Dynamics of Functioning of Thyroid Gland Transplant under Conditions of Stimulation with Autologous Adherent Bone Marrow Cells

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The efficiency of thyroid tissue autotransplantation was studied in 150 male Wistar rats for 3 months. Comparative analysis of functional competence of the thyroid tissue transplanted into the great omentum showed restoration of the thyroid hormonal activity in animals receiving cell suspension of autologous adherent bone marrow cells.

Key Words: *transplantation; thyroid; stem cells; hormonal system*

Increasing incidence of thyroid gland (TG) diseases necessitates the development of pathogenetically substandiated treatment methods, aimed at preservation of the organ function. One of approaches to thyropathy correction and restoration of thyroid function after total thyroidectomy is transplantation of normal fragments of thyroid tissue into the greater omentum [1-4]. Recent data on the properties and regularities of vital activity of stem cells opened new vistas of their use for combined treatment of thyropathology [3,5-9].

We studied the dynamics of recovery of TG function after its transplantation into a greater omentum strand under conditions of treatment with autologous adherent bone marrow cells.

MATERIALS AND METHODS

Experiments were carried out on 150 certified 6-month-old male Wistar rats (300-350 g) from Breeding Center of Institute of Pharmacology. Thyroidectomy and transplantation of TG tissue (fragment of the lobe or homogenate) into the greater omentum were carried out under ether (mask) narcosis. Manipulations on animals were carried out in accordance with regulations of the European Convention for Vertebrates Protection. The animals were divided into 4 groups: 1) transplantation of TG tissue fragment; 2) transplantation of TG tissue homogenate; 3) transplantation of TG tissue fragment with 500,000 adherent bone marrow cells; 4) transplantation of TG tissue homogenate with 500,000 adherent bone marrow cells. Control group consisted of 30 intact rats.

Autologous adherent bone marrow cells were prepared as follows. A suspension of bone marrow cells in 1 ml preparative medium containing 95% RPMI-1640 and 5% FCS was prepared. After filtration through a capron filter and 10-min centrifugation at 1500 rpm the cell suspension in 5 ml preparative medium was incubated in a Petri dish in a CO_2 incubator (5% CO_2) at 37°C for 90 min. Adherent elements were separated by 0.25% trypsin with 0.02% EDTA (1:1) for 15 min under the same conditions. After double washout the cells were suspended to the needed concentration and 500,000 cells in 0.2 ml preparative medium were introduced into the transplant. The function of transplanted TG tissue was evaluated 1 and 3 months after surgery. Narcotized rats were sacrificed by cervical disloca-

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tion. Blood (5-7 ml) was centrifuged and 3-4 ml serum was taken for routine analysis. Thyrotropin (TSH), thyroxin (T4), triiodothyronine (T3), free T4 (T4f) and T3 (T3f) were measured using commercial enzyme immunoassay kits ThyroidEIA-TSH-1, ThyroidEIA-thyroxin-01, ThyroidEIA-freeT4 (Alkor-Bio), and T3f-EIA (Hema).

The data were processed using Wilcoxon—Mann—Whitney nonparametric *U* test.

RESULTS

Evaluation of the functional competence of the transplant showed low TSH values in all groups at different terms of observation, hormone values were closer to the control after 3 months, especially in group 3 (Table 1). In contrast to the level 1 month after transplantation, the concentration of T4f by the 3rd month was almost 2-fold higher, the maximum values were observed in group 3. The T3f values were virtually the same in groups 1 and 2 and did not differ from the levels 1 month after transplantation. The levels of T3f in groups 3 and 4 receiving autologous adherent bone marrow cells differed appreciably throughout the study, increasing by the 3rd month, but remained within the range of control values. A similar trend was observed for total T4 concentration. By the 3rd month of observation the maximum T4 level was observed in group 2 (90.44±34.08 nmol/liter), which surpassed the control. The levels of total T3 after 1 and 3 months corresponded to control values in all groups, being somewhat lower at the late stage of observation.

Evaluation of the time course of the functional competence of transplanted thyroid tissue and the effects of autologous adherent bone marrow cells on thyroid hormone production indicates that the hormonal profile (T4, T4f, T3f) 3 months after transplantation was significantly higher in groups treated using cell technologies. The dynamics of TSH concentration is worthy of note; it indicates the development of optimal adaptation of the hypothalamus-pituitary-transplant system in group 3. In none groups thyrotoxic reactions were detected. The only exclusion was group 2 with a significant increase of T4f concentration. Bearing in mind the role of T3 as a hormone realizing its effects directly in tissue structures, we should like to draw attention to the increase of T3f concentrations over the course of the study in groups 3 and 4 (transplantats with autologous adherent bone marrow cells), which presumably determines tissue saturation and formation of a status close to euthyroid. This indicates high degree of adaptation of the transplant with

TABLE 1. Thyroid Hormone Status in Experimental Groups 1 and 3 Month after Transplantation ($X \pm m$)

Group	T3f, pmol/liter		T4f, pmol/liter		T3, nmol/liter		T4, nmol/liter		TSH, mU/liter	
	1 month	3 months	1 month	3 months	1 month	3 months	1 month	3 months	1 month	3 months
Control	12.11±1.96		63.87±10.32		1.93±0.52		62.23±7.13		0.059±0.008	
1	10.34±1.10	9.15±0.07	15.18±2.60*	34.84±2.60*	3.14±0.46*	1.43±0.09	37.32±5.30*	59.02±3.16	0.02±0.01*	0.037±0.005
2	7.14±0.38*	9.54±0.21	28.63±2.80*	44.22±3.06	2.02±0.09*	1.50±0.06	46.11±5.70	90.44±34.08	0.02±0.01*	0.038±0.008
3	4.71±0.05*	8.88±0.07	29.00±4.00*	52.76±3.63	1.73±0.21	1.49±0.16	42.96±5.20*	53.50±1.39	0.01±0.01*	0.047±0.003
4	4.68±0.10*	8.92±0.21	37.04±1.90*	43.68±4.98	1.83±0.20	1.40±0.07	38.84±3.70*	56.36±3.39	0.03±0.01*	0.033±0.004*

Note. *$p<0.05$ compared to the control.

autologous adherent bone marrow cells and, consequently, the possibility of normalizing the function of transplanted thyroid tissue by means of cell stimulation.

Hence, the use of autologous adherent bone marrow cells in autotransplantation of thyroid tissue reduced manifestations of thyroid insufficiency, providing the formation of full-value functional activity of the transplant.

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