DOI 10.26724/2079-8334-2020-4-74-215-219
UDC 618.11-006.04-092.001.57

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STRUCTURAL CHANGES IN RATS' OVARIES AFTER HORMONAL THERAPY FOR PRECANCEROUS CONDITIONS

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Ovarian cancer is a principal cause of death and accounts for 25% among all gynecological tumors. The aim of this study is to establish efficacy of hormonal treatment for precancerous conditions in rats' ovaries. We used modified Biskind's method and made intramuscular injections of gonadotropin-releasing hormone agonist for precancerous conditions treatment. We described morphological changes in rats' ovaries in different terms of hormonal treatment. Thus, from the 30th day, this leads to total hydropic degeneration of ovarian cells or atrophy of the ovary. From the 60th day the treatment is still effective, however we can observe collection of luteal cells with normal cytoplasm. From the 90th day, it is no longer effective and doesn't lead to degeneration of cells, we observe follicular and lutein elements in the ovary with cellular atypism signs. Thus, from the 90th day, the ovarian tissue becomes resistant to hormonal influence and the treatment is ineffective.

Key words: precancerous conditions, ovary, Biskind`s model, hormonal therapy, gonadotropin-releasing hormone agonist, sex-cord tumor

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СТРУКТУРНІ ЗМІНИ В ЯЄЧНИКАХ ЩУРІВ ПІСЛЯ ГОРМОНАЛЬНОГО ЛІКУВАННЯ ПЕРЕДПУХЛИННИХ СТАНІВ

Рак яєчників є найбільш частою причиною смерті і становить 25% серед всіх гінекологічних пухлин. У попередніх дослідженнях ми описували морфологічні зміни в яєчниках щурів на різних строках (17, 27, 75, 120, 223 дня) після операції Biskind. Метою даного дослідження є встановити ефективність гормонального лікування передпухлинних станів в яєчниках щурів. Після двосторонньої оваріектомії ми пересадили один яєчник в селезінку і виконували внутрішньом'язові ін'єкції агоніста гонадотропін-рилізинг-гормону (бусерелін-депо 0,15 мг) для корекції передпухлинних станів. Ранній початок лікування (з 30-го дня) призводив до повної гідропічної дистрофії клітин яєчника або його атрофії. З 60-го дня після трансплантації яєчника, лікування все ще було ефективним, проте ми спостерігали скупчення лютеїнової клітин з нормальною цитоплазмою і ядрами серед клітин з гідропічною дистрофією. Пізній початок терапії (з 90-го дня) був неефективним і не призводив до дистрофії клітин - ми спостерігали фолікулярні і лютеїнові елементи в яєчнику з ознаками клітинного атипізму, а тканина яєчника ставала стійкою до гормонального впливу.

Ключові слова: передпухлинні стани, передракові стани, яєчник, модель Biskind, гормональна терапія, агоніст гонадотропін-рилізинг-гормону, пухлина статевого тяжу.

The study is a fragment of the research project "Changes in internal organs and regulatory systems in the conditions of experimental damage and historical aspects of histology, cytology and embryology development in Ukraine", state registration No. 0116U000121

Ovarian cancer (OC) is a principal cause of death that accounts for 25% of all types of gynecological tumors. According to data from International cancer agency, about 225 000 of new OC cases are registered every year and about 140 000 women die every year due to OC [6]. While cancer is considered to be multifactorial disease, the endocrine and metabolic disorders play an important role in tumor development from the glandular epithelium (e.g. tumors of endometrium, mammary gland, ovary) [7, 8, 10].

More than 32% of females do not survive more than 1 year after the diagnosis establishment, what is due to the following reasons: 1) the patients admitted for the treatment with III and IV stages of OC. Usually, disease is asymptomatic or appears with unspecific symptoms; 2) fast clinical manifestation and early beginning of metastatic process.

Biskind M.S., 1944 has confirmed the theory of hormonal cancerogenesis by developing a method of transplantation of one ovary on the spleen after bilateral ovaryectomy [5]. Thus, the transplanted ovary of the sterilized female rat was vascularized at the expense of the spleen and produced estrogens that fell into the vein and were inactivated in the liver without influence on the pituitary gland. As a result, the mechanism of negative feedback was violated, which provide to hyperproduction of gonadotropic hormones of pituitary gland and constant stimulation of follicular epithelium of the ovaries and formation of atypical cell complexes.

However, the question of carcinogenicity of gonadotropic hormones remains open because it was not possible to get ovarian tumors in the experiment by simple administration of gonadotropins, which is explained by the antigenic properties of these hormones [1].

Pathogenesis of OC includes following factors: metabolic (hypercholesterinaemia) and dishormonal disorders (hyperestrogenemia, disorders of hypothalamo-hypophisial system), disorders of compensation mechanisms (hyperconversion of androgens into estrogens in adipose tissue, muscles, hyperfunction of hypophisis during puberty period), disorders of immune function, genetic predisposition to cancer [2, 11]. Therefore, early diagnostics of OC is crucial for successful treatment.

The purpose of the study was to establish efficacy of precancerous conditions prevention in rats' ovaries by using gonadotropin-releasing hormone agonist, to study structural changes in rats' ovaries after treatment.

Materials and methods. The study carried according to ethical standards of" European Convention for the protection of vertebrate animals used for experimental and scientific purposes" (1985), and Directive EU No. 609 (1986).

The animals were divided into control group (10 rats without treatment), and experimental group (30 rats with treatment). The animals from both groups were operated according to Biskind`s model in our modification. After bilateral ovariectomy, one of the ovaries was transplanted to the spleen. In this case estrogens and progesterone, produced by ovarian tissue, enter the liver via portal vein. These hormones are inactivated in the liver and do not reach hypophysis and hypothalamus. Consequently, hypophysis and hypothalamus fail to determine the presence of the ovary in rat's organism. Increased level of gonadotropic hormones created conditions for ovarian tissue proliferation and precancerous conditions development [7].

On the 120th day we performed laparotomy for rats in control group and took ovaries for morphological investigation. The animals from experimental group received GnRH agonist (Buserelin-depo) 0.15 mg intramuscularly in different terms of postoperative period: on 30th day (1 time per month, totally 3 injections, subgroup II-1), on 60th day (1 time per month, totally 2 injections, subgroup II-2), and on 90th day (1 time, subgroup II-3). The laparotomy and excisional biopsy to the experimental group of rats were performed on 120th day after the surgery.

Preparation of the tissue sample: pieces of tissue 0.3–0.5 cm thick were cut out from the ovary and fixed in 10% neutral formalin solution. Alcohols of different concentrations were used to dehydrate the fixed material, gradually increasing from 50-70 to 100 degrees. Then the tissue was sealed in paraffin and impregnated with xylene. After impregnation of the object with liquid paraffin at the temperature of 55-56 ° C the tissue samples were allowed to harden at room temperature with paraffin in paraffin block.

In order to undertake a histological examination, histogram sections of 7 μm size were made from paraffin blocks, cut out on a HM 340E rotary microtome. To study the microscopic structure of the ovary, the sections were stained with hematoxylin and eosin.

The monoclonal antibodies were used to identify rats' tumor nature from control group without treatment, such as: Ingibin-alfa (DAKO, clon R1), Calretinin (DAKO, clon DAK-Calret), Melan A (DAKO, clon A103).

Results of the study and their discussion. In rats' ovaries from control group the following changes were observed on the 120th day of postoperative period: ovaries were enlarged, there were few cystic lesions with dark liquid inside (fig. 1). While cutting out ovary from the spleen we observed significant bleeding.

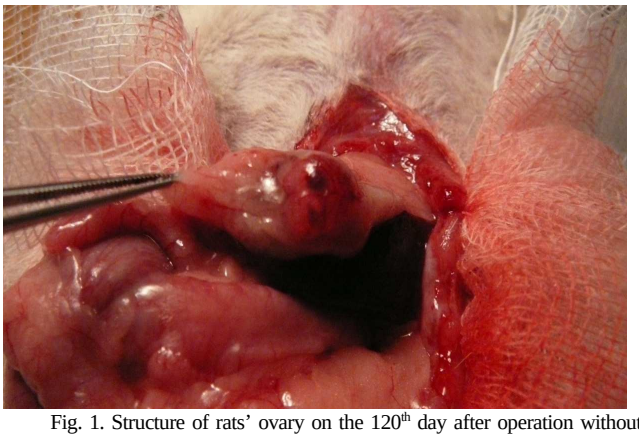


Fig. 1. Structure of rats' ovary on the 120th day after operation without treatment. Enlarged ovary with few cystic lesions inside.

Morphological examination revealed the following changes in the microstructure of the ovary in all animals of the subgroup: no differentiation between cortical and medulla substance. Ovarian tissue is represented by numerous luteal bodies in active and reduction phase. A follicular cyst was also detected in one rat from the same experimental group.

There was a difference in another animal, namely: numerous luteal bodies in reduction phase with large amount of hemosiderin in the cytoplasm of cells. The simple cysts were revealed in the ovarian parenchyma lined by the flattened epithelium. In addition, the follicular cysts with eosinophilic content were found. The development of follicular cysts on the 120th day of the postoperative period we associate with the operation performed in proestrus.

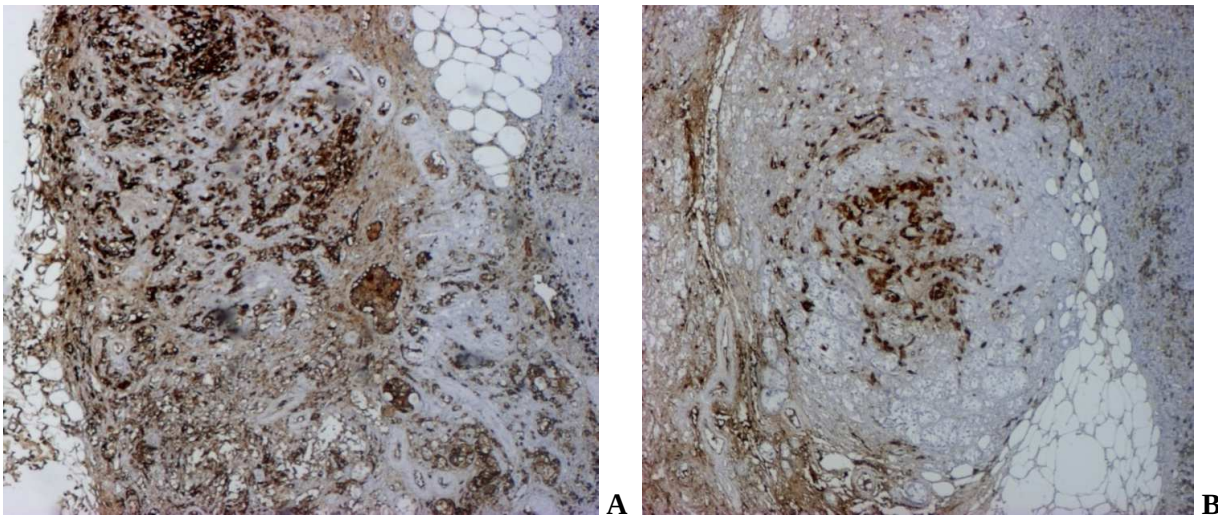
Nuclei has ovoid shape, are basophilic, clearly visualized, centrally located. Figures of mitosis are not detected. Hemosiderin deposits are found in the stroma of tumor cells. In the cytoplasm of some tumor cells, a similar pigment was detected.

The nature of the tumor was confirmed by immunohistochemical investigation: there was positive response to inhibin-alpha (DAKO, clone R1) and calretinin (DAKO, clone DAK-Calret 1) (fig. 2).

Inhibin-alpha is a heterodimeric protein that inhibits or activates pituitary FSH secretion. A positive response to inhibin-alpha can be found in Sertoli cells, granulose, adrenocortical and some other tumors. Calretinin is a calcium-binding protein that is structurally similar to inhibin and protein S100; is used for differential diagnosis of many tumors, particularly between sex cord tumors (Sertoli-Leydig ovarian tumors) from Sertoli-like endometrioid carcinoma.

A positive reaction to melan A (DAKO, clone A103) is observed as well. Melan A stands for Melanoma Antigen and is used for immunohistochemical confirmation of melanoma, differential diagnosis between adreno-cortical, renal cell tumors, and as an additional antibody in the diagnosis of sex cord tumors. In this case, there was Sertoli-Leydig tumor.

Immunochemical analysis confirmed that histological structure of tumor corresponded to tumor developed from Sertoli-Leydig`s cells: positive reaction with Ingibin alpha (DAKO, clon R1) and Calretinin (DAKO, clon DAK-Calret 1) in Sertoli`s cells; and positive reaction Melan A (DAKO, clon A103) with Leydig`s cells.



A **B**

Fig. 2. Microstructure of rats' ovary on the 120th day after operation without treatment. Positive response to Inhibin alfa (A) and Calretinin (B) in tissue sample, oc.10 ob.40.

Rats from Group II received injections of gonadotropic hormone agonist (0.15 mg GnRH agonist Buserelin-depo). Mechanism of GnRH agonist action is following: medication competes with the receptors of the cells of the anterior pituitary gland, what causes a short-term increase of sex hormones level in blood. Continued use of therapeutic doses of the drug results in complete blockade of gonadotropic function of the pituitary gland in 12-14 days, thus inhibiting the secretion of LH and FSH. As a result, we observe the inhibition of sex hormone synthesis in the gonads, which is manifested by a decrease in the concentration of estradiol in the blood plasma to post-menopausal values in women and a decrease in testosterone content to post-castration levels in men. Bioavailability is high, maximum plasma concentration is reached in 2-3 hours after intramuscular injection and is maintained at a level sufficient to inhibit pituitary gonadotropin synthesis for at least 4 weeks [7]. Ovaries of rat's in subgroup II-1 were significantly decreased in size and had features of atrophy. Microscopic examination revealed the disorder of cytoarchitectonics of ovarian tissue, differentiation into cortical and medulla layers is absent, no follicles in sample. The rat ovarian parenchyma is represented by a cellular infiltrate consisting of two degenerated cells populations: 1) granulosa-luteal cells are located in the center of tissue sample; 2) theca-luteal cells are localized peripheraly and have signs of hydropic dystrophy (fig. 3).

Dystrophy is a typical pathomorphological process, indicating ischemic, toxic cell damage, or early autolysis. Hydropic dystrophy is usually a reverse process and when the factor ceases, the cell recovers. On the basis of microscopy it is impossible to determine the degree of damage to the organelles of a cell in a state of hydropic dystrophy.

Dystrophy reflects impaired protein-water-electrolyte metabolism, accompanied by changes in colloid-osmotic pressure in the cell. Increased membrane permeability, disintegration of lipoprotein complexes, activation of lysosomal system enzymes may result in cell death (fig. 4).

Tissue atypia was characterized by absence of cortical and medullar parts of the ovary, formation of atypical collections of cells, large cysts with cylindrical and squamous epithelial lining, absence of folliculs. Cell atypia was characterized by cellular polymorphism: different sizes and shapes of cells, increased and hyperchromic nuclei, double-nuclei cells**.**

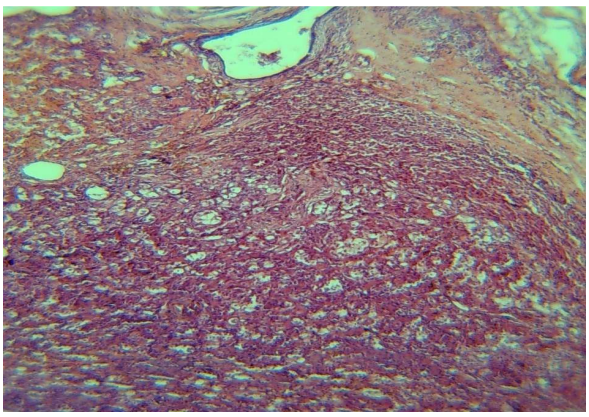


Fig. 3. Microstructure of rat ovary subgroup II-1 after 120 days of the experiment. Hematoxylin-eosin, oc.10, ob.40.

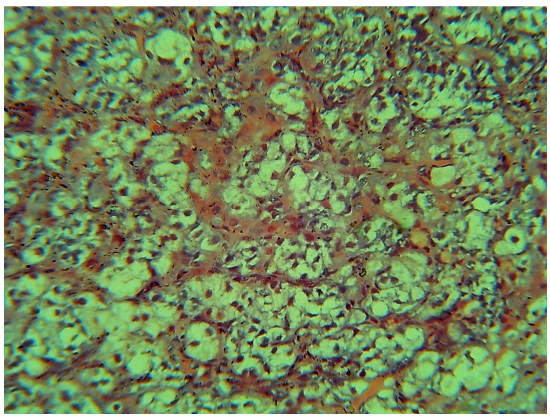


Fig 4. Microstructure of rat ovary subgroup II-2 after 120 days of the experiment. Hydropic dystrophy. Hematoxylin-eosin, oc.10, ob. 40.

In animals from subgroup II-3 (with late beginning of treatment - from 90th day), 2 variants of ovarian structure were observed during histological examination. In 6 of 10 animals, the ovarian parenchyma is represented by a cell infiltrate consisting of two degenerated granulosa-luteal cell populations in the center and theca-luteal cells peripherally. Part of the parenchyma is replaced by a cystic mass with an atrophic lining, cystic mass is surrounded by theca cells. Aslo we observed capsule around the ovary and connective-tissue septs going inside ovarian parenchyma and separating clusters of luteocytes.

In 4 out of 10 animals from subgroup II-3, the ovarian tissue was separated from the splenic pulp by connective tissue with lymphoid infiltration. In some places there was no clear boundary in the contact area between two organs. The ovarian parenchyma consists of luteal bodies, primordial, primary, secondary and atretic follicles. Some follicles have cystic component. A follicular cyst with eosinophilic content was found.

So, if drug administration is started lately, the prevention of precancerous conditions is not effective. In 4 of 10 animals, viable follicular and luteal elements were observed [4]. As shown above, follicular elements develop into follicular cysts and atypical cell complexes, and theca-granulosa cell tumors can develop from luteal bodies with a favorable hormonal background. At the same time, the histological picture of the ovaries 6 of 10 animals is similar to the histological picture of the ovaries from rats' subgroups II-1 and II-2. This situation probably occurs due to different phase of estrous cycle, when operation was performed.

Thus, with late onset of hormonal correction, atypical processes in ovarian tissue progress, follicular and luteal elements are present simultaneously. Microscopic examination revealed cytoarchitectonics abnormalities in all subgroups of experimental animals; the parenchyma of rat's ovary was represented by a cell infiltrate consisting of two degenerated granulosa-lutein cell populations located at the center with signs of hydropic dystrophy and atrophy. The hydropic dystrophy reflects a disorder of protein-water-electrolytic metabolism, which is accompanied by changes in colloid-osmotic pressure in the cell [7, 8]. We recorded single cystic formations with a flattened atrophied epithelial lining adjacent to the connective tissue strands that penetrate the parenchyma.

Long-surgery GnRH agonist can be used to correct the ovarian pretumor conditions, which are competitively associated with GnRH receptors and transitoryly increase the secretion of lutheinizing (LH) and follicle-stimulating hormones (FSH). In results, desensitization and decrease in the number of GnRH receptors of gonadotropic cells of pituitary gland, which leads to decreased synthesis and secretion of gonadotropins, and in the ovary provided of atrophic changes in the tissue.

So, concluding results of the experiment, GnRH agonist can be used for precancerous conditions development after operation according to Biskind model.

Conclusion

We demonstrated the development of precancerous conditions with tissue and cell atypia signs in rats ovaries on the 120th day after ovary transplantation to the spleen. In some cases histological and immunohistochemical picture was similar to Sertoli-Leydig cell tumors.

Usage of gonadotropin releasing hormone (GnRH) agonist is effective for precancerous conditions prevention in the ovary, if treatment is started early.

So, administration of GnRH agonist from 30th day after surgery leads to a decrease secretion of FSH and LH, which are required for proliferation of the structural components of the ovary. As result, degenerative changes of the ovarian cells (total hydropic dystrophy of cells) were observed. Hydropic dystrophy can probably be considered as a therapeutic pathomorphosis as response to GnRH administration.

If we start GnRF agonist administration after 90 day, therapeutic effect of the hormonal treatment is less likely. Follicular and luteal elements are present in the ovary and precancerous conditions develops.

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Стаття надійшла 12.10.2019 р.

DOI 10.26724/2079-8334-2020-4-74-219-223
UDC 611.12-034:591.33-092.9

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CARDIOGENESIS CHANGES AFTER THE PLUMBIC ACETATE IMPACT IN RATS UNDER THE CORRECTION CONDITIONS IN THE EXPERIMENT

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The purpose of the study was to determine the morphogenetic patterns of forming the effects of isolated plumbic acetate impact and the combined action of plumbic acetate with metal citrates on the development of the rat embryo's heart in the experiment. With isolated plumbic acetate administering to pregnant females in a dose of 0.05 mg/kg, the thickness reduction of the compact myocardium, walls of the right and the left ventricles in the embryo's heart occurs. The most sensitive to the plumbic acetate impact is the heart right ventricle wall, where not only the compact myocardium thinning, but also the increased number and diameter of the functioning vessels are observed. The combined administration of plumbic acetate with the gold citrate solution (or iron citrate/silver citrate) prevents the negative effect of plumbic acetate on the overall course of cardiogenesis in rat embryos under the experimental conditions and indicates their bioanagonism.

Key words: embryogenesis, cardiogenesis, plumbic acetate, gold citrate, silver citrate, iron citrate.

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ЗМІНИ КАРДІОГЕНЕЗУ ПІД ВПЛИВОМ ВАЖКИХ МЕТАЛІВ ТА ЗА УМОВ КОРЕКЦІЇ В ЕКСПЕРИМЕНТІ У ЩУРІВ

Метою дослідження було визначення морфогенетичних закономірностей формування ефектів ізольованого впливу ацетату свинцю та комбінованої дії ацетату свинцю з цитратами металів на розвиток серця зародків щурів в експерименті. При ізольованому введенні вагітним самицям ацетату свинцю в дозі 0,05мг/кг відбувається зменшення товщини компактного міокарду стінки правого та лівого шлуночків серця ембріонів. Найбільш чутливою до дії ацетату свинцю виявляється стінка правого шлуночку серця, де спостерігається не тільки витончення компактного міокарду, а й збільшення кількості та діаметру функціонуючих судин. Комбіноване введення ацетату свинцю з розчином цитрату золота (або цитрату заліза/цитрату срібла) попереджує негативний вплив ацетату свинцю на загальний хід кардіогенезу ембріонів щурів в експериментальних умовах та свідчить про їх біоантагонізм.

Ключові слова: ембріогенез, кардіогенез, ацетат свинцю, цитрат золота, цитрат срібла, цитрат заліза.

The work is a fragment of the research project "Biological foundations of the organ and animal morphogenesis under the nanometals impact in the experiment", state registration No. 0118U006635.

Despite the significant achievements in the diagnosis and treatment of many cardiovascular system diseases, there is a tendency for their growth in Ukraine, both in adults and children [4, 5]. Over the past 25 years, the cardiovascular pathology among the population of Ukraine has grown thrice, and according to the World Heart League, Ukraine occupies one of the first places among the European states in terms of mortality from blood diseases and strokes; in 2009, deaths from cardiovascular diseases amounted to 65.2% of the total number of deaths [2]. The results of numerous studies confirm that one of the etiopathogenetic causes of