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Oophorectomized Rat Fetal Tissue Transplantation Estrogenicity Index

Bekjanova Gulnara Markabaevna
Candidate of medical sciences Lecturer, Life safety center at the Ministry of Health of the Republic of Karakalpakstan

Summary: The article deals with the post-castration syndrome is characterized by a significant deficiency of estrogens and a violation of the mechanism of negative feedback between them and the hypothalamic-pituitary region.

Keywords: Syndrome, transplantation, tissues, radioimmunological method, estrogenicity, progesterone.

It is known that post-castration syndrome is characterized by a significant deficiency of estrogens and a violation of the mechanism of negative feedback between them and the hypothalamic-pituitary region. Unfortunately, despite the widespread use of hormone replacement therapy (HRT), the problem of treating this syndrome still remains quite urgent. This is due to the identification of a number of side effects and contraindications with the use of synthetic and natural estrogen-progestagenic drugs. In this regard, alternative methods that affect the receptors of not only estrogens, but also most CNS mediators may become more promising in the prevention and treatment of syndrome after ovariectomy. However, to date, not a single pharmacological or physiotherapeutic effect has been developed that could, without the addition of estrogens, have a complete therapeutic effect for all patients after ovariectomy. One of the contenders for this may be the recently proposed method of fetal tissue transplantation for such a purpose. Since we have not identified in the available literature data on the variety and number of fetal tissues used for transplantation in patients with post-castration syndrome, in this work we set ourselves the goal of studying the estrogenic saturation of the body of ovariectomized rats when they transplant placental, liver and brain tissue.

Material and methods. In the experiment, white female rats of a mixed population were used, which were ovariectomized through the abdominal incision a month before the start of the experiments. The animals were then divided into several groups of the first injected tissues of the placenta, the second-tissues of the fetal liver, the third-fetal brain, the fourth-complex of these tissues. Ethynyl estradiol was administered for 7 days in a separate group of ovariectomized animals. Rat fetuses were obtained on day 20 of gestation by Caesarean section. Brain, liver, and placenta fetus tissues were crushed in Hanks medium to sizes that allowed tissue fragments to pass through the injection needle. Cell suspensions were microscopic and their viability determined by trypan blue exclusion test. Estradiol and progesterone were also studied in the preparations by radioimmunological method.

To each test rat, depending on body weight (160-200 gr 200-240 gr), a corresponding tissue suspension obtained from 3 or 4 fetuses was injected into the muscular pocket made on the foot. To avoid fluid loss at the time of injection (excess pouring), under mild ether anesthesia, a small muscle pocket with a capacity of up to 1 ml was built in the outside of the thigh or front leg by cutting the skin and aponeurosis, as well as blunt extension of the muscle layers 0.5 cm lateral to the incision. After the suspension was injected into the pockets, the aponeurosis and skin were sutured.

Morphometric studies of the uterus of ovariectomized animals were performed one week after fetal tissue transplantation. The isolated uterus were weighed, tissue sections stained with hematoxylin and eosin, then viewed under a light microscope. Myometrial thickness and endometrial stroma as well as epithelial cell height were evaluated using a micrometer. Eosipophilic infiltrates in the endometrial stroma were calculated by the number of eosipophils in each field at an increase of x 80. Estrogenicity index was calculated by the sum of the following parameters converted on a scale from 1 to 7, uterine weight divided by 100 mg, eosipophil count, myometrial thickness and endometrial stroma, as well as epithelial height multiplied by 10 mm.

Results and discussion. Our choice of these three fetal tissues for transplantation is due to the fact that post-castration syndrome is characterized by the appearance of vegetative-neurogenic and metabolic-endocrine disorders, in the mechanism of which the CNS plays a key role, and especially the hypothalamohypophyseal region of the brain, as well as the liver, as the center of metabolism of the vast majority of chemicals in the body, including steroid hormones. The placenta was considered by us the source of estrogens and progestins.

Fetal tissue isolation conditions were adjusted to maintain the maximum number of living cells. Microscopy of the obtained suspension samples showed that the isolated placental tissue consists mainly of large conglomerates of cyto and syncytiotrophoblast cells, among which isolated blood cells (lymphocytes and red blood cells) are occasionally detected. The remaining cells are represented with destruction of varying degrees. Brain preparations are presented in the form of conglomerates of cells consisting of neurons similar to pyramid stars, and multipolar neurons with a large number of semicircular cells of glial nature.

The fetal liver suspension contains hepatocytes two-thirds of the total number of cells detected. (In total, 5-7 x 108 cells were detected). The rest were nucleated hematopoietic cells, the bulk of which were erythroblasts. Up to 90% of all cells had an undestructed cytoplasm and cell membranes, judging by the impoverishment of trepan blue.

In the largest amount, these hormones are contained in the suspension of the placenta. Almost 3 times less progesterone was found in the tissue of the fetal liver and only 28% less estradiol. The data obtained do not differ much from those of human fetal tissues. The suspension of brain tissue contained such a meager amount of these hormones that the reliability of their detection was overlapped by a permissible error in the implementation of the methods. Based on the absolute content of hormones in the animal dose (0.6 ml) of suspension), it can be seen that their amount is incomparably low relative to the conventional dosage of substitution therapy. According to various authors, for ovariectomized female rats, the replacement dose ranges from 0.5-5.0 mg. day of ethypylestradiol, which exceeds estradiol content in transplanted amount of placenta by almost 3 x 105-106 times. According to Z.M. Alikhanova, a single IM administration of 1.0 ml of fetal tissue suspension was sufficient to normalize the level of estrogens in the blood of women with post-castration syndrome. It was noted that the concentration of estradiol in such preparations was 24 times, and progesterone - 42 times higher than their indicators in the blood of healthy women. However, if we recalculate the obtained data for absolute indicators, it will become obvious an extremely large excess of HRT dosages relative to the amount of estradiol contained in transplanted tissues (105-106 times).

Taking the above into account, we were interested in the question of whether the estrogenic background of ovariectomized rats will be normalized after transplantation of fetal tissues isolated by us?

Ovariectomy leads to a decrease in uterine weight by more than half of the level of intact control. No significant changes in such rats occur after transplantation of fetal brain tissue, whereas

transplantation of fetal liver and placenta tissue leads to a reliable nose increase in uterine weight. However, the values of intact control in these cases are still not achieved, remaining lower by only 27% and 21%, respectively. Statistically significant differences relative to the control level disappear only when fetal tissue complex is transplanted. The introduction of EE to ovariectomized animals also leads to a complete restoration of uterine weight.

Ovariectomy leads to sharp changes in the tissue structure of the uterus of animals. Epithelial cells shrink in size and become more cuboidal with a small dark nucleus. Their height becomes more than half below the conial indicators. The average thickness of the myometrium and endometrial stroma decreases by 30-35%, while the stromal eosinophilicity decreases by almost 6 times. When EE is administered to the rat uterine endometrium, the number of epithelial cells with typical elongated bodies and nuclei increases. The average epithelial height increased significantly and not only relative to ovariectomized animals, but even almost twice the values of intact control. The same was observed in the evaluation of stromal eosinophilicity. At the same time, the endometrial stroma thickness indicator only slightly exceeded the reference level. A similar pattern was found with respect to myometrial thickness.

Brain tissue transplantation did not have a statically significant effect on any studied morphometric parameter of ovariectomized animals. In contrast, fetal song tissue transplantation contributed to the normalization of stromal thickness and eosinophilicity, as well as an almost threefold increase in the height of the epithelial layer. Along with this, the increase in the thickness of the myometrium was not so pronounced and, due to significant static deviations, did not reach reliable values. Complete recovery of all parameters to intact control values was observed in transplantation of both single placental tissue and in combination with brain and liver tissue.

The sum of all studied parameters converted on a scale from 1 to 7 gives an integrated estrogenic saturation index - estrogenicity index, the data of which are presented in Figure.

It shows that the estrogen index decreases by almost half in the absence of ovaries in rats. The exchange rate dose of EE, along with the reimbursement of estrogen deficiency, even contributes to the emergence of some tendency to exceed the reference level of the estrogen index. Restoration of the latter is also demonstrated in transplantation of placental tissues and fetal liver, as well as in their joint introduction with brain tissue. At the same time, fetal brain tissue transplantation leads only to a tendency to increase the estrogen index relative to the index of ovariectomized rats.

Based on the results obtained, it can be assumed that the main contribution to the process of normalization of the estrogen index of ovariectomized animals is still the estrogens contained in these tissues. Besides, not only estradiol, but also its other derivatives possessing estrogenic activity. However, it is impossible that such an important factor as receptor sensitivity is also involved in this process. Indeed, only with their increase, the integral indicator of estrogen saturation of the body can be normalized even with a low absolute content of estrogens in the blood.

Thus, despite the low absolute estradiol content in the fetal liver and placenta, their transplantation to ovariectomized animals leads to the restoration of estrogenic background. At the same time, the maximum effect can be achieved when they are combined with a suspension of fetal brain tissue.

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