

The combination of cryoendoscopy with the thymic hormones in treatment for uterine myoma and endometrial hyperplasia

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The paper is a clinical and experimental study. The experimental part was made up of 60 female guinea-pigs with endometrial hyperplasia induced by intramuscular introduction of hexestrol. Fifteen animals received a thymic hormone-thymalin that resulted in appearance of areas with the regular structures in the endometrium. The above fact enabled us to apply thymalin in clinic in treatment of 55 patients with uterine myoma and endometrial hyperplasia. Thymalin application resulted in the decrease of the number of after-effects after intrauterine cryosurgery improvement of the patients' general well-being and the decrease of the number of the disease relapses. We also obtained more steady and long-term normalization of the immune system indices. [Turk J Med Res 1993; 11(2): 70-74]

Key Words: Uterine myoma, Endometrial hyperplasia, T- and B- lymphocytes, FSH, LH, Progesterone, Estradiol, Thymalin

Nowadays, the advantage of the methods enabling a surgeon to preserve the organs is considered undoubtedly in all fields of medicine. One of these methods is cryosurgery, as the latter meets the above requirements (1). Cryosurgery is free of blood and free of pain, it involves a small number of after-effects and promotes normalization of the estrogen-receptor system of the endometrial cells (2). Cryosurgery now is the only method of treatment for hyperplastic uterine processes that does not suppress, but stimulates the immune system. Though cryosurgery does not greatly affect the dynamic characteristics of T-cells that are most important in determining the tenseness of the antitumoral immunity. Following the above-stated, humoral factors (hormones) of the thymus gland now are rising interest of the researchers. The principal feature of those hormones is the fact that they are produced by the epithelial cells of the thymus gland and do not require antigenic stimulation. The thymic hormones, particularly the Tp4 fraction, stimulate the synthesis of the luteinizing hormone (LH) and the LH-releasing factor, this fact testifying to close ties between the immune and endocrinal systems. It is also promising to study the possibilities of this preparation

application in treatment for uterine myoma and hyperplastic endometrial processes (3).

The objective of this paper is to study an immunomodulator thymalin as to its efficiency and mechanisms of its effect in experiment and in clinic in uterine myoma and endometrial hyperplasia and to compare its efficiency and mechanisms of its effect in its isolated application with those in its combination with cryosurgery.

MATERIALS AND METHODS

The experimental part was made up of 60 puberal female guinea-pigs ranging in weight from 350 to 420 gs. In the experimental animals, glandular endometrial hyperplasia was induced by means of subcutaneous injection of hexestrol butyric solution in 1 mg a week for 19 weeks (4). At the next stage, 15 animals with endometrial hyperplasia underwent intramuscular injections of thymalin daily for 5 days in 0.4 mg along with 0.2 ml sodium chloride solution; 15 animals received 0.2 ml sodium chloride solution at the same terms (the control group). As the experiment results were studied, the animals were exterminated under full anaesthetic.

At the same time, we examined and treated 55 patients who had uterine myoma and hyperplastic endometrial processes, their average age being 43.9 years. All the patients underwent colpo- and hysteroscopy, diagnostic curettage of the uterine mucosa with the subsequent histological examination of the scraping and ultrasonic scanning of the uterus and its ap-

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pendages. The assessment of the endocrinal pattern was based on the study of the rectal temperature graphs and measurement of the steroid hormones (LH, FSH, estradiol, progesterone) content in the blood serum by the radioimmune method. The dynamic condition of the immune system was assessed by measurement of the total number of T-lymphocytes, their active fraction (Tact), T-cells with receptors for rabbit's erythrocytes (Tab), T-cells containing receptors to Fc-fragment of immunoglobulin G (Ty - suppressors) and immunoglobulin M (Tji - helpers). The total number of B-cells and active B-cells were measured in the reaction of rosette formation. Immunoglobulins of the three main classes, i.e. A, M, G, were measured in the blood serum according to Mancini et al. (6). To perform the immunological and radioimmune examination, the blood was taken out of the cubital vein either on the 5th to the 7th and on the 20th to the 24th days of the menstrual cycle or, in the absence of the latter, at the same terms, considering the first day of the menstrual-like reaction to be the first day of the menstrual cycle. All the patients were divided into two groups. The first group comprised 30 patients. They underwent only cryosurgery with a cryosurgical probe making in diameter 4 mm. Cryosurgery with a cryosurgical probe making in diameter 4 mm. Cryosurgery was performed on the 20th to 24th day of the menstrual cycle after diagnostic curettage. Freezing of the uterine walls was either aimed or total depending on hyperplastic process localization. The second group comprised 25 patients. They underwent cryosurgery according to the above method and were also administrated an immunomodulator thymalin since the first day after cryosurgery. The assessment of general clinical effects was being made as treatment went on and also in 3, 6, 12 and 24 months after treatment in all the patients. The results obtained were treated according to the conventional methods of mathematical processing.

RESULTS

In the experimental animals, hexestrol resulted in deviations in the twin and azygous parts of the uterus and in development of glandular endometrial hyperplasia. This condition is characterized by a steep increase of the glandular crypt's relative volume at the expense of proliferation of epitheliocytes covering the crypts and dilation of the glandular lumens. The endometrial stroma was edematic. The lumens of most blood vessels were dilated some of them displayed thickened walls and thrombosis. There was found cyst-like dilation of the glandular crypt's lumen along with the areas of planocellular metaplasia of the columnar epithelium. It is worthwhile mentioning that the uterine mass of the experimental animals, initially making 2.0 ± 0.21 g, increased up to 3.2 ± 0.04 g after hexestrol ($p < 0.01$). Introduction of thymalin resulted in the decrease of the uterine mass down to 2.6 ± 0.17 g. Histological examination of the uterus found areas of regular morphological structure side by side with endometrial hyperplasia in the uterine mucosa. The

glands became shorter and tubular. Thymalin resulted in the decrease of the relative volume occupied by the glands by $18.6 \pm 3.2\%$ ($p < 0.05$) and the increase of the stroma volume by $18.4 \pm 5.3\%$ ($p < 0.05$). At the same time, the glandular crypt's lumens decreased more than twice ($8.1 \pm 0.8\%$, $p < 0.05$). They looked like narrow fissural spaces covered with epitheliocytes whose relative volume reduced by $10.5 \pm 1.2\%$ ($p < 0.05$). Animals with endometrial hyperplasia who received sodium chloride solution demonstrated no statistically reliable deviation of the endometrial morphological indices. Thymalin normalizing effect on the structure of hyperplastic endo- and myometrium in experiment enabled us to apply this preparation in clinic in treatment for uterine myoma and hyperplastic endometrial processes.

In our examination, we found high degree of hereditary aggravation in the first and the second generations in 55 (40%) women. Their relations mainly suffered tumours of different localizations. Uterine myoma and hyperplastic endometrial processes occurred in relations of every third patient. In colposcopy, we found leukoplakia in 12 women, endometriosis following electrosurgery of the cervix in five women, ectopy in three women and a zone of transformation of the cervix in nine women. The study of the endocrinal pattern demonstrated the presence of the anovular cycle in 19 patients and the ovulatory cycle in 36 patients. In all the women whose diseases progressed on the background of the two-phase cycle, there occurred the increase of FSH content at the first phase in comparison with the control group. In the women whose cycle was anovular, the similar pattern was observed in case uterine myoma was associated with endometrial hyperplasia. The deviations found in the hypophysial hormones involved the function of the ovaries, too, developing in violation of the ratio between estrogens and progesterone in all the patients who uterine myoma and endometrial hyperplasia.

At the second phase of the menstrual cycle, E2 content inclined to rise ($p < 0.05$) while that of progesterone simultaneously had a reliable drop ($p < 0.001$). The same ratio was observed in the patients with uterine myoma and endometrial hyperplasia associated with the anovular cycle.

According to the ultrasonic data, the average uterine sizes made 73.9 ± 2.55 mm and 64.6 ± 2.1 mm.

In hysteroscopy and in examination of the endometrial histological structure before treatment, in the group of patients who underwent cryosurgery we found, total hyperplasia in 21 patients, focal hyperplasia in three patients and endometrial polypi in six patients.

In the group of patients who underwent combined treatment, we found total endometrial hyperplasia in 20 women, focal hyperplasia in two women and endometrial polypi in three women (see Fig. 1,2. The data are given in percentage).

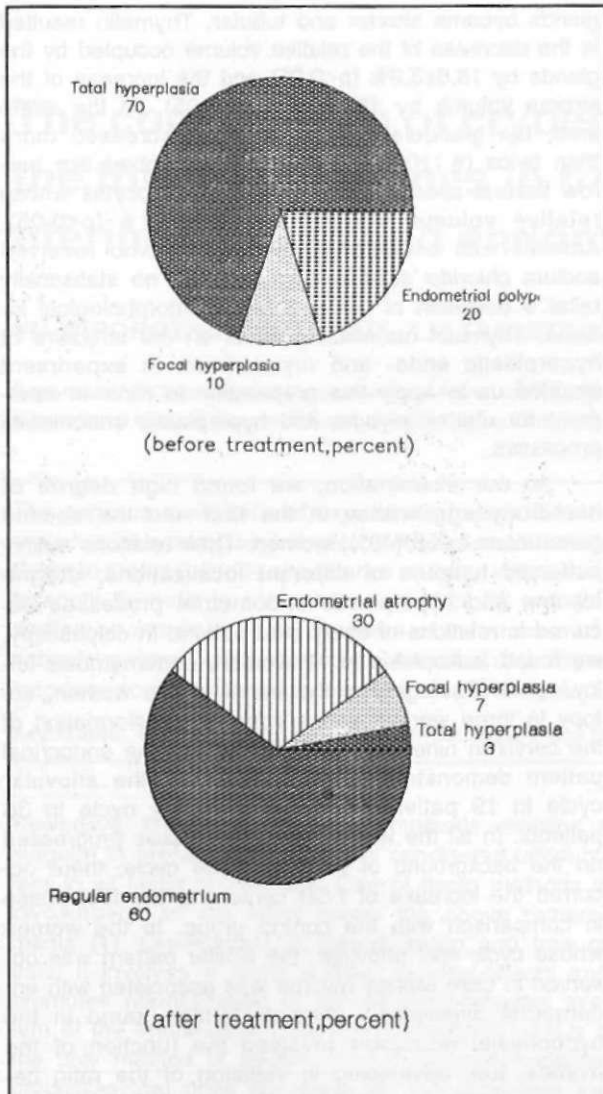


Figure 1. Endometrial morphology before and after cryo in uterine myoma and endometrial hyperplasia patients.

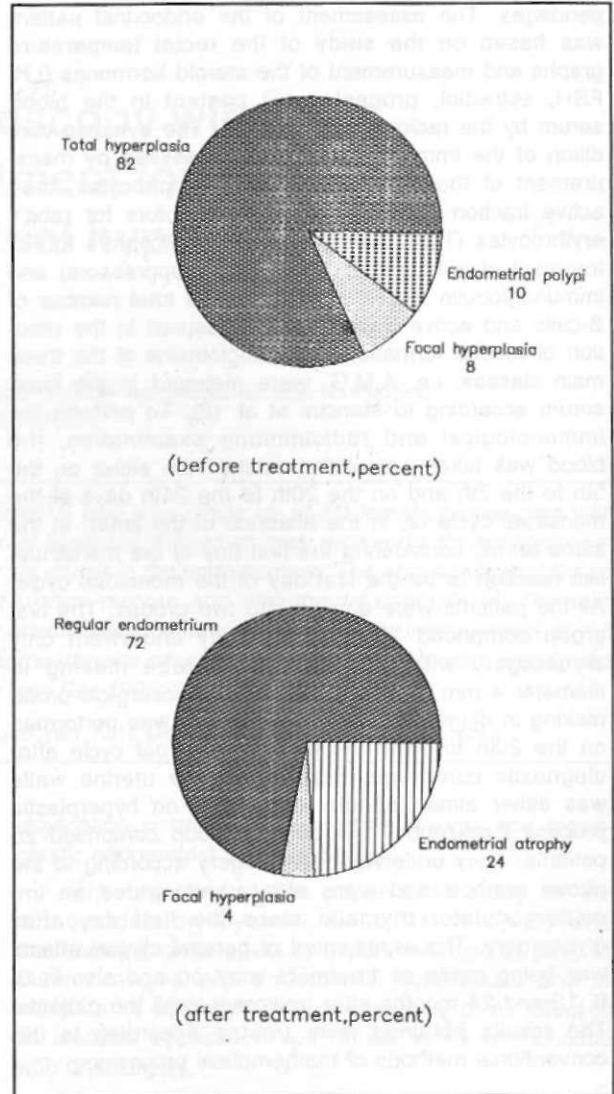


Figure 2. Endometrial morphology before and after cryo and thymalin in uterine myoma and endometrial hyperplasia patients.

The study of the condition of the T-cell system in patients having uterine myoma and endometrial hyperplasia associated with the preserved menstrual cycle showed the reliable decrease of the rate of the T-lymphocytes total number at the first as well as at the second phase of the cycle (from $53.8 \pm 3.9\%$ up to $69.6 \pm 4.8\%$ and from $49.8 \pm 4.1\%$ up to $71.7 \pm 5.8\%$, $p < 0.05$, $p < 0.01$ respectively). At the same time, the absolute content of these cells decreased at the first phase (2.76 ± 0.46 G/l and 1.5 ± 0.16 G/l, $p < 0.05$). The lack of balance between T-cells, helpers (Tu) chiefly, and T-cells, suppressors (Ty) chiefly, was also notable. In apparently healthy women, the ratio between absolute indices Tu/Ty ranges from 1.0 to 0.8 ($p < 0.01$) depending on the phase of the menstrual cycle, while in the patients this ratio ranges from 0.2 to 0.4 ($p < 0.001$) (see Fig. 3).

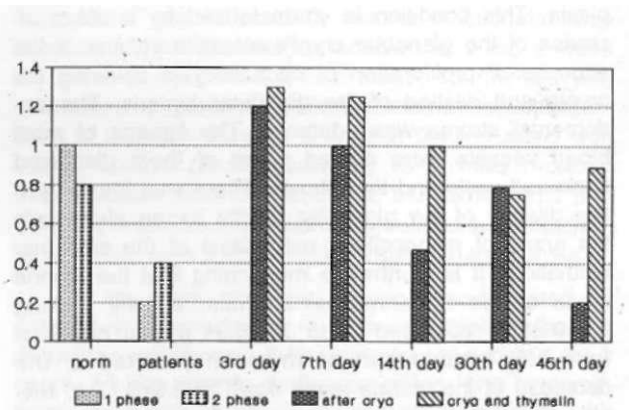


Figure 3. T_{μ}/T_{γ} index after cryo alone and cryo and thymalin in uterine myoma and endometrial hyperplasia patients.

Within the period of from the 3rd to the 7th day after intrauterine cryosurgery, the patients complained of weakness and indisposition which could be conditioned by the loss of the big amount of trace elements together with exudate, potassium in particular. In 13 of 30 patients, the temperature rose by 0.6 to 0.9°C and remained so for 5 to 7 days of the post-operative period. There were no such complaints by the 14th to the 16th day while the exudate discharge was over by the 28th to 30th day. It is also worth mentioning that, on the first day after cryosurgery, six women experienced spasm of the os uteri internum, exudate retention in the uterine cavity, the rise of temperature up to 37.5 to 38°C following it and aggravation of pains in the lower part of the abdomen. In 9 of 30 patients who had chronic inflammations of the adnexa uteri in anamnesis, there occurred aggravation of pains in the lower part of the abdomen and sensitive, enlarged appendages were felt in examination. Therapeutic measures undertaken in proper time, i.e. bougieurage of the cervical canal, administration of spasmolytic medicines in complex with anti-inflammatory therapy, enabled us to cope with these after-effects within 7 days.

Thymalin application considerably affected the degree of expression and frequency of appearance of complaints and after-effects in the patients. The women belonging to this group did not complain either of weakness or indisposition after surgery. At the same time, the amount of exudate reduced during the postoperative period. Their body temperature rose by 0.3 to 0.4°C on average. Of 25 women, just one experienced retention of exudate in the uterine cavity which required administration of subsidiary therapy. The stay in hospital reduced by 5 days in the patients of this group.

Though it should be mentioned that the patients displayed the following reactions to thymalin: in 5 minutes after the first injection, one patient experienced fever sensation without the rise of temperature, in one patient, who had chronic inflammation of the appendages, the temperature rose up to 37.2°C and remained so for 3 hours accompanied by aggravation of pains in the inflammation focus. One patient experienced giddiness in 10 minutes. After the second injection, two women experienced spasmodic pains in the lower part of the abdomen for 1 hour; pains in the area of the heart occurred in one patient in 30 minutes, those pains being over in 10 minutes; one patient experienced weakness and giddiness for 5 hours. There was noted no reaction after other injections.

In 3 months, uterine bleedings and smeary bloody discharge were over in all the patients. The regular menstrual cycle resumed in the women at the reproductive age. In 12 months, follow-up hysteroscopy and biopsy found relapses of endometrial hyperplasia after isolated cryosurgery in 10% of cases while after cryosurgery combined with thymalin, relapses were found in 4%.

The study of the hysteroscopic pattern and the histological structure of the endometrium after treatment demonstrated the presence of regular endometrium in 18 patients, atrophied endometrium in nine patients, relapses of focal hyperplasia in two patients and total hyperplasia in one woman in the group of patients who underwent cryosurgery alone.

In the group of patients who underwent cryosurgery together with thymalin, we found regular endometrium in 18 women, atrophied endometrium in six women and focal hyperplasia in one woman (see Fig. 1, 2. The data are given in percentage).

The study of the immune system condition in both groups showed the preferential effect of thymalin on the subpopulations of T-lymphocytes, i.e. T_p , T_y , A-RFC. Thus, in the women who underwent cryosurgery alone, there occurred a reliable increase of the rate of T_y -cells ($p < 0.001$) even on the 7th day of the post-operative period. However, the above increase became most significant on the 45th day, i.e. the absolute number of T_y -cells reached the value of 2.27 ± 0.53 G/l. The similar pattern was observed in $T_{a,b}$. In the group of women who received thymalin during the post operative period, the opposite situation could be seen, i.e. the number of both T_y and $T_{a,b}$ decreased. Though it should be noted that, in all the patients, there occurred simultaneous decrease of T_j -cells as well, the said decrease becoming reliable ($p < 0.01$) on the 7th day of the post-operative period in the group of women who received thymalin.

Following the above-stated, we studied the alteration of ratio between subpopulations T_p and T_y , this ratio being more demonstrative than comparison of the absolute values. On the third day of the post-operative period, this index got normalized in both groups of women (1.3 ± 0.1 , $p < 0.05$). Though in patients belonging to the first group, the 7th day was notable for the decrease of this value down to 1.0 ± 0.1 and this decrease went on steadily as further examinations were performed (on the 14th day - 0.47 ± 0.08 , on the 30th day - 0.79 ± 0.09 and the 45th day - 0.2 ± 0.02). In the women who received thymalin, the ratio T_p/T_y was equal to 1.25 ± 0.01 on the 7th day, 1.0 ± 0.09 on the 14th day, 0.75 ± 0.09 on the 30th day and 0.6 ± 0.08 on the 45th day (see Fig. 3). Side by side with it, there took place an irreversible decrease of the absolute number of cells forming rosettes with their own erythrocytes (A-RFC). It is worthwhile mentioning that, after cryosurgery and thymalin, the above decrease becomes reliable even on the 3rd day of the post-operative period ($p < 0.05$), while after cryosurgery alone, the reliability is obtained only on the 45th day ($p < 0.01$).

Therefore ten women received the repeated course of thymalin according to the previous scheme. All the patients noted the improvement of general well-being. Four women who had expressed premenstrual syndrome noted considerable improvement of their condition during 3 months after the repeated course of the preparation. Ultrasonic scanning found reduction of

the uterine sizes by 10.5 ± 1.1 mm on average in all the patients for a year after the preparation taking.

DISCUSSION

The search and study of the immunomodulators' characteristics as to their ability to render selective effect on some cells' subpopulations of the immune system is undoubtedly of great interest. The thymic hormones and thymalin, the latter being the extract of calves' thymus glands are among such substances. Thymalin positive effect on the reproductive system function is to conditioned by this preparation effect on both the central links of the reproductive system and its subordinate structures, the uterus in particular. This preparation renders the regulating effect on the ribosomes' function and on the ability of a body to preserve and restore the regulating mechanisms of protein synthesis (7.8).

The present paper is a clinical and experimental study. The experimental part was made up of 60 female guinea-pigs in whom hyperplastic endometrial processes were induced by introduction of hexestrol in 1 mg a week for 9 weeks. Part of the animals underwent injections of thymalin on the therapeutic purpose and thymalin was proved to render normalizing effect on hyperplastic structure of the endo- and myometrium right up to the appearance of the areas with regular morphology in the uterine mucosa.

The results obtained in experiment enabled us to apply this preparation along with cryosurgery in treatment for uterine myoma and endometrial hyperplasia. We examined 55 women having the above diseases. Thirty patients underwent cryosurgery alone and 25 patients underwent cryosurgery together with thymalin. Ten patients had the course of thymalin repeated in a month.

As has been mentioned above, most patients complained of their menstrual cycle disturbances. After our treatment, uterine bleedings and smeary bloody discharge were over in almost all the patients. The regular menstrual cycle resumed in the women at the reproductive age. In three patients whose cycle had been anovular the latter became ovulatory; in four patients who had had insufficiency of the lutein phase the cycle aquired its full value. In comparison with the control group, where the patients underwent cryosurgery alone, in the second group, thymalin application resulted in more expressed and long-term normalization of the immune system indices, especially the ratio Tu/Ty, the latter being principal in the tension of the antitumoral immunity. Moreover, thymalin application promoted reduction of the number of post-operative after-effects and remote relapses of the diseases and also shortening the periods of the patients' stay in hospital.

This result is likely to be obtained through several mechanisms of the preparation effect on both the immune and the endocrinal systems. On the one hand, it can render regulating effect on the intracellular

biochemical processes and expression of the differentiated antigens on the lymphocytes' surface and stimulating effect on the interferon production in a body. On the other hand, it renders inhibiting effect on the glucocorticoid function of the adrenal glands. One can also suppose that thymalin, as well as the other thymic hormones affects the function of the pituitary gland, stimulating the production of the luteinizing hormone and the luteinizing releasing-factor.

The results obtained made us consider the application of thymalin together with cryosurgery to be promising in the above pathologies.

Uterus myomu ve endometrial hiperplazi tedavisinde kryoendoskopi ve timik hormon kombinasyonu

Bu araştırma klinik ve deneysel bir çalışmadır. Deneysel kısmında intramüsküler hexestrol ile indüklenen endometrial hiperplazili 60 dişi kullanıldı. Bir timus hormonu olan timulin alan 15 hayvanda endometriumda düzenli yapılar içeren alanlar izlendi. Bu gözlem timulini uterus myomu ve endometrium hiperplazisi olan 55 hastanın tedavisinde kullanmaya yöneltti. Timulin uygulaması hastalarda cryocerrahiden sonra izlenen olumsuz etkilerde azalma, genel durumlarında iyileşme ve hastalık nükslerinde azalmayla neticelendi. Ayrıca immün sistem göstergelerinde de daha kararlı ve uzun süreli bir normalleşme elde edildi.

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