-induced endometrial proliferation. The increase in AR consequent to SPRMs could produce these antiproliferative effects. This effect appears to be the mechanism explaining the antiproliferative effect, although it could also be related to the fact that PR-A isoform inhibits estrogen-receptor gene transcription induced by progestins and progesterone antagonists[(4)].

Aromatase expression is significantly increased in endometriotic implants compared to eutopic endometrium and this leads to an increase in estradiol. Mifepristone blocks medroxyprogesterone acetate-induced aromatase activity in endometrial stromal cells[(5)].

The susceptibility of endometrial tissue to spontaneous apoptosis is lower in women with endometriosis than in healthy controls. One of the apoptotic pathways involves bcl-2/bax family of proteins, increased expression of bcl-2 protein has been observed in the proliferative eutopic endometrium from patients with endometriosis compared with controls. This suggests a protective effect in the endometriotic cell facilitating its survival[(6)]. Mifepristone has been shown to promote apoptosis by over-expressing bax and down-regulating bcl-2[(7)].

Angiogenesis is also involved in the pathogenesis of endometriosis and its inhibition is regarded as a novel therapeutic approach. Estradiol is a potent stimulus for angiogenesis through the direct increase of Vascular Endothelial Growth Factor (VEGF) expression, which is expressed in endometriotic implants and is elevated in the peritoneal fluid of women affected by the disease. It is shown that SPRMs suppress VEGF in human and cynomolgus endometrial tissue samples[(8)].

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For all these reasons, SPRMs could represent a new and promising therapeutic option for endometriosis. Even if there is only a small number of clinical trials reported with mifepristone and asoprisnil, there were encouraging results obtained[9,10]. According to our knowledge at this moment no clinical or experimental study was published about the effect of Ulipristal on endometriosis.

CDB-2914, also called VA-2914, and now named Ulipristal acetate (UPA) derives from 19-norprogesterone (17 alpha-acetoxy-11-[4-N, N-dimethylaminophenyl]-19-norpregna-4,9-diene-3,20-dione) and is an antagonistic, partial agonistic, progesterone receptor modulator currently undergoing clinical investigation.

Ulipristal has potential clinical applications for regular and emergency contraception, the treatment of fibroids and endometriosis, cervical ripening for induction of labor and the treatment of breast cancer and gliomas[11,12].

To test Ulipristal in endometriosis treatment an experimental animal model for endometriosis is useful. Nonhuman primates are ideal for this study, but they are expensive. In the mean time, the rat endometriosis model has several advantages like limited cost, possibility of studying the endometriotic peritoneal implants, the investigation of different therapeutic effects.

Methods

Animal Model

Sixty female Wistar albino rats, non-pregnant, nulligravid, aged 10-12 weeks, weighing 180-200 g, obtained from the animal Laboratory of the Experimental Research Center and Practical Skills of the "Iuliu Hatieganu" University of Medicine and Pharmacy from Cluj-Napoca, Romania, were used in this experiment. The rats were caged individually in a controlled environment (room temperature 21±2 °C and humidity 60±5 %) with 12 hours light/dark cycles, and were fed ad libitum. All experiments were performed in compliance with international guidelines on the ethical use of animals and the study was approved by the Ethics Committee of the "Iuliu Hatieganu" University of Medicine and Pharmacy from Cluj-Napoca.

Experimental Design and Surgical Procedures

The experiment had four steps (Figure 1). The first was the surgical induction of endometriosis in 50 Wistar albino rats. We transplanted fat tissue in 10 rats. The second step was the laparotomy made after 7 weeks from induction, when we randomly sacrificed 10 rats with previously induced endometriosis and the rats with fat tissue transplant. The third step was the treatment period with Ulipristal which was administered to 20 rats with surgical induced endometriosis. The other 20 were given only the vehicle, without Ulipristal. The rats were divided in the two groups by randomization (using a randomization table). The fourth step was the second laparotomy and the sacrifice of all rats after the three months of treatment.

First step: Induction of Endometriosis

Endometriosis was induced using homologous uterine horn transplantation as proposed by Vernon and Wilson[13]. All 60 rats were anesthetized with an intramuscular injection of ketamine hydrochloride (60 mg/kg) and xylazine hydrochloride (7 mg/kg). Before surgery, the abdominal skin was shaved and the antisepsis was obtained by using a 10% povidone iodine solution. Using sterile techniques a vertical midline incision was made, about 5 cm long, and the uterus was exposed. A distal segment about 1 cm long was removed from the left uterine horn, the fragment was placed in a phosphate-buffered saline 37°C and was split longitudinally, obtaining two pieces and revealing the endometrium. These two pieces were sutured separately, on the bowel mesentery, close to a vessel, with the endometrial layer of the uterine fragment facing the serosa. A single nonabsorbable 6-0 polypropylene suture was used for fixing (Figure 2 A, B). For the

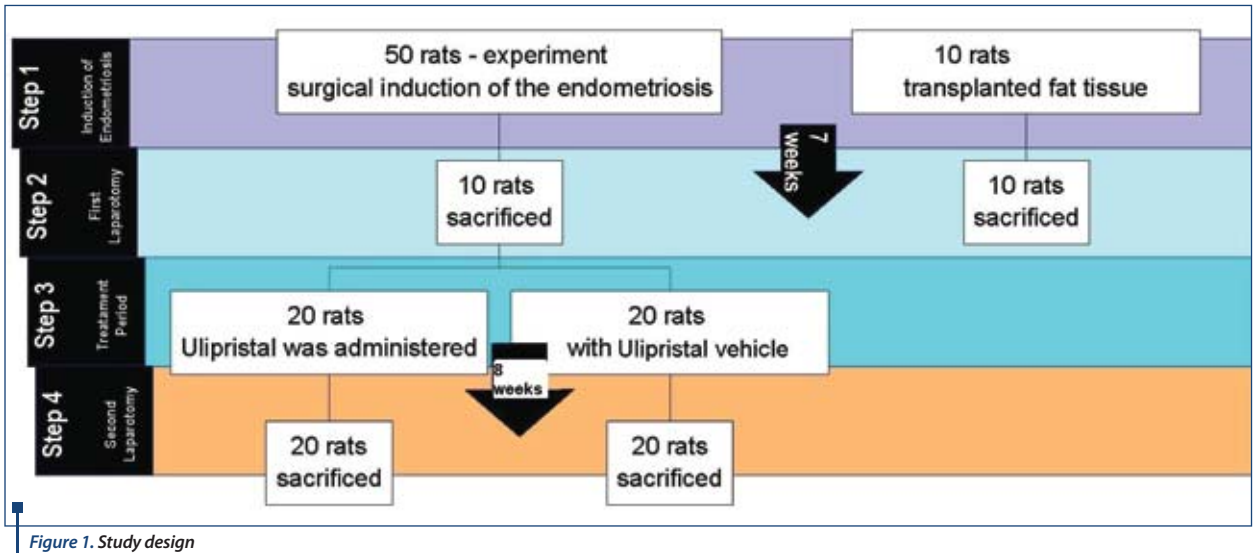

Figure 1. Study design

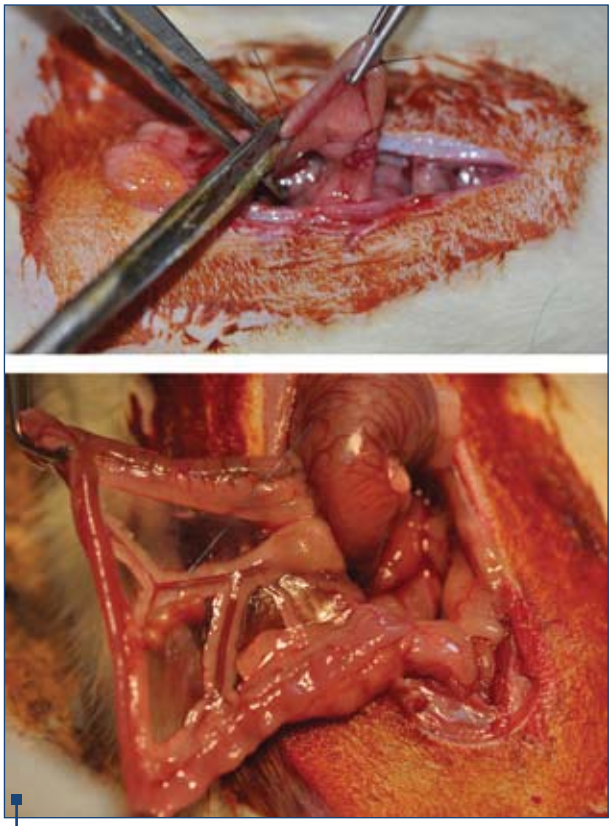

Figure 2 (A and B). Experimental induction of endometriosis

other 10 rats we used two fragments of properitoneal fat tissue, which were also attached separately, on the bowel mesentery, close to a vessel. The peritoneal cavity was kept moist with saline solution during the surgery. The abdominal incision was closed using 4-0 chromic suture. The operation was limited to 30 minutes and the external wound was protected with an antibiotic cream. The animals were individually caged after the intervention and left for recovery.

Second step: first laparotomy

In this step we wanted to evaluate endometriotic lesions, so we sacrificed 10 rats from the experimental group and the 10 rats from the group with transplanted fat tissue fragments. We made a vertical incision, discovered the place of the implants, excised them and weighed them (in milligrams). We measured the ectopic uterine tissue in three dimensions: length, width, height in millimeters using a caliper. This group of rats with experimental induced endometriosis was named Group E1 and the group with transplanted fat tissue fragments was named Group M1. The pieces were fixed in 10% buffered formaldehyde. Formalin fixed specimens were paraffin-embedded, cut into 5-µm sections, and stained with hematoxylin and eosin (H&E). Sections were examined microscopically for the presence of histologic hallmarks of endometriosis.

Third step: treatment period

We divided the remaining 40 rats with experimental induced endometriosis, using a randomization table in two groups of 20 rats each. We treated one group with Ulipristal Acetate (commercial product EllaOne - HRA Pharma) for 8 weeks. The vehicle used for Ulipristal was a solution of distilled water and ethyl alcohol (4/1). The other group, used as control, received only the vehicle without Ulipristal. The daily dose was 0.1 mg per day, per rat, administered p.o. The rats were monitored daily, no evidence of toxicity was noted at this dose, based on body weight, food consumption, grooming behavior when compared with controls.

Fourth step: second laparotomy

All 40 rats were sacrificed at the end of the treatment. Same as in the case of the first laparotomy, endometriotic implants were excised from both groups, weighed and measured. We calculated the volume of each ectopic implant using the same formula. The treatment group was named Group E2 and the rats with surgically induced endometriosis that received only the vehicle were part of Group M2. The specimens were fixed and stained with H&E after the same method. The same pathologist examined microscopically the sections for the presence of histological hallmarks of endometriosis.

Statistical Analysis

Statistical analysis was performed on a personal computer using GraphPad InStat 3.06 for Windows (32

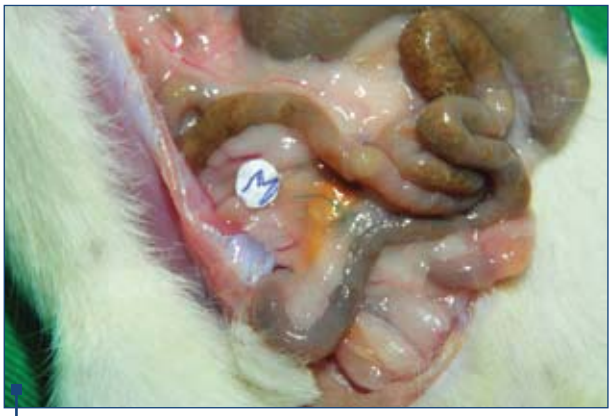

Figure 3. Adipose tissue implant at group M1 at 7 weeks after the implantation - macroscopic aspect

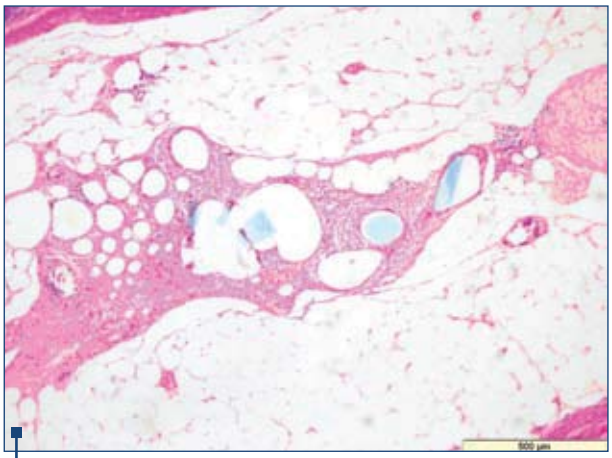

Figure 4. Adipose tissue implant at group M1 at 7 weeks after the implantation-microscopic aspect H&E(x200)

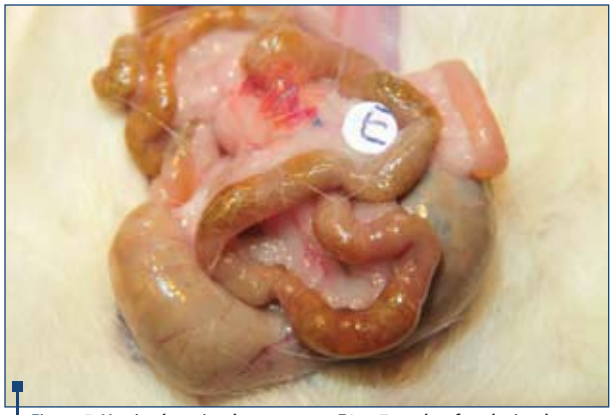

Figure 5. Uterine horn implant at group E1 at 7 weeks after the implant-macroscopic aspect

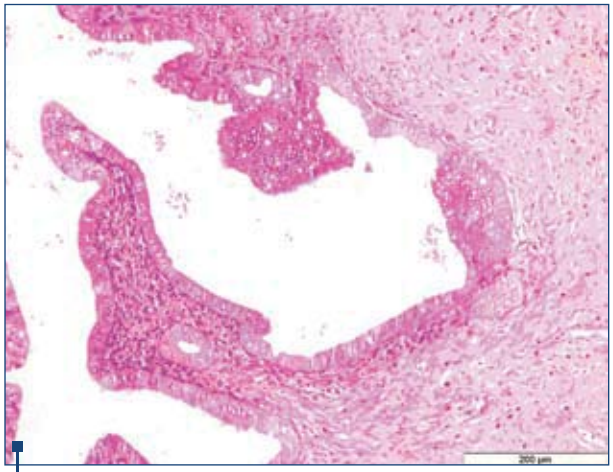

Figure 6. Uterine horn implant at group E1 at 7 weeks after the implant-microscopic aspect H&E(x40)

bit). The Kolmogorov-Smirnov test was used to show that the variables (volumes and masses) were normally distributed. Results were given as mean ± standard deviation for volumes and masses. The unpaired t test with Welch correction was used to analyze the populations and the obtained one-tailed P value <0.01 was considered statistically highly significant.

Results

Histological Analysis

The histopathological examination was made for each of the mentioned groups.

In group M1 we observed a good integration of the abdominal fat tissue that we implanted, with a chronic inflammatory reaction around the implant (Figures 3, 4).

In group E1 we also observed a very good integration of the implant that was fixed to the mesentery through the granulation tissue. In the endometrium there is an inflammatory reaction and the endometrial glands are well defined, in some places they are disposed on many layers even presenting papilliferous growth (Figures 5, 6).

In group M2, that received only the vehicle, we observed the persistence of the disease, the implants were still present, well vascularised, the inflammatory reaction persisted along with the endometrial glands.

In group E2, that received Ulipristal for 8 weeks we analyzed its effect over the morphology of the implanted uterine horn. We observed a severe atrophy of the uterine wall, which was much thinner than in M2 group. In the endometrium the stroma is very poor and the endometrial glands are in small number and atrophied (Figures 7, 8).

Measurements and statistical data processing

In order to compare the effect of the treatment over the two groups (induced endometriosis with treatment and without treatment) the volumes of the surgically induced endometriosis were determined using the box volume formula V=l x h x w, where the length (l), the height (h), and the width (w) were measured on the sacrificed rats as shown in the following table. All geometrical dimensions are given in millimeters thereby all volumes will be obtained in cubic millimeters, while the M, mass units are in milligrams. For each variable we calculated the mean and standard-deviation (Table 1).

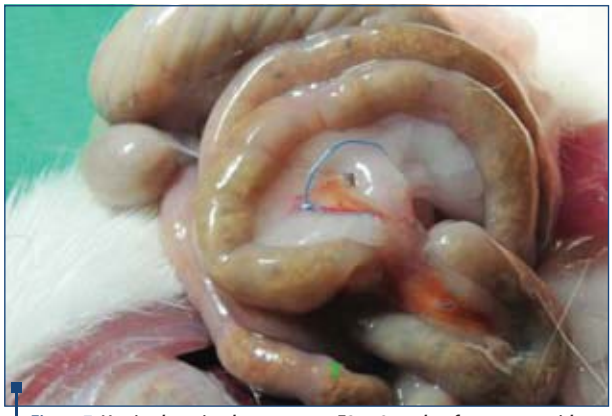

Figure 7. Uterine horn implant at group E2 at 8 weeks of treatment with Ulipristal macroscopic aspect

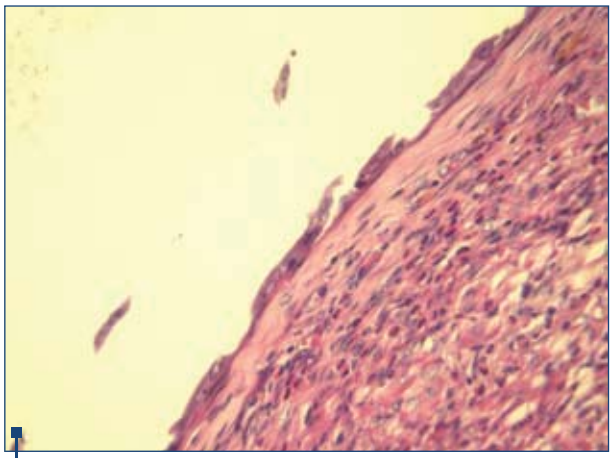

Figure 8. Uterine horn implant at group E2 at 8 weeks of treatment with Ulipristal microscopic aspect H&E(x200)

Table 1 Comparison of the posttreatment measurements of volumes and masses in the control and Ulipristal-treated groups

	Control group (n=20)	Ulipristal group (n=20)	P value
Volume (mm^3)	120,7±27,8	56,4±18,7	< 0,01
Mass (mg)	150,975±28,7	69,4±22,8	< 0,01
Values are mean ± standard deviation.			

The P value obtained for volumes is under 0.01 as well as for the masses, the test is "highly significant", so the decrease of the volumes and masses in the case of the treatment did not happen by chance.

Discussion

The ideal medical treatment for endometriosis has yet to be developed. Our study investigated the effect of Ulipristal, a selective progesterone receptor modulator, on surgically induced endometriosis in a rat model. In this experimental, prospective, controlled, randomized study, the volume and weight of implanted uterine horn decreased significantly after 8 weeks of treatment.

In both cases, volumes and masses, the treatment reduced the average values and this means that the treatment is effective (P=0.01).

The decrease of the mass average is less significant compared to the volume because the measurement accuracy for the mass was 1 mg while that of the distance was of 1 mm. Masses were measured more precisely as this is a direct measurement affected only by the precision of the balance, while volumes are determined by a formula where each length is affected individually, then the three values are multiplied.

In this study we used CDB-2914 a novel SPRM that compared with RU-486, a well-known and widely used progesterone antagonist, shows improved specificity and efficacy due to its lower antiglucocorticoid activity and better binding affinity to progesterone receptors[14,15]. According to our knowledge at this moment no clinical or experimental study was published about the effect of Ulipristal on endometriosis.

The progesterone receptor modulator Mifepristone acts as an abortifacient and postcoital contraceptive in women 16 and when given chronically it also ameliorates the size of uterine leiomyomata and endometriomas, releasing the pain associated with endometriosis[17].

Ulipristal, marketed as EllaOne, has been recently approved as an emergency contraceptive due to its ability to delay ovulation beyond the life span of the sperm[18]. Also, Ulipristal under the commercial product Esmya, has been approved for the treatment of uterine leyomioma. The treatment consists of one tablet of 5 mg taken orally once daily for up to 3 months, and it should be started during the first week of a menstrual cycle. There is no data available on treatment with duration longer than 3 months or on repeat courses of treatment.

The benefits with Esmya are its ability to reduce fibroid-related bleeding, anemia and fibroid size. Ulispristal showed better efficacy compared to placebo (a dummy) at reducing bleeding and anemia, but only moderated efficacy with regards to fibroid volume reduction[19].

The effect of Ulipristal and related compounds in endometriosis are difficult to predict since the physiopathology of this disease remains unclear. Further more the effect on the endometriotic implants and on the eutopic endometrium can be different. The beneficial effect in endometriosis may be related to its antiproliferative effect as well as to its apoptosis-promoting effect[20]. We believe this is the principal mechanism of action of Ulipristal on the endometriotic lesions in this experiment.

In this study Ulipristal induced a reduction of more than 50% of the volume and masses of endometrial implants and the histological findings correspond to those described previously. It remains to be seen if the response of endometriotic implants to Ulipristal follows a dose depending manner.

Progesterone antagonists have been shown to reduce endometriotic lesions, onapristone (ZK 98 299, 2mg/day for 1 month) induced a 40-50% reduction of lesions while ZK 136 799, 0,4 mg/day induced a 63-75% reduction[21].

The regression of endometriotic lesions seems comparable to that obtained by other authors that evaluated the effect of dienogest and buserelin on experimental endometriosis in rats, but without the decrease in bone mineral density associated with GnRH[22,23].

Conclusions

The results presented are encouraging, hence they support continued experimental and clinical investigation of Ulipristal as a novel treatment for endometriosis. According to our knowledge at this

moment no clinical or experimental study was published about the effect of Ulipristal on endometriosis. Further studies are needed in order to establish the correlation between the dose, treatment duration and effect of regression on the endometriotic implants. If confirmed in humans this potent selective progesterone modulator, with increased clinical applications, could also be an option in the treatment of endometriosis. ■

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