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Original Research

Histological pattern of leiomyomas after ulipristal acetate treatment in women: a case-control study

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Summary

Purpose of Investigation: Assessing cytological and extracellular matrix histological patterns of uterine leiomyomas, in women previously treated with ulipristal acetate (UPA). *Materials and Methods:* Patients underwent surgical intervention for uterine leiomyomas between 2017 and 2018 were screened for previous use of UPA and were considered as cases. Controls were matched for race and non-menopausal status among patients with surgeries for leiomyomas. Fibrosis, hyalinosis, edema, necrosis, cellularity, phlogosis, and mitosis were assessed in the specimens of fibroids by a semiquantitative score. The rates of scores for each histological pattern were compared among cases and controls, and described in a two-dimensional correspondence analysis. *Results:* There is not significance among scores between cases and controls fibroids, except than for the hyalinosis of moderate grade, that seems more likely in the ulipristal acetate group. The fibroids after ulipristals appear to have less mitosis and cellularity, while it seems to have more hyalinosis, edema and, in case of two cycles of ulipristal, fibrosis. *Conclusions:* Histological changes of leiomyomas after UPA therapy are heterogeneous. UPA mostly acts on extracellular matrix and, to a lesser extent, on histological parameters of smooth muscle cell proliferation.

Key words: Leiomyomas; Ulipristal acetate; Fibroids; Extracellular matrix; Histological pattern.

Introduction

Uterine fibroids (UFs) are tumors that originate from the smooth muscle cells of the myometrium. They affect more than 70% of reproductive-age women and 25% of pre-menopausal patients and are a cause of heavy menstrual bleeding, iron deficiency, pelvic pressure and pain, thereby worsening the quality of life [1, 2].

Therapeutic options for UFs include surgical interventions (hysterectomy or myomectomy), so that leiomyomas represent one of the main cause of hospitalization and the most common indication for hysterectomy in the USA, producing a heavy economic impact on healthcare systems [3-6].

The need of uterine-sparing and less invasive therapies, that may improve fertility outcomes [7] and reduce morbidity rate associated with surgery, drove gynecologists to apply for medical treatment as first-line approach. Beside hormonal methods, selective progesterone receptor modulators (SPRMs) represent a new pharmacological option which have been proven to be effective in the interference with the mechanism of tumorigenesis without inducing common GnRH-agonists side effects (hypoestrogenic state associated with significant menopausal symptoms, such as vasomotory symptoms and increased osteoporotic risk) [8].

Ulipristal acetate (UPA), a high-selective progesterone-receptors (PRs) ligand with both antagonist and partial agonist activity [9], has been proved to reduce leiomyoma volume, uterine size, and menstrual blood loss, thereby resulting in increasing serum hemoglobin levels, reducing pelvic pain, and improving the quality of life [10-14].

Specific and reversible histological endometrial effects of UPA *in vivo* are known [15], and the impact of the drug on the estrogen receptors (ERs), PRs-induced genes, and cellular proliferation rate have been studied *in vitro* [16]. On the contrary, less clear are the changes induced on UFs elements. Assays revealed that UPA changes the expression of genes involved in the production of extracellular matrix (ECM) proteins [17, 18] such as metalloproteinases (MMP) [19] and growth factors [20, 21], and influences cellular proliferation index and apoptosis [22]. The pathological pattern of the smooth muscular cells, ECM and vascular grid arranged in fibroids, have not been reported *in vivo* from a histological point of view.

The aim of this study was the *in vivo* assessment of the cytological and extracellular matrix histological patterns of uterine leiomyomas, in women who underwent surgery after a preoperative treatment with UPA.

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Table 1. — *Descriptive statistics and univariate analysis.*

	UPA (Cases: 14)	No UPA (controls: 15)	*p*
Age (median, limits)	45.5 (27-51)	46 (29-53)	n.s.
Body mass index (median, limits)	1.6 (1.55-1.80)	1.6 (1.57-1.75)	n.s.
Multiple UFs (crude number, rate)	3 (21.4%)	4 (26.7%)	n.s.
Sub-mucous UF (crude number, rate)	0	0	/
Intramural UF (crude number, rate)	12 (85.7%)	9 (60.0%)	n.s.
Sub-serosal UF (crude number, rate)	2 (14.3%)	6 (40.0%)	n.s.
*Volume mm^3 (median, limits)	229 (21-628)	113 (33-314)	n.s.
Fibrosis (crude number, rates)			
- absence	6 (42.9%)	5 (33.3%)	n.s
- low grade	4 (28.6%)	8 (33.3%)	n.s
- moderate grade	1 (7.1%)	2 (6.7%)	n.s.
- high grade	3 (21.4%)	0	n.s.
Hyalinosis (crude number, rates)			
- absence	3 (21.4%)	6 (40.0%)	n.s.
- low grade	3 (21.4%)	6 (40.0%)	n.s.
- moderate grade	7 (50.0%)	2 (13.3%)	0.05
- high grade	1 (7.1%)	1 (6.7%)	n.s.
Oedema (crude number, rates)			
- absence	1 (7.1%)	3 (20.0%)	n.s.
- low grade	5 (35.7%)	8 (53.3%)	n.s.
- moderate grade	5 (35.7%)	4 (26.7%)	n.s.
- high grade	3 (21.4%)	0	n.s.
Cellularity (crude number, rates)			
- absence	0	0	/
- low grade	1 (7.1%)	5 (33.3%)	n.s.
- moderate grade	8 (57.1%)	5 (33.3%)	n.s.
- high grade	5 (35.7%)	5 (33.3%)	n.s.
Phlogosis (crude number, rates)			
- absence	12 (85.7%)	15 (100%)	n.s.
- low grade	2 (14.3%)	0	n.s.
- moderate grade	0	0	/
- high grade	0	0	/
Necrosis (crude number, rates)			
- absence	14 (100%)	15 (100%)	n.s.
- low grade	0	0	/
- moderate grade	0	0	/
- high grade	0	0	/

** Volume has been calculated by multiplying three dimensions*0.5 [23].*

Materials and Methods

Patients underwent surgical intervention for uterine leiomyomas at the Obstetric and Gynecologic Unit of the Saint Anna University Hospital of Ferrara between January 1, 2017 and March 31, 2018 were screened for previous UPA use cross-linking information from medical chart and from the outpatient gynecological assessments. UPA was administered in-label according to Italian Government indication until the February 19, 2018, when the drug had not to be prescribed in Italy due to the need to collect more information on its side-effects. Prescription criteria of UPA during the recruitment period were the pre-operative therapy with one month or two months drug cycles in pre-menopausal women affected by symptomatic UFs. Five mg/die for one or two therapy cycles of UPA was administered.

After carefully screening of cases of patients underwent surgery for UFs and to whom the UPA was administered, the whole surgical database was screened looking from controls. In the same time frame in which cases were collected, controls were also collected basing on the Internationa Classification of Disease 9 (ICD-9) codes related to leyomiomas (from 218.0 to 218.9) reported in the on-theater surgical registry. As cases were all Caucasian and not menopausal, controls were matched excluding non-Caucasian and non-menopausal patients.

Indication for surgery, as a common policy of this setting, was overall assessed by general clinic interview to check symptoms of UFs, and by gynecological visit and transvaginal ultrasound (TV-US) evaluation. The gynecological US was finalized to record uterine volume, number, size and localization of the three major fibroids, following the Morphological Uterus Sonographic Assessment (MUSA) group criteria [23]. Patients with one or more leiomyomas were enrolled. At least one single leiomyoma of diameter ≥ 3 cm had to be detected, independently from its position, that made the women deserving from conservative (myomectomy) or demolithic (hysterectomy) surgery.

Women who took part in the UPA-sample, underwent regular clinical evaluation at the end of each treatment cycle (13^{th} and 29^{th} week, respectively), which comprehended the interview, the gynecological visit, and the TV-US scan. The evaluation was carried

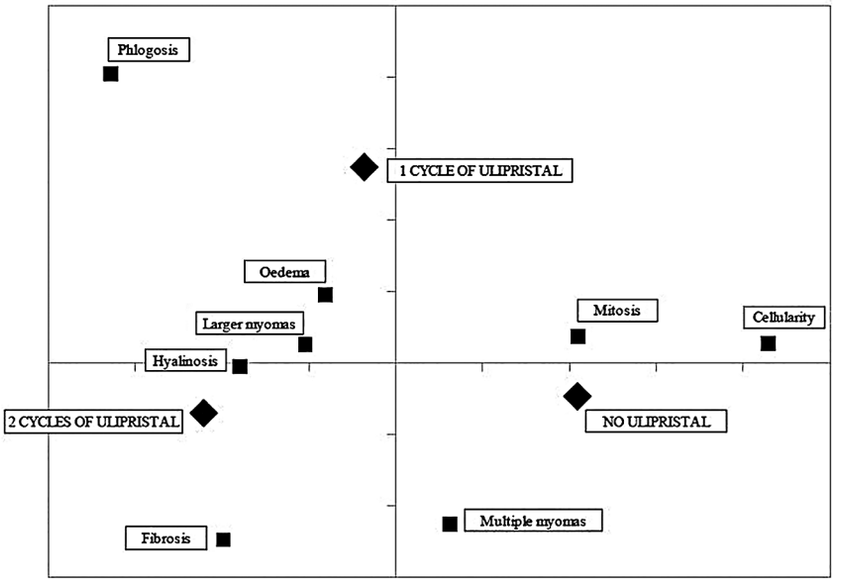

Figure 1. — Associations among myoma characteristics and ulipristal administration.

out with particular attention to the histological differences between UFs responder and not responder to UPA intake, assessed with US in term of tumor shrinkage. PEARL IV study [13] and its protocol extension [14] describes a reduction of the UFs volume of at least 38% after the first UPA cycle and of at least 54.1% after the second drug assumption cycle. Patients were classified as responders only if the percentage of shrinkage of the fibroids were more or equal to the 50% of the volume calculated before initiating the therapy.

Tissue samples, composed by leiomyomas, in case of myomectomy, or by the uterus, after hysterectomy, were immediately fixed in formalin overnight and transferred to the Department of Surgical Pathology, Sant'Anna University Hospital of Ferrara. After 24 hours, a macroscopic analysis of the specimens was finalized to primarily assess uterine and fibroids volume and appearance. Then leiomyoma nodules were sectioned into 1 cm-thick slices, embedded in paraffin and Haematossilin & Eosin stained.

The architectural analysis of the nodule was realized with optical microscope at ×2-4 magnification with the aim of describing areas of fibrosis, hyalinosis, edema, and necrosis. Mitosis count, cellularity, phlogosis was assessed at high-power (×40) field magnification. Every pattern was examined following four parameter scale: absence, low, moderate, and high-grade presence. All the pathological analysis were realized by a single pathologist who was blinded from the treatment with UPA.

Statistical analysis was drawn by comparing rates among case and controls of the modalities "absence", "low", "moderate", and "high-grade" for each pathological item (fibrosis, hyalinosis, edema, necrosis, cellularity, mitosis, and phlogosis). Fisher's test and Mann Whitney's test was used for univariate comparisons. To best describe the histological pattern of UFs in patients under UPA therapy, a two-dimensional correspondence analysis was built, assessing if UPA administration and other parameters of the fibroids (volume, number) are more related to histological parameters than no administration of UPA. SPSS 16.0 was used for calculations. *P* value was set at 0.05 for significance.

The Ethical Committee of Ferrara District approved the study. The original idea of the study was to collect data on UFs and UPA in a time frame larger than the one reported above. Because the Government temporary stop to the UPA administration in Italy has been noticed after the Ethical Committee approval, the authors also were forced to stop the study, accepting the risk of the beta error for a small sample size by reporting the results.

Results

During the time frame in which the study was conducted, 29 patients underwent surgery for UFs. Among them, 14 patients (cases) assumed UPA (four patients only a cycle; ten patients two cycles), and 15 did not assume UPA. Descriptive statistics were reported in Table 1. The median time from the last administration of the UPA to the surgery was 53 days (limits 9-463). Three patients had a volumetric reduction of the myoma size of more than 50% after two cycles of UPA, while one patient had a reduction of 41%, The remaining patients, have had a reduction of the myomas of less than 38% or have not had a reduction of the myomas or have had a growth of the myomas. Table 1 reports also univariate comparison among the two arms. There is not significance among histological parameters. Only the hyalinosis of moderate grade seems more likely in the UPA group.

Figure 1 depicts the associations among myomas characteristics and UPA administration after the two dimensional correspondence analysis. The squares are the items of pathological characteristics of the myomas along with the number of myomas (multiple myomas) and larger myomas (over the median size: 45.5 mm^3). The diamonds represents the items cycles of UPA: "no cycle", "1 cycle", and "2 cycles". The closer the squares are to the diamonds, the higher the association between the items represented by squares and diamonds is. "Edema" and "hyalinosis" are closer to the items "1 cycle" and "2 cycles" of UPA, while "fibrosis" is closer to the items "2 cycle" of UPA. "Mithosis" and "cellularity" are closer to the items "no cycle". "Larger myomas" is closer to the item "1 cycle" of UPA and "2 cycles" of UPA.

Discussion

This study reports the characteristics of pathological specimens of leiomyomas under UPA therapy and compares them to specimens of leiomyomas removed from patients who were not under UPA therapy before surgery. Morphological assessment of specimens of leiomyomas, described in a semi-quantitative way by the pathologist, does not result in a typical pattern in this small number of patients under UPA treatment. However, there is an overall trend to reduce cellularity and mithosis, by producing a variable amount of edema, fibrosis, and particularly, hyalinosis. This is what appears after univariate analysis and correspondence analysis. This behaviour can be biased by subjective assessment of the pathologist, despite subjectivity would be able to produce unrealistic differences between the two arms. Additionally, patients underwent surgical operation after UPA treatment would be more likely to be unresponsive to the drug, or they have already planned the surgery after UPA. The mild changes in pathological pattern are in agreement to what already reported by other immunhistochemical studies [17-19, 22]. The poor differences found in pathological patterns of leiomyomas found in this study lead us to feel that leiomyomas have an heterogeneous behaviour after UPA administration. Additionally, it seems that administering two cycles of UPA are more likely to produce pathological changes (Figure 1). This interpretation could explain why only one patients has had a reduction of more than 38% in the leiomyoma size and only three patients have had a reduction of more than 50%.

It has been reported that UPA could be an option for avoiding surgical operations if it would be administered for more cycles [24]. Currently, in Italy, UPA cannot be administered for more than one cycle for the risk of hepatic side effects and discontinuously in patients ineligible to surgery. Basing on such indication, it is difficult to hypothesize that UPA could be currently administered to the patients aiming to avoid surgeries. However, if UPA can be proven to be safe in selected patients, it can be proposed that the ideal candidate of a long-lasting therapy should be a patient with a wide volumetric reduction of the leiomyoma after one cycle. Such a policy could avoid unnecessary administration of UPA to all patients with leiomyomas for more cycles.

Conclusion

Histological changes of leiomyomas after UPA therapy is mostly on ECM and, to a lesser extent, to the smooth muscle cells proliferation parameters. However, the effect of the UPA seems heterogeneous, suggesting heterogeneous susceptibility of patients to the drug.

References

[1] Stewart E.A., Cookson C.L., Gandolfo R.A., Schultze-Rath R.: "Epidemiology of uterine fibroids: a systematic review". *BJOG*, 2017, *124*, 1501.

[2] Ciebiera M., Lukaszuk K., Męczekalski B., Ciebiera M., Wojtyla C., Słabuszewska-Jóźwiak A., et al.: "Alternative oral agents in prophylaxis and therapy of uterine fibroids — an up-to-date review". *Int. J. Mol. Sci*., 2017, *18*, 2586.

[3] Wise L.A., Laughlin-Tommaso S.K.: "Epidemiology of uterine fibroids: from menarche to menopause". *Clin. Obstet. Gynecol.*, 2016, *59*, 2.

[4] Williams A.R.W.: "Uterine fibroids - what's new?" *F1000Res.*, 2017, *6*, 2109.

[5] Sparic R., Mirkovic L., Malvasi A., Tinelli A.: "Epidemiology of uterine myomas: a review". *Int. J. Fertil. Steril.*, 2016, *9*, 424.

[6] Mazzon I., Favilli A., Grasso M., Horvath S., Di Renzo G.C., Gerli S.: "Is cold loop hysteroscopic myomectomy a safe and effective technique for the treatment of submucous myomas with intramural development? A series of 1434 surgical procedures". *J. Minim. Invasive. Gynecol.*, 2015, *22*, 792.

[7] Ali M., Al-Hendy A.: "Selective progesterone receptor modulators for fertility preservation in women with symptomatic uterine fibroids". *Biol. Reprod.*, 2017, *97*, 337.

[8] Kashani B.N., Centini G., Morelli S.S., Weiss G., Petraglia F.: "Role of medical management for uterine leiomyomas". *Best Pract. Res. Clin. Obstet. Gynaecol.*, 2016, *34*, 85.

[9] Ferrero S., Vellone V.G., Barra F.: "Pharmacokinetic drug evaluation of ulipristal acetate for the treatment of uterine fibroids". *Expert Opin. Drug Metab. Toxicol.*, 2018, *14*, 107.

[10] Donnez J., Tatarchuk T.F., Bouchard P., Puscasiu L., Zakharenko N.F., Ivanova T, *et al.*: "Ulipristal acetate versus placebo for fibroid treatment before surgery". *N. Engl. J. Med.*, 2012, *366*, 409.

[11] Donnez J., Tomaszewski J., Vázquez F., Bouchard P., Lemieszczuk B., Baró F., *et al.*: "Ulipristal acetate versus leuprolide acetate for uterine fibroids". *N. Engl. J. Med.*, 2012, *366*, 421.

[12] Donnez J., Vázquez F., Tomaszewski J., Nouri K., Bouchard P., Fauser B.C., *et al.*: "Long-term treatment of uterine fibroids with ulipristal acetate". *Fertil. Steril.*, 2014, *101*, 1565.

[13] Donnez J., Hudecek R., Donnez O., Matule D., Arhendt H.J., Zatik J., *et al.*: "Efficacy and safety of repeated use of ulipristal acetate in uterine fibroids". *Fertil. Steril.*, 2015, *103*, 519.

[14] Donnez J., Donnez O., Matule D., Ahrendt H.J., Hudecek R., Zatik J., *et al.*: "Long-term medical management of uterine fibroids with ulipristal acetate". *Fertil. Steril.*, 2016, *105*, 165.

[15] De Milliano I., Van Hattum D., Ket J.C.F., Huirne J.A.F., Hehenkamp W.J.K.: "Endometrial changes during ulipristal acetate use: a systematic review". *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 2017, *214*, 56.

[16] Whitaker L.H., Murray A.A., Matthews R., Shaw G., Williams A.R., Saunders P.T., *et al.*: "Selective progesterone receptor modulator (SPRM) ulipristal acetate (UPA) and its effects on the human endometrium". *Hum. Rep.*, 2017, *32*, 531.

[17] Cox J., Malik M., Britten J., Lewis T., Catherino W.H.: "Ulipristal acetate and extracellular matrix production in human leiomyomas in vivo: a laboratory analysis of a randomized placebo controlled trial". *Reprod. Sci.*, 2018, *25*, 198.

[18] Courtoy G.E., Donnez J., Ambroise J., Arriagada P., Luyckx M., Marbaix E., et al.: "Gene expression changes in uterine myomas in response to ulipristal acetate treatment". *Reprod. Biomed. Online*, 2018, *37*, 224.

[19] Courtoy G.E., Henriet P., Marbaix E., de Codt M., Luyckx M., Donnez J., et al.: "Matrix metalloproteinase activity correlates with uterine myoma volume reduction after ulipristal acetate treatment". *J. Clin. Endocrinol. Metab.*, 2018, *103*, 1566.

[20] Ciarmela P., Carrarelli P., Islam M.S., Janjusevic M., Zupi E., Tosti C., *et al.*: "Ulipristal acetate modulates the expression and functions of activin a in leiomyoma cells". *Reprod. Sci.*, 2014, *21*, 1120.

[21] Ciebiera M., Włodarczyk M., Wrzosek M., Słabuszewska-Jóźwiak A., Nowicka G., Jakiel G.: "Ulipristal acetate decreases transforming growth factor β3 serum and tumor tissue concentrations in patients with uterine fibroids". *Fertil. Steril.*, 2018, *109*, 501.

[22] Courtoy GE, Donnez J, Marbaix E, Dolmans MM. In vivo mechanisms of uterine myoma volume reduction with ulipristal acetate treatment. *Fertil. Steril.*, 2015, *104*, 426.
[23] Van den Bosch T., Dueholm M., Leone F.P., Valentin L., Rasmussen C.K., Votino A., *et al.*: "Terms, definitions and measurements to describe sonographic features of myometrium and uterine masses: a consensus opinion from the Morphological Uterus Sonographic Assessment (MUSA) group". *Ultrasound. Obstet. Gynecol.*, 2015, *46*, 284.
[24] Olejek A., Olszak-Wąsik K., Czerwinska-Bednarska A.: "Long-term intermittent pharmacological therapy of uterine fibroids – a possibility to avoid hysterectomy and its negative consequences". *Prz. Menopauzalny*, 2016, *15*, 48.

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