EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Proliferative Activity and Level of Steroid Hormone Receptors in the Myometrium and Myoma Nodes in Different Phases of Menstrual Cycle

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We compared proliferative activity and levels of steroid receptors in the myometrium and myoma nodes in patients with uterine myoma in different phases of menstrual cycle. Maximum proliferative activity was observed at the periphery of myoma nodes in the the secretory phase of the cycle. The content of progesterone receptors at the periphery and in the center of myoma nodes was lower in the proliferative phase of the cycle than in the secretory phase. Steroid regulation of proliferative activity in the myoma nodes in the the secretory phase through modulation of the content of progesterone receptors is hypothesized.

Key Words: *uterine myoma; myometrium; proliferative activity; progesterone receptors; estrogen receptors*

Uterine myoma is the most prevalent tumor of the reproductive system and the cause of interventions on the female pelvic organs [4].

Proliferative activity, one of the most important processes reflecting the function of cell population, can be objectively evaluated using ki-67 antibodies to nuclear protein of proliferating and mitotic cells [6].

The concept of predominant estrogen regulation of the uterine myoma growth was recently revised. The important role of progesterone in the initiation of molecular reactions during uterine myoma growth was proved, which necessitated revision of the philosophy of drug therapy in this condition. Steroid receptors play an important role in the regulation of myoma growth [5,12]. Differences in the expression of markers of different processes in the proliferative and secretory phases of the menstrual cycle can be indicative of their hormonal regulation. It was shown that proliferative activity in uterine myoma peaks in the secretory phase of the cycle [7,10].

We studied proliferative activity and content of steroid hormone receptors in the myometrium and different areas of uterine myoma in the proliferative and secretory phases of the cycle.

MATERIALS AND METHODS

The study was carried out in 23 patients of reproductive age (mean age 38.4±1.1 years). None of these patients received hormones for at least 3 months before the intervention. The patients with intramural intermuscular myoma of 3-7 cm in diameter were selected. The diagnosis of uterine myoma before surgery was based on clinical and ultrasonic findings and was confirmed by morphological findings in all cases.

The phase of the cycle was determined from the first day of the last menstruation, ultrasonic data, hormone level in peripheral blood, and (in some patients) results of morphological study of the endometrium.

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Before surgery the patients gave informed consent to the utilization of tissue biopsy specimens for scientific purposes.

Tissue specimens were collected from the same patients during laparoscopic myomectomy: specimens of the myometrium were obtained by biopsy, myoma nodes using the first perforating excoriation with a morcellator. The samples were immediately frozen in liquid nitrogen and stored until the study. In cases of multiple myoma biopsy specimens were collected from the largest node. According to pathomorphological findings, the samples represented myometrial or myoma node tissue of common structure without signs of necrotic or trophic changes.

Immunohistochemical analysis was carried out using avidin-biotin-immunoperoxidase method. Antibodies ki-67 (MIB-1, Immunotech) to prolipherating cell nuclear antigen served as the first antibodies, anti-murine biotinated antibodies (Vectastain®, Vector Laboratories) as second antibodies. Diaminobenzidine (Vector Laboratories) was used for specific staining. Hematoxylin was used for contrast staining. Human ovarian carcinoma cells served as positive control samples, samples incubated with 2% BSA in phosphate buffer instead of first antibodies served as the negative control. Stained cells were counted under a microscope (total number of stained cells in randomly selected 10 visual fields at ×400). Proliferative index was calculated as the percent of positively stained cells from the total number of cell nuclei.

Steroid receptors were detected in tissue specimens (85-115 mg) dissected from the bulk material at dry ice temperature with a cold scalpel. Estrogen (RE) and progesterone receptors (PR) in tissue homogenates were measured under standard conditions using monoclonal test systems for measurements of ER and PR (Abbott RE-EIA Monoclonal, Abbott RP-EIA Monoclonal, Abbott Diagnostica) in accordance with manufacturer recommenations.

Cytosol protein was measured using Bio-Rad Protein Assay (Bio-Rad). The results were analyzed using SPSS for Windows software. The intergroup differences were considered significant at $p<0.05$.

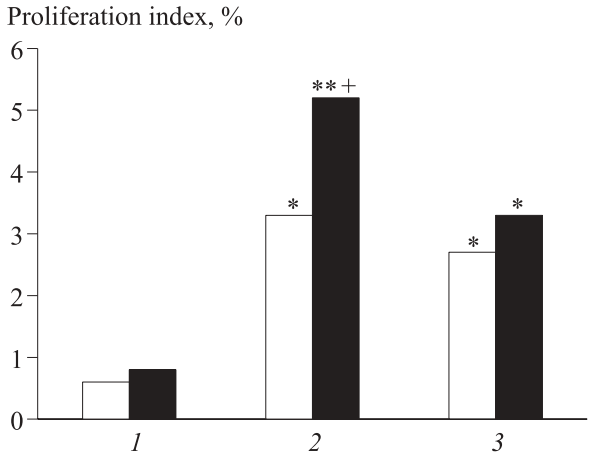

Fig. 1. Proliferation index in the myometrium, peripheral and central parts of myoma nodes in proliferative (light bars) and secretory phases (dark bars) of the cycle (%). *1*) myometrium; *2*) peripheral parts of myoma nodes; *3*) central parts of myoma nodes. *$p<0.01$, **$p<0.05$ compared to myometrium; $^{+}p<0.05$ compared to central part of myoma nodes.

The study was carried out at Research Center of Obstetrics, Gynecology, and Perinatology and at Department of Obstetrics and Gynecology, University of Uppsala (Sweden).

RESULTS

Proliferative activity in myoma nodes was higher than in the myometrium (Fig. 1). The detected differences were characteristic of both peripheral and central parts of myoma nodes and were present in both phases of menstrual cycle. Significant differences between the levels of proliferative activity in the peripheral and central parts of myoma nodes were revealed in the secretory phase of the cycle. Proliferative activity in the central and peripheral parts of the myoma nodes were in positive correlation in both proliferative ($r=0.6$, $p=0.02$) and secretory phases of the cycle ($r=0.6$, $p=0.01$).

The level of PR in peripheral and central parts of myoma nodes significantly decreased (Table 1). In the secretory phase the content of PR at the periphery of nodes positively correlated with their level in the myo-

TABLE 1. Content of ER and PR in the Myometrium, Peripheral and Central Parts of Myoma Nodes in Proliferative and Secretory Phases of the Cycle (*M*±*SD*, fmol/mg protein)

Cycle phase	Myometrium		Myoma parts			
			peripheral		central	
	ER	PR	ER	PR	ER	PR
Proliferative (n=15)	35.9±29.9	387.4±301.2	46.3±52.8	639.4±552.4	23.6±27.7	436.0±284.5
Secretory (n=8)	21.3±12.3	231.5±210.3	25.6±46.9	272.5±194.9*	18.6±26.7	215.5±152.9*

Note. *$p<0.05$ compared to proliferative phase of menstrual cycle.

metrium and in the center of the nodes (r=0.77, p=0.024 and r=0.72, p=0.043, respectively). The content of ER in the myometrium correlated with the content of PR in the myometrium and peripheral and central parts of myoma nodes (r=0.73, p=0.041; r=0.73, p=0.04, and r=0.91, p=0.002, respectively). A relationship between ER level in the center and at the periphery of myoma nodes was detected (r=0.73, p=0.041). Proliferative activities in central and peripheral parts of myoma nodes and in the myometrium were never compared. We first detected differences in proliferative activity at the periphery of myoma nodes in comparison with their center in the secretory phase of the cycle. The positive correlation between proliferative activity in the central and peripheral parts of myoma nodes and the absence of correlations between proliferative activity in the myometrium and myoma nodes suggest that proliferative processes in myoma nodes are to a certain extent autonomous.

Our data on high proliferative activity in myoma nodes in the secretory phase of the cycle and changes in PR level in myoma nodes are in line with the results of previous studies of the role of progesterone in the genesis of uterine myoma and suggest that regulation of progesterone and its receptors can serve as an obligatory components in conservative therapy of myoma. The best studied drugs used in the therapy of uterine myoma are gonadotropin releasing hormone agonists (GRHa) [1-3]. However, these drugs produce pronounced side effects determined by hypoestrogenia. Therefore the use of GRHa in patients with uterine myoma is limited by several months before surgery; they are also used in perimenopausal patients. Antiprogestines can become perspective drugs in the treatment of uterine myoma. It was shown that clinical efficiency of mifepristone is comparable to that of GRHa [11]. Mifepristone reduces the size of myoma nodes and blood flow in these nodes due to a decrease in the content of steroid receptors in vascular smooth muscles and endothelium. Clinical efficiency of antiprogestines was also demonstrated in other studies [8,9].

Hence, proliferative activity in myoma nodes is maximum at their periphery in the secretory phase of the menstrual cycle and is paralleled by reduction of PR content. These findings attest to possibility of using antiprogestines for the therapy of patients with uterine myoma.

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REFERENCES

1. N. I. Volkov, D. P. Kamilova, and I E. Korneeva, *Akush. Ginekol.*, No. 3, 49-50 (2002).
2. V. I. Kulakov, T. V. Ovsyannikov, M. N. Shilova, and N. I. Volkova, *Probl. Reproduktsii*, No. 3, 34-37 (1997).
3. N. F. Chavez and E. A. Stewart, *Clin. Obstet. Gynecol.*, **44**, No. 2, 372-384 (2001).
4. D. W. Cramer, *Semin. Reprod. Endocrinol.*, No. 10, 320-324 (1992).
5. K. Englund, A. Blanck, I. Gustavsson, *et al.*, *J. Clin. Endocrinol. Metab.*, **83**, 4092-4096 (1998).
6. J. Gerdes, L. Li, C. Schlueter, *et al.*, *Am. J. Pathol.*, **138**, No. 4, 867-873 (1991).
7. K. Kawaguchi, S. Fujii, I. Konishi, *et al.*, *Virchows Arch. A. Pathol. Anat.*, **419**, No. 4, 309-315 (1991).
8. A. A. Murphy, L. M. Kettel, A. J. Morales, *et al.*, *J. Clin. Endocrinol. Metab.*, **76**, No. 2, 513-517 (1993).
9. A. A. Murphy, A. J. Morales, L. M. Kettel, and S. S. Yen, *Fertil. Steril.*, **64**, No. 1, 187-190 (1995).
10. M. Nisolle, S. Gillerot, F. Casanas-Roux, *et al.*, *Hum. Reprod.*, **14**, No. 11, 2844-2850 (1999).
11. R. C. Reinsch, A. A. Murphy, A. J. Morales, and S. S. Yen, *Am. J. Obstet. Gynecol.*, **170**, 1623-1628 (1994).
12. X. Wu, *Molecular Mechanisms Involved in the Growth of Human Uterine Leiomyoma*, Stockholm (2002).