RESEARCH ARTICLE

Eutopic/ectopic endometrial apoptosis initiated by bilateral uterine artery occlusion: A new therapeutic mechanism for uterus-sparing surgery in adenomyosis

Li Chen¹,², Caixia Li¹,², Jing Guo¹,², Ning Luo¹,², Xiaoyan Qu¹,², Le Kang¹,², Mingmin Liu¹,², Zhongping Cheng¹,²∗

¹ Department of Obstetrics and Gynaecology, Yangpu Hospital, Tongji University School of Medicine, Shanghai, PR China, ² Institute of Gynaecologic Minimally Invasive Medicine, Tongji University School of Medicine, Shanghai, PR China

* mdcheng18@263.net

Abstract

The objective of the present study was to investigate differences in the expression of apoptosis-related factors in the eutopic and ectopic endometrium (EuE/EE) in women with adenomyosis before and after laparoscopic bilateral uterine artery occlusion (LUAO). Ten patients with uterine adenomyosis who received LUAO were selected as the research subjects, from whom EuE and EE tissues were obtained before and after LUAO and detected for the expression of apoptosis-related molecules in EuE and EE by PT-PCR and Western blot, and changes in the mitochondrial structure by electron microscopy. Normal endometrial stromal cells (NESC), and EuE/EE stromal cells in women with adenomyosis were cultured in a 1% O2, 5% CO2 incubator to establish a physical anoxia state in an in vitro stromal cell model. The expression of apoptosis-related molecules was observed at 0, 6, 12, 24 and 48h of hypoxic. The results showed that the expression of apoptosis-related factors in EuE and EE were increased significantly after LUAO and under hypoxic conditions in vitro, suggesting that transient ischemia and hypoxia were involved in the apoptosis of adenomyosis lesions, and that uterine artery occlusion could remove adenomyosis lesions on tissue/cell level by cytoreduction, thus reaching the goal of treating adenomyosis effectively.

Introduction

Adenomyosis is a common chronic disease primarily diagnosed in childbearing females. It is defined as the presence of endometrial glands and stroma causing reactive hyperplastic or hypertrophic myometrium, surrounded by chronic inflammation in the endometrium [1,2,3,4]. Uterus-sparing surgery is the current trend in the treatment of uterine adenomyosis to enable women to preserve future fertility and avoid the impact of a hysterectomy on sexual function and mental discomfort. Several new methods and techniques have been tentatively used in treatment of adenomyosis, including uterine artery embolism (UAE), high frequency
ultrasound (HIFU), balloon endometrial thermoablation and hysteroscopic endometrial resection [5]. A study by Nijenhuis et al. [6] reported that UAE using polyzene F-coated hydrogel microspheres showed good clinical outcomes in 28 (97%) of their 29 patients with treatment-resistant adenomyosis, and hysterectomy was required in only one patient. UAE was used as a potential therapy for adenomyosis in all related publications from 1999 through 2010. Long-term data are available from 511 affected females from 15 studies, with an improvements rate of 75.7% (378/511) during a median follow-up period of 26.9 months [7]. Based on the UAE method, researchers have begun performing laparoscopic uterine artery occlusion (LUAO) for uterine fibroids with satisfactory outcomes [8,9,10,11]. From 2003 to 2005, we utilized LUAO combined with partial resection for the treatment of 182 eligible patients with symptomatic adenomyosis [12]. The result showed that the postoperative menstrual quantity was decreased significantly and the uterus volume was reduced by 58.3% in these patients during the 36-months follow-up period. Their health-related quality of life was also improved significantly as compared with that before treatment. Postoperative recurrence occurred in only three (1.7%) patients, for which hysterectomy was required. Our preliminary clinical practices have demonstrated that LUAO combined with partial resection for the treatment of adenomyosis is safe and effective, and the overall outcome is superior to that reported in the literature [13]. This technology has been incorporated into the uterus adenomyosis classification treatment as an independent operation [5].

Based on a series of LUAO treatments of uterine fibroids, we originally propose the hypothesis of ‘Single organ uterus shock’ in our previous study, which may ideally explain the UAO mechanism in theory [14]. After LUAO, the uterus and myomas underwent ischaemia and hypoxia. The uterus survived due to restoration of its blood supply, but the myomas ‘died’ due to lack of blood supply and the duration of hypoxia. During the process, the uterus underwent pathophysiological changes of hypoxia-reperfusion similar to the course of shock [15]. Our previous study showed that LUAO as one kind treatment of adenomyosis achieved satisfactory clinical efficacy. Nevertheless, the exact mechanism underlying LUAO remains to be further elucidated. In this study, we observed an apoptosis phenomenon in the eutopic endometrium (EuE) and ectopic endometrium (EE) in patients with adenomyosis after LUAO, and then established a hypoxia cell models to validate this phenomenon.

Materials and methods

Reagents

Antibodies for Apaf-1, CHOP, TRADD, Bcl-2 and Bax were purchased from Santa (Dallas, USA). Antibodies for Endo-G, Cyt-c, AIF, GRP78, Caspase3, Caspase4, Caspase8 and Caspase-9 were purchased from Abcam (Cambridge, USA). Antibodies for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were purchased from Cell Signaling Technologies (Danvers, USA). Secondary antibodies of goat anti-mouse FITC, goat anti-rabbit HRP and goat anti-mouse HRP were purchased from the Beyotime Institute of Technology (Shanghai, China).

Subjects

Ten patients with uterine adenomyosis who received LUAO combined with partial resection of adenomyosis were recruited in this study. The preoperative diagnosis of adenomyosis patients based on typical clinical symptoms of dysmenorrhea and/or menorrhagia and pelvic pain, physical examination findings, and imaging results including transvaginal ultrasound and MRI. The ages of the patients ranged from 34 to 45 years with a mean of 39.67±5.01 years. All control women and adenomyotic women had regular menstrual cycles (28–35 days). The
exclusion criteria were patients who were diagnosed with uterine fibroid and subsequently received hormonal therapy within the past 6 months; patients with reproduction tract infections or immune system and endocrine diseases; and patients whose preoperative hysteroscopy excluded the diagnosis of endometrial lesions. The phase of the menstrual cycle was the early proliferative phase. Fresh EuE and EE tissues before and after LUAO were collected, and EuE and EE tissues obtained before LUAO were used as control. Part of them were stored at 4°C in phosphate-buffered saline (PBS) (100 U/mL penicillin, 100 μg/mL streptomycin added) and processed within 2–18 h, and the remaining part were stored at -80°C for RNA and protein extraction. In hypoxia cell models experiment, normal cell control was from patients who voluntarily required to place intrauterine device (IUD) excluding other diseases, without the use of hormone drugs nearly 3 months. Samples of endometrium obtained by pipelle or curette before place the IUD were cultured. Details of the LUAO procedure and the sampling time were recorded. All cases included in this study were confirmed by postoperative pathology.

**Ethics statement**

The study was approved by the Ethics Committee of Yang-Pu Hospital, Tongji University School of Medicine (Shanghai, China; Registration No.: LL-2014-WSJ-006), and in accordance with the tenets and guidelines of the Declaration of Helsinki. All patients provided written informed consent.

**Electron microscopy reveals histomorphological changes of EuE and EE after LUAO**

EuE and EE samples were fixed in glutaric acid and osmic acid, dehydrated with pyruvic acid, epoxy resin embedding, sliced into ultra-thin sections, stained with uranyl and lead citrate, and finally observed under an electron microscope for mitochondrial morphological changes and cell apoptosis such as vague or disappearing mitochondria cristae, mitochondrial swelling or cavity changes.

**Real-time polymerase chain reaction (PCR)**

Liquid nitrogen-preserved tissues were homogenized, and total RNA was extracted using the TRizol method (Invitrogen, CA, USA). A reverse transcription kit was used to prepare cDNA. The following PCR primer sets were used in this study: Apaf-1, forward primer: 5’ TCTACTGCTGACAAG 3’, reverse primer: 5’ CACCGTTTGAGACATTCC 3’; CHOP, forward primer: 5’ CAGGAAACGGAACAGAG 3’, reverse primer: 5’ CACCATCCTGCAATCAG 3’; TRADD, forward primer: 5’ CCAGCCCTTTACAGTTTCAC 3’, reverse primer: 5’ GCCAGGCAAAGTGGATTCC 3’; Bcl-2, forward primer: 5’ AGACCGAAGTCCGCGAACC 3’, reverse primer: 5’ GAGACCCACACTGCCCTGTTG 3’; Bax, forward primer: 5’ AGCTGAGCCGAGGTCTCAAG 3’, reverse primer: 5’ TGGCCAGCCCATGATGTTTC 3’; Endo-G, forward primer: 5’ TGCTGCAAGGCCGCTTACCTTC 3’, reverse primer: 5’ GCCTCCAGGACTAGCCTTGG 3’; Cyt-c, forward primer: 5’ TTGCTGTGCGAGAAAGACC 3’, reverse primer: 5’ CAGGGGACTACACAGCTAAC 3’; AIF, forward primer: 5’ GCTACAGCAGCTCTTACAC 3’, reverse primer: 5’ GCCCAATCCTCGAGAAG 3’; GRP78, forward primer: 5’ CCGTCGCCAGAAGGTGTG 3’, reverse primer: 5’ CAGACCGCTGCAATCAG 3’; Caspase3, forward primer: 5’ GCTACTCAGGCTAGCTACAG 3’, reverse primer: 5’ AGGCTGAGCCAGGACG 3’, reverse primer: 5’ TGGCCAGCATCACACGACCTG 3’; Caspase4, forward primer: 5’ AATCGGACTGACTTTGAC 3’, reverse primer: 5’ AGCTATTGGCGACAGCGTGG 3’; Caspase8, forward primer: 5’ CTGGGAGGAAAGGTGG 3’, reverse primer: 5’ GGGTCGCTATGCTGTTTC
3', reverse primer: 5' TGCTAAGAGCCTGTCTGTC 3'; GAPDH, forward primer: 5' CACCCACTCCTCCACCTTTG 3', reverse primer: 5' CCACCACCCTGTTGCTGTAG 3'. The SYBR Green kit was used, and GAPDH served as an internal control. The mRNA expression level of the apoptosis-related genes Apaf-1, CHOP, TRADD, Bcl-2, Bax, Endo-G, Cyt-c, AIF, GRP78, Caspase3, Caspase4, Caspase8 and Caspase-9 in EuE and EE tissues following LUAO was detected by real-time PCR. The 2-ΔΔCT method was used to calculate the mRNA levels [16].

Western blotting analyses

Western blotting analyses was performed to detect protein expression. Primary antibodies were incubated overnight with the appropriate primary antibody and secondary antibody. Finally, the optical density of protein signal strength was determined, using GAPDH as an internal standard to determine the protein level. The primary antibodies included anti-rabbit Bcl2 (PBST 1: 200, Santa Cruz Biotechnologies), anti-rabbit Bax (1: 200, Santa Cruz Biotechnologies), anti-rabbit cyt-c (1: 1000, Cell Signaling Technology), anti-rabbit AIF (1: 1000, Abcam), anti-rabbit caspase3 (1: 1000, CST), anti-rabbit caspase 4 (1: 1000, Abcam), anti-rabbit Caspase 8 (1:2000, Abcam), anti-rabbit Caspase 9 (1: 1000, Abcam), anti-rabbit EndoG (1: 2000, Abcam), anti-rabbit Apﬁ (1: 1000, Abcam), anti-rabbit GRP78 (1: 1000, Abcam, Cambridge, MA), anti-rabbit CHOP (1: 1000, CST), anti-rabbit TRADD (1:500, Abcam), and anti-rat GAPDH (1: 1500, CST).

Primary cell culture

Three samples of fresh EE in Hank’s Balanced Salt Solution (HBSS) containing double resistance (penicillin: 1000 U/ml, streptomycin: 1000 µg/ml) were obtained and rinsed 2–3 times. Next, 8 ml 0.4% collagenase (containing DNase I: 20 µg/ml) and 2 ml 0.05% pancreatic enzyme were added to the samples and digested at 37˚C with shaking for 2–3 hours. Then, the tissues were cultured in DMEM-F12 medium (10% FBS, 100 U/ml penicillin and 100 µg/ml streptomycin) in a 37˚C incubator. According to the adherent time differences of the stromal and epithelial cells, we obtained stromal cells. According to the identification of the cell morphology, stromal cells exhibited flat, spindle-shaped, fibroblastic-like morphology, and interstitial cell surface marker (vimentin) of the separated stromal cells, we established a separate cell culture system. Using this preparation method, three groups (EuE, EE and NE) were established[17]. Three patients with early proliferative phase of the menstrual cycle were used as the control group, from whom a small amount of endometrial tissue was obtained before the intrauterine device was placed.

Construction of the hypoxia mode

An in vitro stromal cell model was established, and then the experiment cells, including normal endometrial stromal cells (NESC), EuE stromal cells (EuESC) and EE stromal cells (EESC) were cultured in a 1% O2, 5% CO2 incubator to establish a physical anoxia state, and a normal oxygen (21% O2) condition for 0, 6, 12, 24 and 48h. Next, the cells were collected, and Western blot was performed to identify the protein expression of the main apoptosis-related factors Bcl-2, Bax, caspase-3, Endo-G, caspase 8 and GRP78. The obtained results were compared between the groups.

Statistical analysis

Using GraphPad Prism 4 (GraphPad software), data were expressed as the mean ± SD. Tissue samples were analysed using the paired t-test, and cell specimens were analysed using an independent t -test. P<0.05 was considered statistically significant.
Results

Electron microscopic observation of changes in EuE and EE before and after LUAO

Electron microscopy (30000×) revealed that EuE and EE tissue in adenomyosis underwent apoptosis after LUAO. The mitochondria cristae looked vague or disappeared with mitochondria swelling and a cavitation change. In addition, there was a nuclear chromatin edge and cell membrane reflex. The cytoplasmic concentration and the formation of apoptotic bodies were observed (Fig 1).

The mRNA expression of apoptosis-related factors in EuE and EE before and after LUAO

RT-PCR was used to detect the gene expression of Apaf-1, CHOP, TRADD, Bcl-2, Bax, Endo-G, Cyt-c, AIF, GRP78, Caspase3, Caspase4, Caspase8 and Caspase-9 in 10. As shown in Fig 2, the expression level of apoptosis-related factor Bax, AIF, caspase-3, caspase-4, caspase-8, caspase-9, Endo-G, GRP78, Apf-1, CHOP and TRADD was increased significantly in EuE and EE after LUAO (p<0.05). After LUAO, cyt-c gene expression was increased significantly in EuE (P < 0.005), but no significant increase in EE (P>0.05); the expression of apoptosis-inhibiting gene Bcl2 was decreased significantly in the EE after LUAO (P < 0.01), but no significant
decreased in EuE (P > 0.05), suggesting that LUAO could induce an apoptosis effect in EuE and EE.

Western blot results

Western blot was performed to detect the protein expression of apoptosis-related factors in EuE and EE tissues after LUAO in the 10 specimens. EuE and EE tissues obtained before LUAO were used as the control group, and those obtained after LUAO were used as the experimental group. The protein expression of Bax, AIF, caspase-3, caspase-4, caspase-8, caspase-9, GRP78, Apf-1, CHOP and TRADD was detected by Western blot. (Fig 3).

Validation of the protein expression level of the apoptosis-related factors in vitro

Primary NESCs, EuESCs and EESCs were cultured for in vitro experiments. The detailed procedures for the isolation, identification and cultivation of the NESCs, EuESCs and EESCs were described previously [18].

Western blot was used to detect the apoptosis-related factors. The result showed that the protein expression levels of apoptosis-related molecules (caspase-3, Bcl2, Bax, EndoG, caspase-8 and GRP78) in NESCs, EuESCs, EESCs was increased significantly at 0, 6, 12, 24 and 48 h of hypoxia in a time-dependent manner (Fig 4).

Discussion

In the present study, we used LUAO to treat patients with adenomyosis and observed changes of EuE and EE tissues by electron microscopy. The result showed that EuE and EE tissues underwent apoptosis after LUAO, presenting typical apoptosis characteristics such as swollen mitochondria, cavitation sample change, nuclear chromatin edge, cell membrane reflex, cytoplasmic concentration and the formation of apoptotic bodies. Then we found the expression of apoptosis-related factors in EuE and EE were increased significantly after LUAO and under hypoxic conditions in vitro.

The main purpose of the current clinical treatment of uterine adenomyosis is to remove the lesions, relieve the symptoms, reduce recurrence, spare the uterus and maintain fertility. By using LUAO combined partial resection, we have achieved good outcome in the clinical
Our practice and experience suggest that use of the ‘Single organ uterus shock’ model is safe and effective in the treatment of adenomyosis. Nevertheless, the action mechanism of this novel therapy has not yet been validated. We hypothesized that LUAO might involve a complex molecular mechanism. In our previous study [14,19], we preliminarily proposed a ‘Single organ uterus shock’ model with LUAO treatment of uterine fibroids, and supposed that the ischemia hypoxia uterus would experience a transient shock after LUAO, resulting in pathological changes, during which mitochondrial apoptotic pathways were initiated, and apoptosis occurred in myometrial cells due to ischemia hypoxia, which compensated for the uterine blood supply. As a result reperfusion began in the ischemic muscular tissue, which helped restore the physiologic activity gradually. As fibroids and smooth muscle tissue have different hypoxia tolerance, irreversible cell apoptosis and death only occurred in the fibroid tissue, and cells in the uterus still survived [14,19].

In the ischemic anoxia-induced intrinsic apoptotic pathway, mitochondria transmit different signals to induce stress, such as molecules from the Bcl-2 and Bax families, to maintain the stability of the membrane. When this balance is impaired, apoptosis-related proteins, such as...
Fig 4. Western Blot showing correlations between protein expression of the apoptosis-related factors. The protein expression of NESC, EuESC, EESC caspase-3 (A), Endo-G (C), caspase-8 (D), GRP78 (E) and Bax (F) was positive.
cyt-c, AIF, and Endo-G, are released from the mitochondria, and cyt-c is transferred into the mitochondrial electron transport chain and released into the cytoplasm where it can bind to Apaf-1 to initiate a downstream caspase cascade, activating apoptosis [20]. In addition, Endo-G, an AIF protein, can directly translocate into the nucleus, causing chromosomal shrinkage of DNA fragments, triggering apoptosis [21]. Thus, we selected Bcl-2, Bax, cyt-c, AIF, caspase-3, caspase-9, Endo-G, and Apaf-1 for analysis. In recent years, evidence has shown that endoplasmic reticulum stress was also involved in the apoptosis pathway, as GRP78 promotes apoptosis, and activated caspase-12 can be translocated into the cytoplasm, resulting in the initiation of the caspase-3 cascade response; CHOP can also directly signal through the nucleus in response to apoptosis [22]. Next, we detected GRP78 and CHOP expression and determined the expression of exogenous channel apoptosis-related proteins caspase-4, TRADD, and caspase-8. Analysis of protein expression revealed that the expression of Bcl-2, Bax, cyt-c, AIF, caspase-3, caspase-9, Endo-G and Apaf-1 was significantly increased, prompting mitochondrial apoptosis, which plays a major role in adenomyosis after UAO treatment, and inducing irreversible apoptosis in many cells, which is consistent with the mechanism shown in elsewhere [15]. It can be concluded that UAO mainly initiates the mitochondrial pathway, accompanied by the expression of endoplasmic reticulum stress factors, causing cellular apoptosis. Our previous research on LUAO treatment of hysteromyoma showed that there is more sensitivity in uterine fibroids in relatively smooth muscle tissue under hypoxic conditions, and more apoptosis, mainly involving the activation of the endogenous mitochondrial apoptosis pathway, occurs during endoplasmic reticulum stress, thereby releasing signalling molecules to activate the caspase signalling cascade, inducing apoptosis [19,23]. The results are consistent with our experimental conclusions.

We constructed a cell hypoxia model in vitro to determine the anoxic conditions at 0, 6, 12, 24 and 48h and selected apoptosis-related proteins in the mitochondria and endoplasmic reticulum to confirm the results. In CESC, EuESC and EESC cells, changes in the expression of caspase-3, Endo-G, caspase-8, GRP78 and Bax showed a positive correlation with the duration of anoxia, particularly for caspase-3, Endo-G and caspase-8 in adenomyosis. However, the expression of Bcl-2 was negatively correlated with hypoxia and inhibited expression. Cytological experiments with prolonged hypoxia confirmed the apoptotic changes and showed a significant difference. The cytology experiments also revealed that with prolonged hypoxia, more significant changes in apoptosis were observed, and these changes were more obvious in the adenomyotic EuE and EE than those in the normal endometrium, although the mechanism remains to be further elucidated. This phenomenon may be correlated with our LUAO solution because the main body combined surgical treatment had a good clinical effect in uterine adenomyosis. However, the effect of LUAO joint lesion resection treatment of adenomyosis on ovarian and reproductive function has been debated. In the early stage, we selected LUAO preoperative patients and followed them up postoperatively for 1, 3 and 6 months to assess the impact of FSH, LH, E2, and INHB (serum inhibin B) on ovarian function, and the results showed no significant difference [24].

In summary, Our study confirms that the EE has experienced more significant apoptosis after LUAO, which may clarify the precise mechanisms so as to lay a theoretical basis for the clinical treatment of the adenomyosis. However, there is still a need for further study of molecular mechanism. In addition, the comprehensive curative effect of this new method and its impact on ovarian function and fertility need to be confirmed in large multicenter randomized clinical trials.
Supporting information

S1 Table. The relative mRNA expression of apoptosis-related factors in EuE and EE before and after LUAO in 10 cases. E1/E2: EuE obtained before and after LUAO; A1/A2: EE obtained before and after LUAO.

S2 Table. Western Blot grey value of the protein expression of apoptosis-related factors in EuE and EE before and after LUAO in 10 cases.

S3 Table. Western Blot grey value of the protein expression of apoptosis-related factors in primary NESCs, EuESCs and EESCs at 0, 6, 12, 24, 48h of hypoxia in 3 cases.

Acknowledgments

We thank all the patients and families who participated in this study.

Author Contributions

Conceptualization: ZpC.
Investigation: XyQ MmL LK.
Resources: XyQ MmL LK.
Writing – original draft: LC.
Writing – review & editing: LC CxL JG NL XyQ LK MmL.

References

1. Benagiano G, Brosens I. History of adenomyosis. Best practice & research Clinical obstetrics & gynaecology. 2006; 20(4):449–63. Epub 2006/03/07.
2. Exacoustos C, Manganaro L, Zupi E. Imaging for the evaluation of endometriosis and adenomyosis. Best practice & research Clinical obstetrics & gynaecology. 2014; 28(5):655–81. Epub 2014/05/28.
3. Garcia L, Isaacscon K. Adenomyosis: review of the literature. Journal of minimally invasive gynecology. 2011; 18(4):428–37. Epub 2011/05/31. PubMed Central PMCID: PMCPMC4014226. PMID: 21622029
4. Zhang Y, Zhou L, Li TC, Duan H, Yu P, Wang HY. Ultrastructural features of endometrial-myometrial interface and its alteration in adenomyosis. International journal of clinical and experimental pathology. 2014; 7(4):1469–77. Epub 2014/05/13. PubMed Central PMCID: PMCPMC4014226. PMID: 24817942
5. Grimbizis GF, Mikos T, Tarlatzis B. Uterus-sparing operative treatment for adenomyosis. Fertility and sterility. 2014; 101(2):472–87. Epub 2013/12/03. PubMed Central PMCID: PMCPMC4289992
6. Nijenhuis RJ, Smeets AJ, Morpurgo M, Boekkooi PF, Reuwer PJ, Smink M, et al. Uterine artery embolisation for symptomatic adenomyosis with polyzene F-coated hydrogel microspheres: three-year clinical follow-up using UFS-QoL questionnaire. Cardiovascular and interventional radiology. 2015; 38(1):65–71. Epub 2014/04/03. doi: 10.1007/s00270-014-0878-1 PMID: 24692030
7. Popovic M, Puchner S, Berzaczky D, Lammer J, Bucek RA. Uterine artery embolization for the treatment of adenomyosis: a review. Journal of vascular and interventional radiology: JVIR. 2011; 22(7):901–9; quiz 9. Epub 2011/05/17. doi: 10.1016/j.jvir.2011.03.013 PMID: 21570318
8. Cheng Z, Yang W, Dai H, Hu L, Qu X, Kang L. Laparoscopic uterine artery occlusion combined with myomecectomy for uterine myomas. Journal of minimally invasive gynecology. 2008; 15(3):346–9. Epub 2008/04/29. doi: 10.1016/j.jmig.2008.01.005. PubMed Central PMCID: PMCPMC18439509
1. Liu WM, Ng HT, Wu YC, Yen YK, Yuan CC. Laparoscopic bipolar coagulation of uterine vessels: a new method for treating symptomatic fibroids. Fertility and sterility. 2001; 75(2):417–22. Epub 2001/02/15. PMID: 11172850
2. Liu WM. Laparoscopic bipolar coagulation of uterine vessels to treat symptomatic leiomyomas. The Journal of the American Association of Gynecologic Laparoscopists. 2000; 7(1):125–9. Epub 2000/01/29. PMID: 10648752
3. Dubuisson J, Ramyead L, Streuli I. The role of preventive uterine artery occlusion during laparoscopic myomectomy: a review of the literature. Archives of gynecology and obstetrics. 2015; 291(4):737–43. Epub 2014/11/14. doi: 10.1007/s00404-014-3546-4. https://doi.org/10.1007/s00404-014-3546-4 PMID: 25391639
4. Kang L, Gong J, Cheng Z, Dai H, Liping H. Clinical application and midterm results of laparoscopic partial resection of symptomatic adenomyosis combined with uterine artery occlusion. Journal of minimally invasive gynecology. 2009, 16(2):169–73. Epub 2009/03/03. doi: 10.1016/j.jmig.2008.12.003. https://doi.org/10.1016/j.jmig.2008.12.003 PMID: 19249704
5. Liu M, Cheng Z, Dai H, Qu X, Kang L. Long-term efficacy and quality of life associated with laparoscopic bilateral uterine artery occlusion plus partial resection of symptomatic adenomyosis. European journal of obstetrics, gynecology, and reproductive biology. 2014; 176:20–4. Epub 2014/03/22. doi: 10.1016/j.ejogrb.2013.11.014. https://doi.org/10.1016/j.ejogrb.2013.11.014 PMID: 24647206
6. Cheng ZP, Tao X, Gong J, Dai H, Hu LP, Yang WH. Early-stage morphological observations of myoma and myometrium after laparoscopic uterine artery occlusion treatment. European journal of obstetrics, gynecology, and reproductive biology. 2009; 145(1):113–6. Epub 2009/05/19. doi: 10.1016/j.ejogrb.2009.03.027. https://doi.org/10.1016/j.ejogrb.2009.03.027 PMID: 19447540
7. Yang W, Cheng Z, Yu J, Yang H, Liu Z, Ren Q, et al. Multicentre study to evaluate the clinical effects of laparoscopic uterine artery occlusion in combination with myomectomy to treat symptomatic uterine leiomyomas. European journal of obstetrics, gynecology, and reproductive biology. 2016; 204:9–15. Epub 2016/07/30. doi: 10.1016/j.ejogrb.2016.05.033. https://doi.org/10.1016/j.ejogrb.2016.05.033 PMID: 27471836
8. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods (San Diego, Calif). 2001; 25(4):402–8. Epub 2002/02/16. doi: 10.1006/meth.2001.1262.
9. Shevelky D, Shushan A, Ben Bassat H, Klein BY, Ben Meir A, Levitzky R, et al. Acetaldehyde differentially affects the growth of uterine leiomyomata and myometrial cells in tissue cultures. Fertility and sterility. 2009; 91(2):575–9. Epub 2008/02/29. doi: 10.1016/j.fertnstert.2007.12.001. https://doi.org/10.1016/j.fertnstert.2007.12.001 PMID: 18304535
10. Guo J, Chen L, Luo N, Li C, Chen R, Qu X, et al. LPS/TLR4-mediated stromal cells acquire an invasive phenotype and are implicated in the pathogenesis of adenomyosis. Scientific reports. 2016; 6:21416. Epub 2016/02/24. doi: 10.1038/srep21416. PubMed Central PMCID: PMCPMC4761971. https://doi.org/10.1038/srep21416 PMID: 26898650
11. Xie Y, Tao X, Cheng Z, Guan Q, Yang W, Zhu Y. Discrepancy of uterine leiomyoma and myometrium to hypoxia-induced endoplasmic reticulum stress after uterine occlusion therapy accounts for therapeutic effect. Archives of gynecology and obstetrics. 2014; 289(5):1039–45. Epub 2013/11/30. doi: 10.1007/s00404-013-3100-9. https://doi.org/10.1007/s00404-013-3100-9 PMID: 24287709
12. Jonas EA. Molecular participants in myometrial cell death channel formation during neuronal ischemia. Experimental neurology. 2009; 218(2):203–12. Epub 2009/04/04. PubMed Central PMCID: PMCPMC2710418. https://doi.org/10.1016/j.expneurol.2009.03.025 PMID: 19341732
13. Susin SA, Lorenzo HK, Zamzami N, Marzo I, Snow BE, Brothers GM, et al. Molecular characterization of mitochondrial apoptosis-inducing factor. Nature. 1999; 397(6718):441–6. Epub 1999/02/16. https://doi.org/10.1038/17135 PMID: 9989411
14. Brozzi F, Nardelli TR, Lopes M, Millard I, Barthson J, Igollo-Esteve M, et al. Cytokines induce endoplasmic reticulum stress in human, rat and mouse beta cells via different mechanisms. Diabetologia. 2015; 58(10):2307–16. Epub 2015/06/24. https://doi.org/10.1007/s00125-015-3669-6 PMID: 26099855
15. Yang W, Cheng Z, Dai H. Calcium concentration response to uterine ischemia: a comparison of uterine fibroid cells and adjacent normal myometrial cells. European journal of obstetrics, gynecology, and reproductive biology. 2014; 174:123–7. Epub 2014/01/11. https://doi.org/10.1016/j.ejogrb.2013.12.013 PMID: 24405728
16. Xu Q, Cheng Z, Yang W, Xu L, Dai H, Hu L. Controlled clinical trial assessing the effect of laparoscopic uterine arterial occlusion on ovarian reserve. Journal of minimally invasive gynecology. 2010; 17(1):47–52. Epub 2010/02/05. https://doi.org/10.1016/j.jmig.2009.10.001 PMID: 20129332