EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Tamoxiphene Therapy of Endometrial Adenomatosis in Patients of Reproductive Age

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Estrogen-dependent hyperplastic processes, significant increase of free estrogen index, and decreased concentrations of globulin binding sex steroids occur in the majority of patients with endometrial adenomatosis develop. Three-month therapy with tamoxiphene was highly effective. The data suggest a relationship between prostaglandins E and pathogenetic mechanisms of development of hyperplastic processes in the endometrium. Tamoxiphene in a daily dose of 30 mg for 3 or 6 months cured adenomatosis and other hyperplastic processes in the endometrium in the overwhelming majority of patients resistant to traditional hormone therapy.

Key Words: *endometrial adenomatosis; tamoxiphene; estrogen receptors; prostaglandins; sex steroid-binding globulin*

Increased incidence of endometrial cancer necessitates improvement of prevention, early diagnosis, and therapy of hyperplastic processes in the endometrium. Such processes are observed in women of all ages and in many cases anticipate endometrial cancer [13,14].

Special attention should be paid to frequently relapsing hyperplastic processes, particularly to their most grave forms, such as endometrial adenomatosis and atypical hyperplasia; their close relationship with invasive cancer of the uterus was noted in 25-50% of cases [1,16]. Today, conservative therapy of these diseases is ineffective: patients often develop relapses and forms resistant to gestagens.

Important role of steroid hormones in regulation of endometrial cell growth, differentiation, and proliferation has been demonstrated [3,8]. Endometrial hyperplasia, specifically, adenomatosis, can be caused by hormone imbalance in patients with hyperestrogenia or hyperandrogenia [5,14,18]. These disorders are observed in patients with chronic anovulation, polycystic ovaries, and hormone-active ovarian tumors [1,2,4]. Recent studies of tumor growth in endometrial cancer led to a hypothesis on the contribution of not only hormones, but also of other bioactive substances, namely, prostaglandins, peptide growth factors, and humoral and cellular immunity, to the pathogenesis of hyperplastic processes in the endometrium [6,7,10,17].

Our insufficient knowledge of pathogenetic mechanisms of endometrial hyperplasias is one of the causes of its ineffective treatment. Therefore, the search for new methods of treating recurrent endometrial hyperplasia, particularly of precancer states, proceeding from our knowledge of factors closely related to the pathogenesis of these processes, is an important task. We mean the use of antiestrogens for the treatment of these diseases.

Our purpose was to improve the treatment of endometrial adenomatosis in patients of reproductive age by using tamoxiphene.

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MATERIALS AND METHODS

Women of reproductive age (n=51, mean age 30.9± 0.9 years) with relapsing hyperplastic processes in the endometrium and adenomatosis, ineffectively treated with hormones (17-hydroxyprogesterone capronate, progesterone, and nonsteroid gestagens) were examined. Control group consisted of 11 age-matched women (mean age 28.6±0.7 years) without endometrial hyperplasia or ovarian dysfunction.

Tamoxiphene (daily dose 30 mg) was administered to patients with endometrial adenomatosis for 3 months. Initial state of the endometrium and cervical canal mucosa was examined before treatment and 3 weeks after the drug had been discontinued, when control hysteroscopy and selective diagnostic scraping of the uterus were carried out in all patients. On days 5-7 of the cycle before tamoxiphene and 3 weeks after the therapy, ultrasonic scanning of pelvic organs and hormone measurements were carried out. Basal levels of blood serum luteinizing hormone (LH), follicle-stimulating hormone (FSH), total 17β-estradiol, and testosterone and estradiol-binding globulin (sex steroid-binding globulin, SSBG) were measured. Free 17β-estradiol was estimated as described elsewhere [6,15] and free estrogen index by a previously described method [9]. Prostaglandins E were assessed in hyperplastic tissue after their extraction from tissue samples in acid medium (pH 3.5-4.0) with ethylacetate as described previously [11] and subsequent radioimmunoassay with Clinical Assays kits was carried out.

The content of 17β-estradiol receptors in the cytosol fraction of hyperplastic endometrial tissue before and after therapy was determined by protamine sulfate precipitation of the hormone receptor complex and expressed in fmoles of receptor-bound ligand of 1 mg total protein per cytosol sample. Hysteroscopy was carried out with a Storz hysteroscope at direct 5-fold magnification. Morphological status of the endometrium and endocervix was assessed by the method used at the Center of Obstetrics, Gynecology, and Perinatology. Serum hormones (LH, FSH, and 17β-estradiol) were radioimmunoassayed using CEA-IRE-Sorin kits, SSBG concentration was radioimmunoassayed using a Farmos Diagnostica kit.

RESULTS

Disorders of lipid metabolism were detected in the majority (76.6%) of patients. The mean body weight index was 29.0±2.09 *vs.* 19-25 in health. Thirty-six (70.5%) patients had hirsutism. Chronic anovulation was detected in all patients. Oligomenorrhea was revealed in 14 (27.5%), oligomenorrhea alternating with metrorrhagias in 32 patients (62.7%), and secondary amenorrhea in 5 (9.8%) patients. Forty-two (82.4%) women suffered from primary sterility and 9 (17.6%) from secondary sterility. Ultrasonic scanning revealed cystous-changed ovaries in 15 out of 51 patients (29.4%).

Results of primary hormone assays are presented in Table 1.

Despite significant differences in the mean concentrations of gonadotropic hormones, the LH/FSH index was higher in 15 (29.4%) patients. Mean concentration of total 17β-estradiol in patients' sera did not differ considerably from that in the control. On the other hand, serum SSBG concentrations were low in 86.3% patients. Its mean value in the patients was significantly lower than in the control (Table 1), and hence, free estrogen fraction was increased. Mean index of free estrogens was almost two times higher than in the control. Estradiol-17β receptors were found in endometrial tissue with signs of adenomatosis in 83.3% cases; in the control they were found in the overwhelming majority of women, but in significantly lower concentrations. The increase in the free estrogen fraction and 17β-estradiol receptors was paralleled by rise of prostaglandin E content in the endometrium of patients, in contrast to that in the controls.

From these data we can hypothesize that hyperestrogenia and estrogen-dependent hyperplastic process develop in the endometrium of the majority of patients with endometrial adenomatosis. This hypothesis prompted us to prescribe the antiestrogen tamoxiphene to women of reproductive age with endometrial adenomatosis.

Two types of menstrual reaction were observed during tamoxiphene therapy: absence of menses in 24 (47.1%) and regular or rare menses in 27 (52.9%) (groups 1 and 2, respectively). No side effects of therapy were observed.

Adenomatosis was arrested by 3-month tamoxiphene therapy in 46 (90.2%) women. In 29 (56.9%) women adenomatosis and all forms of endometrial hyperplasia disappeared, which was regarded as a complete morphological effect. Cases with cessation of adenomatosis and on-going hyperplasia were regarded as incomplete effect.

Histological analysis of the endometrium was carried out after a 3-month tamoxiphene therapy individually for each group (Table 2). In group 1, positive effect (disappearance of adenomatosis) was observed in 100% cases. Endometrial atrophy was detected in 15 (62.5%), uterine coating of uneven thickness with signs of secretory transformation in 2, and focal endometrial hyperplasia in 6 (25.0%) patients. One patient became pregnant after tamoxi-

TABLE 1. Initial Hormone Status of Patients with Endometrial Hyperplasia and of Controls (*M±m*)

Parameter	Control	Endometrial adenomatosis
LH, U/l	11.1±1.7	15.1±2.1
FSH, U/l	7.9±2.0	10.6±1.9
17β-estradiol, pmole/l	420.3±32.3	451.0±57.7
SSBG, nmole/liter	60.5±5.3	41.4±3.1*
Free estrogen index	0.6±0.05	1.1±0.13*
17β-estradiol receptors fmole/mg protein	93.7±7.5	139.1±12.4*

Note. *$p<0.05$ *vs.* control.

phene had been discontinued. Therefore, complete effect consisting in normalization of the mucosa and absence of hyperplasia was attained in 75% of patients in this group.

In group 2, positive effect (disappearance of adenomatosis) was observed in 23 (85.2%) out of 27 patients. Arrest of adenomatosis and all forms of endometrial hyperplasia was observed in 11 patients. Histological analysis of endometrial smears showed no atrophy in any of the 27 patients. Secretory changes in the mucosa were detected in 6 (22.2%), endometrial proliferation in 4 (14.8%), focal endometrial hyperplasia in 9 (33.3%), endometrial hyperplasia with focal adenomatosis in 3 (11.1%), and endometrial adenomatosis in 4 (14.8%) patients. Like in group 1, one patient became pregnant after the drug had been discontinued.

Tamoxiphene therapy was prolonged to 6 months for 22 patients with incomplete morphological affect. After 6 months, complete morphological effect was attained in 18 patients. Focal endometrial hyperplasia persisted only in 3 patients and focal adenomatosis in 1 patients, for whom surgery was proposed.

From histological analysis of endometrial smears after long tamoxiphene therapy we concluded that the best results are attained in cases when there is no menstrual reaction to the drug. Therefore, the absence of menses in such patients can serve as a prognostic criterion of the treatment efficacy.

Analysis of tamoxiphene effect and results of hormone measurements showed the following regularities. There were no essential changes in basal levels of gonadotropic hormones in the patients. In patients with complete morphological effects, the concentrations of total 17β-estradiol after 3-month therapy did not differ from the initial values (420.8± 21.3 and 412.8±18.8 pmole/l, respectively). Serum concentration of SSBG was increased (67.6±4.4 nmole/l) compared with that before therapy ($p<0.05$), and free estrogen index decreased to 0.60±0.06, which was virtually normal. 17β-Estradiol receptors were not detected in endometrial tissue of any of the patients after 3-month tamoxiphene therapy, except one patient. The content of prostaglandins E in hyperplastic endometrium decreased almost by 50% after therapy in comparison with initial values and became 5207± 327 and 9728±483 pg/g tissue ($p<0.05$). In contrast to group 1, in patients without effect or with incomplete effect SSBG concentrations did not increase after treatment (31.5±4.4 and 36.8±6.4 nmole/liter, $p<0.05$). The total 17β-estradiol concentration in the blood increased after tamoxiphene in some patients (405.1±10.4 pmole/liter before and 808.0±25.3 pmole/liter after therapy). A transitory increase in ovarian size was observed for 10-16 days.

These data indicate an estrogen-dependent course of hyperplastic process in the majority of patients with endometrial adenomatosis. A significant increase in free serum estrogens in patients with endometrial adenomatosis confirms this despite the absence of significant differences in blood concentrations of total 17β-estradiol in controls and patients. An in-

TABLE 2. Endometrial Status after 3-Month Tamoxiphene Therapy

Endometrial morphology	Group 1		Group 2	
	abs.	%	abs.	%
Fine mucous membrane of corpus uteri	15	62.5	—	—
Mucous membrane of irregular thickness with signs of secretory transformation	2	8.3	6	22.2
Proliferating mucous membrane	—	—	4	14.8
Focal endometrial hyperplasia	6	25.0	9	33.3
Hyperplasia with focal adenomatosis	—	—	3	11.1
Pregnancy after treatment	1	4.2	1	3.8
Endometrial adenomatosis	—	—	4	14.8

crease in free estrogen fraction may be associated with a decrease of SSBG concentration in patients with endometrial adenomatosis. High content of 17β-estradiol receptors in endometrial tissue in the majority of patients before treatment, probably permitting estrogen stimulation of hyperplastic processes through the receptor mechanism, is one more proof of the important role of hyperestrogenemia in the development of endometrial adenomatosis. Three-month therapy with tamoxiphene was effective in the majority of patients. However, analysis of the treatment efficacy and blood concentrations of 17β-estradiol and SSBG permits us to conclude that proliferative effect of estrogens can be blocked not only at the level of 17β-estradiol receptors in the endometrium, but also by estrogen binding to their main transporting protein in the blood, SSBG, whose concentration is increased under the action of tamoxiphene. This results in a decrease of free estrogen fractions capable of affecting target cells, specifically, endometrial. 17β-estradiol stimulates the production of prostaglandins in glandular cells of the endometrium. Prostaglandins, in turn, stimulate adenylate cyclase by increasing the content of cAMP and normally promoting mitosis. A relative decrease of prostaglandins E in endometrial tissue after tamoxiphene therapy suggests a relationship between prostaglandins E and pathogenetic mechanisms of development of endometrial hyperplasia. However, tamoxiphene not always exerted positive effect. Apparently, hyperestrogenemia does not play the leading role in hyper- and neoplastic processes in the endometrium in all patients, and other factors can be involved, specifically, polypeptide growth factors [7,12,17].

Thus, 3-month (or 6-month, if necessary) therapy with tamoxiphene led to disappearance of endometrial adenomatosis and other hyperplastic processes in the overwhelming majority of patients in whom traditional hormone therapy failed. Therapeutic effect of the drug was associated with changes in specific estrogen binding to receptor proteins in the cytosol fraction of the endometrium, a decrease of prostaglandins E in endometrial tissue, increase of SSBG concentration, and decrease of bioactive fraction of free estrogens in the serum.

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