Effect of Xenotransplantation of Cell Cultures Enriched with Stem and Progenitor Cells on Hormonal Profile of Rats with Abdominal Cryptorchism

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We present the results of application of cell cultures enriched with stem and progenitor cells for the treatment of experimental abdominal cryptorchism in outbred albino rats. Xenotransplantation of human fetal enriched cell cultures was performed to animals with experimental cryptorchism during orchiolysis. Total testosterone, luteinizing and follicle-stimulating hormones were assayed by immunochemiluminescent method. It was found that xenotransplantation of cell cultures enriched with stem and progenitor cells normalized the level of total testosterone, decreased the concentrations of gonadotropic hormones, reduced hyperplasia of Leydig cells and the number of chromaffin granules, and restored normochromism of Leydig cells nuclei.

Key Words: *Leydig cells; stem cells; cryptorchism; hypogonadism*

Previous studies showed that reproductive disorders in 5-25% cases are caused by endocrine factors [1,2,6]. The pathogenesis of endocrine disturbances in men is complex, because it involves not only central nervous system, endocrine glands, and gonads, but also other organs of the neuroendocrine system (thyroid glands, adrenals, sympathoadrenal system, subcutaneous fat); the role of the later organs is now actively studied [6]. Disorders in the hypothalamus—pituitary gland—testes regulatory system irrespective on the level of damage lead to functional disturbances in the gonads. Disturbances in hormone metabolism developing under these conditions manifest in qualitative and quantitative changes in the production of bioactive substances in various glands, which can lead to their dysfunction. Changes in the production of total testosterone are the most typical indicator of testicular dysfunction. In elderly men, androgen deficiency is determined by hypofunction of the testes and adrenal glands due to disturbances in circadian rhythm of testosterone production and changes in its metabolism in the target organs. This factor is responsible for reduced fertility and sexual activity in these patients [2,6].

Unique properties of stem and progenitor cells playing an important role in reparative processes attract much attention of medical community and are the objects of clinical and experimental studies.

There are publications devoted to transplantation of stem and progenitor cells as the experi-

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mental method of treatment and correction of androgen-deficient states. However, the obtained results are controversial. There is no consensus on the use of this method for the correction of the level of sex hormones. The efficiency of transplantation of spermatogonial stem and progenitor cells in animals varies from 10 to 80%. It was reported that fertility recovery is usually accompanied by restoration of adequate level of sex hormones [5,8, 9,12]. It was demonstrated that transplanted stem and progenitor cells promote normalization of spermatogenesis against the background of normal concentrations of total testosterone produced by Leydig cells (LC). Experimental transplantation restores the population of hormone-producing cells regulating the function of spermatogenesis and the processes of differentiation into spermatozoa in the damaged testis [11].

There are published reports on transplantation of rat spermatogonial stem and progenitor cells to mice with busulfan-induced infertility [10,14]. Busulfan administered in a concentration of 40 μg/kg induced death of the total population of spermatogonial stem cells after 4 weeks and emptied the biological niche in the zone of the basal membrane of the seminiferous tubule, which created conditions for xenotransplantation of donor spermatogonial stem cells. Xenotransplantation of these cells into the testes of mice with artificially disrupted spermatogenesis restored this process against the background of normal species-specific levels of total testosterone. The progeny obtained after these manipulations was viable and fertile [8,14]

Transplantation of spermatogonial stem and progenitor cell culture obtained from 4-week calves and containing gonocytes and undifferentiated spermatogonia led to an increase in testis weight and diameter of seminiferous tubules in recipients. It was noted that cell transplantation in these animals restored the population of LC and normalized testosterone level during weeks 4-24 after transplantation [13].

Here we studied hormonal profile in animals with experimental hypogonadism before and after transplantation of cultured fetal cells of different types.

MATERIALS AND METHODS

The study was performed on 3-6-month-old outbred albino rats weighing 250-400 g (n=30).

The animals were divided into 3 groups (10 rats per group). Xenotransplantation of fetal testis culture enriched with spermatogonial stem and progenitor cells and transplantation of mononuclear fraction of the bone marrow (BM) were performed in groups 1 and 2, respectively. Controls received physiological saline.

The method of modeling of bilateral abdominal cryptorchism consisted in fixation of the testes near the lateral channels of the abdominal cavity for 3 weeks followed by their displacement into the scrotum with dissection of the formed adhesions. Xenotransplantation of enriched cell cultures was performed during orchiolysis [4]; to this end, the culture suspension was injected into avascular zone between the capsule and seminiferous tubules [5].

The cells for transplantation were isolated from human fetuses (11-13-week gestation) obtained from licensed clinics according to the current legislation of the Russian Federation (Order of Ministry of Health No.302, December 28, 1993; Suppl. No. 3, April 5, 1994).

BM cells were washed out with DMEM containing 2 mM EDTA as the anticoagulant. The suspension was layered onto Ficoll-urografin density gradient (1.077 g/ml) and centrifuged at 2000g for 30 min. A fraction of mononuclear cells at phase interface was collected, resuspended in the medium, and recentrifuged at 1500g for 5 min. The sediment was resuspended in complete nutrient medium DMEM/F12 (1:1) supplemented with 10% FCS and 0.02% gentamicin, the suspension was stored at 4°C not longer than 24 h. The presence of stem cell pool in the obtained BM and testicular cultures was proved in previous studies [3].

The culture of stem and progenitor cells of fetal testis (gestation week 16) was cultured in standard 75-cm^2 plastic flasks (Corning) at 37°C and 5% CO_2 in DMEM/F12 (1:1) medium supplemented with 10% fetal calf serum and 20 ng/ml epidermal growth factor. The medium was replaced every 3-5 days, the cells were subcultured after attaining a monolayer.

Passage 3 cell culture was used for transplantation. Cell viability was 98% (determined by trypan blue or propidium iodide staining).

In group 1, each rat received ~946,000 spermatogonial stem and progenitor cells; in group 2, each rat received at least 150,000-200,000 stem and progenitor BM cells.

Hormonal profile was studied at the stage of orchiolysis (baseline) and then on days 14 and 28 after orchiolysis. The serum levels of gonadotropin and total testosterone were measured by immunochemiluminescent method on an Access 2 immunochemical analyzer (Beckman Coulter).

Histological preparations (paraffin sections) were stained with eosin. LC in 30 sites between the seminiferous tubules with strictly perpendicular sections

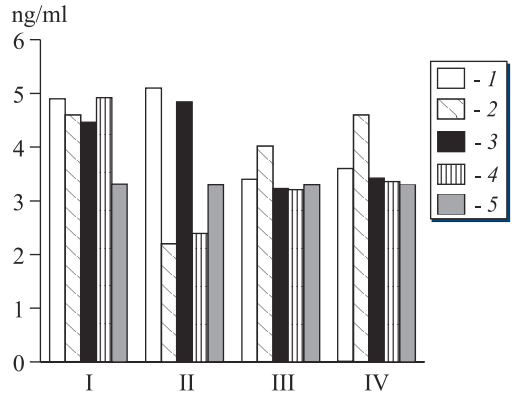

Fig. 1. Dynamics of mean levels of testosterone and LH in the experimental and control groups. *1*) LH in the experimental group; *2*) total testosterone in the experimental group; *3*) LH in the control group; *4*) total testosterone in the control group; *5*) lower limit of normal for total testosterone in animals population. *I*: initial level; *II*: orchiolysis+transplantation, *III*: day 14, *IV*: day 28.

were counted and their number was divided by the number of adjacent seminiferous tubules. The number of LC, chromism of their nuclei, and the state of chromaffin granules were evaluated.

RESULTS

Evaluation of the hormonal profile in rats 3 weeks after cryptorchism modeling revealed decreased level of total testosterone compared to its initial level. The concentration of gonadotropic hormones was elevated. Hyperplasia of LC manifested in their increased number, enhanced chromism of nuclei, and increased number of chromaffin granules. In experimental animals on day 14 after orchiolysis, the concentration of total testosterone returned to normal and remained at this level until the end of the study. In controls, the concentration of total testosterone recovered slowly and on day 28 attained the lower limit of normal (Fig. 1). After transplantation of cell cultures in experimental animals, the concentration of gonadotropins decreased to normal. In the control group, the level of gonadotropins also decreased, the dynamics of the content of luteinizing hormone (LH) being most representative (Table 1, Fig. 1).

The number of LC between the seminiferous tubules increased after orchiolysis (number of LC per 1 tubule), we also observed hyperchromism of nuclei and increase in the number of chromaffin granules. In the experimental groups, the number of LC per 1 tubule decreased 2 weeks after orchiolysis. The cells contained normochromic nuclei and lower number of chromaffin granules. In the control, the number of LC with hyperchromic nuclei and the content of chromaffin granules increased. After 28 days, the number of LC in the experimental groups decreased, they contained normochromic nuclei and moderate number of chromaffin granules. In the control group, the number of LC with reduced chromism of the nuclei and lower content of chromaffin granules continued to increase (Table 2).

Hierarchy and feedback are the most important characteristics in the regulation of the function of endocrine glands. The principle of interaction in this context is based on the fact that the increase in the level of peripheral hormones leads to inhibition of gonadotropin production, while decreased concentration of peripheral hormones stimulates gonadotropin synthesis. Feedback system also implies indirect effect of hormones produced by endocrine glands on the function of the pituitary gland via the hypothalamis—pituitary gland system or other struc-

TABLE 1. Changes in Hormonal Profile (ng/ml, $M \pm m$)*

Group	Hormones	Initial	Orchiolysis and transplantation	Time after orchiolysis, days	
				day 14	day 28
Control (0.9% NaCl)	Testosterone	4.92±0.50	2.39±0.20	3.21±0.30	3.36±0.30
	LH	4.46±0.60	4.84±0.80	3.22±0.20	3.42±0.40
	FSH	5.04±0.80	5.46±0.60	4.05±0.90	4.32±0.80
Culture of fetal testis	Testosterone	5.11±1.08	2.74±0.70	4.38±0.70	4.67±0.90
	LH	6.67±1.30	7.1±1.2	4.52±0.60	4.36±0.60
	FSH	4.96±0.60	5.26±0.60	3.13±0.46	3.03±0.60
Culture of bone marrow cells	Testosterone	4.25±0.65	1.78±0.50	3.67±1.40	4.57±0.80
	LH	3.13±0.46	3.26±0.70	2.37±0.70	2.83±0.70
	FSH	4.79±0.80	4.44±0.53	2.47±0.20	2.09±0.20

Note. FSH: follicle stimulating hormone. *$p<0.05$.

TABLE 2. Characteristics of LC Populations (*M*±*m*)*

Time of observation, day	Control group			Spermatogonial stem and progenitor cells			BM cells		
	1	2	3	1	2	3	1	2	3
Orchiolysis	7.8±2.4	+++	+++	7.9±2.2	+++	+++	6.5±1.9	+++	+++
day 14	9.5±1.3	+++	+++	6.2±1.1	+++	++	4.5±0.8	+++	++
day 28	9.8±1.1	++	+	6.3±2.5	++	++	4.5±1.9	++	++

Note. *1*) number of LC per 1 seminiferous tubule; *2*) chromism of nuclei; *3*) chromaffin granules in LC. *$p<0.05$.

tures of the central nervous system [6,7]. Normally, the closed circuit of regulation of hormone production with feedback mechanisms and circadian principle of hormone secretion provide stable cycle of the development of male sex cells due to maintenance of the mean normal level of hormones and depend on cyclic changes in the regulatory centers.

Analysis of experimental data showed that hormonal changes specific for hypergonadotropic hypogonadism developed 2 weeks after cryptorchism modeling. They were characterized by decreased production of testosterone by the testes against the background of their hyperstimulation with gonadotropins. These changes led to hyperplasia of LC characterized by the increase in the number of these cells, content of chromaffin granules per cell, and hyperchromism of their nuclei, probably due to stimulatory influences of enhanced LH secretion. After removal of the damaging factor, the severity of hypergonadotropic hypogonadism decreased and the level of tropic hormones of the pituitary gland returned to normal. At the same time, the concentration of testosterone attained only the lower limit of normal despite still increased content of LC in the testes. Hyperchromism of LC nuclei was associated with reduced content of chromaffin granules. These findings probably attest to exhausted functional reserves of cell producing testosterone as a result of their excessive stimulation.

In animals receiving cell therapy, the concentration of gonadotropins decreased on day 14 after orchiolysis, which was probably a result of feedback regulation by high testosterone concentrations. The concentrations of follicle-stimulating hormone (FSH) and LH remained at a low level to the end of the experiment (day 28). In rats receiving transplantation of cell cultures, the level of total testosterone recovered sooner than in the control group and the changes were more significant (Fig. 1). Transplantation of stem and progenitor BM cells produced more pronounced effect on the level of testosterone than transplantation of specific spermatogonial stem cells (Table 1). The concentration of LH linearly decreased; in parallel, LC hyperplasia became less pronounced, LC nuclei became normochromic, and the number of chromaffin granules decreased. Moreover, the number of LC in the examined preparations decreased against the background of normal population level of testosterone.

The mechanisms underlying the effects of transplanted stem cells on LC and accelerated normalization of the disturbed hormonal profile remain unclear. It can be mediated by their paracrine influences. Previous studies showed that transplanted stem cells intensively secrete growth factors and other cytokines modulating functional activity of cells. The possibility of direct transformation of stem and progenitor cells into testosterone-producing cells (which is more likely for stem and progenitor cells of fetal testis) is a subject of discussions and requires comprehensive experimental verification.

Experimental primary hypogonadism in animals with bilateral abdominal cryptorchism develops due to direct damage to the testicular tissue and is characterized by testicular hypoplasia, increased blood levels of gonadotropins, and decreased concentrations of total testosterone. Under these conditions, LC are in a state of hyperplasia manifested in the increase in their number, high content of chromaffin granules, and hyperchromism of LC nuclei.

After removal of the damaging factor and cell transplantation, normal levels of testosterone were attained as soon as after 2 weeks against the background of reduced content of gonadotropic hormones.

Xenotransplantation of cultures enriched with stem and progenitor cells led to more rapid and complete recovery of the total testosterone level and normalization of the levels of tropic hormones produced by the pituitary glans.

The level of total testosterone is determined by the state of LC populations. In experimental groups, parameters of total testosterone returned to normal due to hyperfunction of the remaining stable population of LC with normochromic nuclei and medium content of chromaffin granules, while in the

control group this was attained at the expense of quantitative parameters of hyperplastic LC population; the state of these cells attested to extreme functional strain (the parameters approached the lower limit of normal).

The results obtained in this study attest to serious progress in the field of transplantation of cell cultures enriched with stem and progenitor cells, a promising trend in modern experimental medicine. Further studies will allow to improve reproductive technologies aimed at solving the problems of male infertility.

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