CONDITION OF GLUTATHIONE (GSH) METABOLISM SYSTEM AT ALLOTRANSPLANTATION OF EMBRYONIC MUSCULE TISSUE AT RATS

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Abstract

This article presents the results of determination of the of reduced glutathione content, glutathione reductase and glutathione peroxidase during allotransplantation of the embryo muscle tissue. During the research there was carried out 2 kinds of surgery: 1st - allotransplantation of embryo muscle tissue; 2nd - surgery without replanting. To obtain embryos we used female rats from gestation 3-4 weeks. We took an abdominal muscle tissue from embryo that was hemmed to homologous tissue of the adult rat. The same procedure was carried out with femoral muscle tissue.It was found that allotransplantation of the embryo muscle tissue leads to an increasing of restored glutathione at day 7 of experiment in femoral and abdominal muscle of adult rat, leads the increasing of glutathione glutathione reductase activity in all researched tissues on day 7 of the experiment, except the femoral muscle of the adult rat. At experiment day 7 during surgery without replanting glutathione increased in femoral and abdominal muscle tissue, and activity of glutathione reductase increased only in the abdominal muscle, while activity of glutathione peroxidase in the abdominal muscle tissue decreased.

Keywords: Allotransplantation, reduced glutathione, glutathione reductase, glutathione peroxidase, femoral and abdominal muscle tissue, embryo

Introduction

Allotransplantation of embryonic tissue is one of the actual directions in modern theoretical and medical biochemistry, which is developed for stimulation and restoration functions (Shumakov V.I., 2002). Allotransplantation of embryonic tissue is considered as a possible alternative for traditional, conservative treatment methods, as well as the methodological base of experimental development (Stankov D.S.).

This fact underscores the urgency of the problem in experimental and clinical biochemistry, and perspectives of embryonic tissues transplantation (Bjorklund A., 1998).

One of the most important issues, that has medical and biological importance is the question of functioning and increasing of vital activity of the graft (transplant). In this matter, it is known that after transplantation of fetal tissue, the graft grows and differentiates, sprouting by blood vessels, thus forming conditions for oxygenation and energy supply of the activity (Gaillard A., 2000).

The main device (mechanism) of the answer on the stress-factors activity in cell is activation of the peroxide oxidation of lipids (LPO) processes (Baraboi V.A., 1992). The most important system of the cell protection from oxidative destruction is an antioxidant system (Lushak V.I.., 2007). Antioxidant protection represents multicomponent complex of different links and their systems, that provides connection or restoration of free radicals, breaking chain of free radical oxidation process (Vladimirov Y.A., 1972).

One of the important antioxidant cell system is glutathione system. This system contains from renovated glutathione (GSH) and some enzymes connected with it - glutathione peroxidase, glutathione reductase, glutathione transferase, and also NADP. GSH is a key metabolite in the glutathione system, and it has independent antioxidant GSH value inactivates OH^-, O_2, H_2O_2, lipid hydroperoxides, involved in the stabilization of membranes and protection of cell DNA (Menshikov E.B., 1993).

Materials and methods

The main aim of our research was to define amount of glutathione, glutathione reduktase and glutathione peroxidase activity during allotransplantation of embryo muscular tissues. During our research 2 types of surgery was carried out 2: the first – allotransplantation of embryo muscular tissue; second – without grafting surgery.

Experiments were carried out on the basis of the Biochemistry Department laboratory ONU I.I. Mechnikov. Allotransplantation was performed at white nonlinear rats - male, weighing 180-300 g. In this research all requirements of the European Convention on the protection of animals that used for experimental purposes - were complied.

Surgery was performed under sterile conditions. Operative (surgery) field was treated with Jodobac. Source of fetal tissue were taken from embryos of adult pregnant rat females with gestational 3.5-4 weeks. The abdominal muscle tissue was taken from embryo, and this tissue was stitched to the homologous tissues of adult rat. The same procedure was made with the femoral muscle tissue.

The surgery without replanting was performed for comparing the effect of changes in the number of indices of the surgical exposure. The tissue that was not involved to any surgical procedures was used as control.

Determination of reduced level of glutathione in the tissues was performed by E. Butler method, A. Dubonnet, B. Kelly (Goryachkovsky A.M. , 2005). Statistical processing was performed using Student's t test (Rokitsky P.F., 1967). All mathematics calculations were performed using the computer program Excel.

Results and discussion

From Table 1 the data shows that in determining the level of reduced glutathione at allotransplantation of embryo femoral muscle in the femoral muscle of adult animal, compared with controls, there is a significant increase of this indicator in all research terms. Allotransplantation of embryo abdominal muscle leads to a significant increase in all researched parameters during experience both at adult animal and at embryos.

Table 1. The level of reduced glutathione at allotransplantation of embryonic muscle tissue (mM/g of tissue)

Tissue / Day	Femoral muscle tissue of adult animal	Femoral muscle tissue of the embryo	Abdominal muscle tissue of adult animal	Abdominal muscle tissue of the embryo
Control (without replanting)	0,075±0,019	0,674±0,046 **	0,055±0,001	0,860±0,111 **
1st day	0,383±0,109 *	0,710±0,053 **	0,517±0,108 *	0,323±0,014 *
3rd day	0,250±0,029 *	0,781±0,155 **	0,202±0,001 *	0,273±0,026 * **
7 day	0,217±0,017 *	0,559±0,205	0,183±0,017 *	0,255±0,005 * **

Note: * $t \geq 2,3$ - significantly relative to control

** $T \geq 2,3$ - significantly between embryonic and formed muscle tissue

If we compare the results between the formed and embryonic tissue it should be noted that in embryonic muscle tissue such as in femoral the level of reduced glutathione significantly exceed the level of the adult rat. After allotransplantation of embryo femoral muscle we found a significant increase in the amount of reduced glutathione at first and third day of experiments at embryonic tissue and at day 7 this figure decreased almost to the control value.

After allotransplantation of fetal abdominal muscle tissue a significant increasing of the researched (studied) parameter occurred in the embryo tissue on day 3rd and 7 of the research.

Table 2 shows data at the level of reduced glutathione during surgery without replanting, this data shows that in the femoral muscle tissue this figure significantly increased relative to the control on day 7 of the research.

Table 2. The level of reduced glutathione during surgery without replanting (mM/g of tissue)

Tissue / day	Femoral muscle tissue of adult animal	Abdominal muscle tissue of adult animal
Control (without replanting)	0,075±0,019	0,055±0,001
1st day	0,033±0,003	0,023±0,007 *
3rd day	0,055±0,029	0,037±0,007 *
7 day	0,201±0,002 *	0,220±0,015 *

Note: * t ≥ 2,3 - significantly relative to control

At rat abdominal muscle during surgery without replanting at 1st and 3rd days of the experiment was discovered a significant decrease in the level of the studied parameters and on day 7 increasing relative to the control.

In this case, our obtained data shows that allotransplantation of embryonic muscle leads to an increasing of renovated glutathione at 7 day of experiment at femoral and abdominal muscle male. At day 7 of experiment during surgery without replanting the level of reduced glutathione increased in femoral and abdominal muscle.

During next series of experiments we studied the allotransplantation effect on the glutathione peroxidase activity at the embryo muscle tissue and adult animals (Table 3)

The data from Table 3 shows that allotransplantation of femoral muscle compared with control, leads to increasing of this figure at day 7 of the research in all studied tissues, except the femoral muscle of the adult rat.

Table 3. Activity of glutathione peroxidase at allotransplantation of embryonic muscle (c.u. / min. /mg protein)

Tissue / Day	Femoral muscle tissue of adult animal	Femoral muscle tissue of the embryo	Abdominal muscle tissue of adult animal	Abdominal muscle tissue of the embryo
Control (without replanting)	0,10±0,04	0,03±0,004 **	0,02±0,003	0,02±0,004
1st day	0,08±0,01	0,02±0,003 **	0,20±0,01*	0,11±0,02 * **
3rd day	0,12±0,01	0,04±0,01 **	0,14±0,01 *	0,10±0,01 * **
7 day	0,16±0,03	0,09±0,01*	0,26±0,04 *	0,18±0,03 *

Note: * t ≥ 2,3 - significantly relative to control

** T≥2,3 - significantly between embryonic and formed muscle tissue

If we compare the index of femoral muscular tissue of the adult rat and embryo, it can be noted that in femoral muscular tissue glutathione peroxidase activity in embryonic tissue femoral almost 3 times less than in adult tissue. After allotransplantation at 1st -3rd day studied parameters at embryonic muscular tissue of the embryo significantly reduced relative to the corresponding figure of the adult rat, but up to day 7 of the experiment glutathione content almost reached the rate of femoral muscle male. In the abdominal muscle the studied

figure is almost the same, as in the adult animal tissue and tissue of the embryo. After allotransplantation this index was increased in embryonic tissues, but was significantly lower than in the abdominal muscle of adult animal at 1st -3rd day of experiment.

Table 4 shows that surgery without replanting at the femoral muscle on the first day after surgery at 3 times exceeds the control value, but at day 3rd -7 of experiment, significant changes wasn`t occurred.

Table 4. Activity of glutathione peroxidase during surgery (operation) without replanting (c.u./min/mg protein)

Tissue / Day	Femoral muscle tissue of adult animal	Abdominal muscle tissue of adult animal
Control (without replanting)	0,10±0,04	0,02±0,003
1st day	0,31±0,04*	0,67±0,03 *
3rd day	0,17±0,02	0,36±0,05 *
7 day	0,13±0,01	0,28±0,01 *

Note: * t ≥ 2,3 - significantly relative to control

At the abdominal muscle tissue during surgery without replanting was significant increasing of studied parameters, relative to control in all studied periods.

Table 5 shows the results of investigations of glutathione peroxidase activity at allotransplantation of embryonic muscle tissue, it should be noted a significant increasing in the studied parameters, relative to control at 1st -3rd day of experiment in femoral and abdominal muscle tissue of the embryo.

Table 5. Activity of glutathione peroxidase in allotransplantation of embryonic muscle (c.u./ min./mg protein)

Tissue / Day	Femoral muscle tissue of adult animal	Femoral muscle tissue of the embryo	Abdominal muscle tissue of adult animal	Abdominal muscle tissue of the embryo
Control (without replanting)	0,05±0,004 **	0,01±0,001	0,07±0,005 **	0,02±0,003
1st day	0,07±0,008 **	0,03±0,004*	0,10±0,009* **	0,05±0,008*
3rd day	0,05±0,008 **	0,02±0,003*	0,05±0,007 *	0,05±0,002 *
7 day	0,04±0,006	0,02±0,007	0,07±0,010	0,05±0,014

Note: * t ≥ 2,3 - significantly relative to control

** T≥2,3 - significantly between embryonic and formed muscle tissue

In the abdominal muscle male on the first day after the operation there was a significant increasing at glutathione peroxidase activity relative to controls at day 3rd, this figure was significantly decreased and at the day 7 again returned almost to the control value.

Investigating the glutathione peroxidase activity during surgery without replanting (Table 6), we noticed a significant increasing of this index, relative to control on the first day of the research, both the femoral and abdominal muscles of male.

Table 6. Activity of glutathione peroxidase during surgery without replanting (c.u./min./mg protein)

Тканини / Доба	Femoral muscle tissue of adult animal	Abdominal muscle tissue of adult animal
Control (without replanting)	0,05±0,004	0,07±0,005
1st day	0,11±0,015*	0,14±0,015 *
3rd day	0,05±0,008	0,05±0,010
7 day	0,05±0,005	0,02±0,005 *

Note: * t ≥ 2,3 - significantly relative to control

At day 7 of research, the activity of glutathione peroxidase during surgery without replanting it was observed a significant decreasing of this index relative to control at the abdominal muscular tissue of male.

Conclusion

So, our obtained data indicate that, allotransplantation of embryonic muscle leads to an increasing of renovated glutathione at day 7 of researching at femoral and abdominal muscle male, also it leads to increasing of glutathione reductase activity in all tissues studied at day 7 of the experiment, except for femoral muscle tissue of the adult rat.

At day 7 of research during surgery without replanting, renovated glutathione increased in femoral and abdominal muscle tissue, but glutathione reductase activity increased only in the abdominal muscle, and glutathione peroxidase activity in the abdominal muscle tissue decreased.

In this case, it can be noted that at the femoral muscle and abdominal muscle of adult animal the level of reduced glutathione at allotransplantation of embryonic tissue and during surgery without replanting increases. Therefore, we can assume that these changes affect primarily surgery.

The activity of glutathione reductase at the abdominal muscle of rats increases as at allotransplantation in embryonic tissue, as during operation without replanting. The activity of glutathione peroxidase at allotransplantation unchanged, while the operation without replanting leads to decreasing of this index in the abdominal muscle of rats.

So, we found that *allotransplantation able* to normalize *only glutathione peroxidase at the abdominal muscle of rats.*

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