The use of the combined method of preservation of liver transplant in the experiment

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Key words: liver transplantation, ischemia-reperfusion injury, hypothermia, custodiol, antioxidant therapy, perftoran

Introduction

Liver transplantation is currently the "gold standard" in the treatment of end-stage liver disease, regardless of etiology. The number of "successful" liver transplantation is associated not with the number of potential donors, and with transplatata quality, its functionality. There are reasons why even the impeccably conducted a liver transplant leads to patient death due to primary non-functioning graft. The main reason is ischemia-reperfusion injury (PRI) [1, 5, 10, 11].

An important role in causing oxidative stress plays a disturbance caused by an imbalance of production and inactivation of reactive oxygen species (ROS), largely due to the oxidative degradation of lipids, proteins and nucleic acids in diseased tissue [1, 13]. Therefore, for the prevention of graft PRI promising technique is drug therapy, intervention which should be focused on the mechanism of formation of free radicals. Free radicals have an extremely short half-life. Terms of intervention are crucial. Some medication drugs may have pro-oxidant effects under certain circumstances [7, 8, 9].

Differences between the dose of drugs, time and route of administration can lead to unexpected results. Evaluation of the impact of medical drugs requires laboratory tests in which the effects of the treatment can be carefully studied, and only then are recommended for clinical use [10, 12, 14]. Given the fact that the problem of antiischemic protection liver transplant is far from perfect, it is enough actual development of modern methods of action to effectively deal with these disorders [7, 5, 15].

The aim of research is to evaluate the application of the combined method of preservation for the prevention of disturbance in the transplantation of the liver in laboratory animals.

Materials and methods

Studies conducted on 60 male rabbits of Chinchilla breed at the age of 6-7 months, weight of 2000±150 g (in accordance with the number of orders in 1179 the Ministry of Health of the USSR from 10.10.1983, № 267 Ministry of Health of the Russian Federation of 19.06.2003, "Rules of work with experimental animals", the principles of the European Convention (Strasbourg, 1986) and the Declaration of Helsinki of the World Medical Association on the humane treatment of animals (1996)). Rabbits were divided into 3 groups of 20 animals. In the first group performed a liver preservation solution NTC. In the second group used a combined method: NTC solution and non-oxygenized perfluororganic emulsion. NTC and emulsions ratio = 4:1. In the third group preservation of liver was conducted in non-oxygenized perfluororganic emulsion. Preservation of liver was carried by

non-perfusion method for 8 hours at a temperature of 4-5°C. To study the biochemical parameters of the hemostatic system and general blood analysis and at explant after reperfusion, blood was taken. Data processing was carried out in the program Statistica 6.0, for descriptive statistics were calculated mean values, standard deviation, the average error. In order to identify differences in the mean values of signs used the Mann-Whitney test, the comparison of interest was carried out using Fisher's angular transformation to identify the relationships built contingency tables and Pearson's test was applied. Differences were considered significant at a significance level of p = 0.05 [3].

Results of research. For descriptive data statistics to the preservation of liver transplants, and after the 8-hour liver transplant preservation rabbits were calculated in groups of averages, standard deviation, standard error. Using the Mann-Whitney test, we compared average values of liver transplants in the groups of rabbits before preservation. Results of comparing the average values of parameters in groups of rabbits liver transplants before conservation are presented in Tables 1, 2 and 3.

Table 1

The results of comparison of the mean values of the indicators in the first and second groups of rabbits liver transplants before conservation

Index	The average value of the index in a group		The level of significance of the differences (p)
	1	2	
Bilirubin total, μmol/L	3.31	3.21	0.989209
Aspartate aminotransferase, U/L	52.09	51.84	0.797197
Alanine aminotransferase, U/L	59.60	58.48	0.675014
Prothrombin index,%	88.25	91.25	0.417078
APTT, second	36.25	35,60	0.279252
Fibrinogen, g/l	2.95	3.00	0.786775

Table 2

The results of comparison of the mean values of the indicators in the first and third groups of rabbits liver transplants before conservation

Index	The average value of the index in a group		The level of significance of the differences (p)
	1	3	
Bilirubin total, μmol/L	3.31	2.73	0.239323
Aspartate aminotransferase, U/L	52.08	55.48	0.481827
Alanine aminotransferase, U/L	59.60	58.62	0.635866
Prothrombin index,%	88.25	87,90	0.978362
APTT, second	36.25	36,40	0.913317
Fibrinogen, g/l	2.95	2.99	0.755086

Table 3

The results of comparison of the mean values of the indicators in the second and third groups of rabbits liver transplants before conservation

Index	The average value of the index in a group		The level of significance of the differences (p)
	2	3	
Bilirubin total, μmol/L	3.21	2.73	0.159544
Aspartate aminotransferase, U/L	51.84	55.48	0.180577
Alanine aminotransferase, U/L	58.47	58.62	0.818150
Prothrombin index,%	91.25	87,90	0.473481
APTT, second	35,60	36,40	0.350703
Fibrinogen, g/l	3.00	2.99	0.755743

For descriptive statistics after 8-hour liver transplant preservation rabbits were calculated in groups of averages, standard deviation, standard error. By using the Mann-Whitney test, we compared the average values in groups of liver transplants in rabbits after 8 hours of preservation. The results of comparison of the mean values of the indicators between the two groups are shown in Tables 4, 5 and 6.

Table 4

The results of comparison of the mean values of the indicators in the first and second groups of rabbits liver transplants after 8 hours of conservation

Index	The average value of the index in a group		The level of significance of the differences (p)
	1	2	
Bilirubin total, μmol/L	5.81	5.44	**0.002086**
Aspartate aminotransferase, U/L	606.73	322.82	**0.000000**
Alanine aminotransferase, U/L	614.17	277,60	**0.000000**
Prothrombin index,%	62,80	74.70	**0.000000**
APTT, second	53,60	45.35	**0.000000**

Table 5

The results of comparison of the mean values of the indicators in the first and third groups of liver transplants in rabbits after 8 hours of conservation

Index	The average value of the index in a group		The level of significance of the differences (p)
	1	3	
Bilirubin total, μmol/L	5.81	6.31	**0.002100**
Aspartate aminotransferase, U/L	606.73	1040.15	**0.000000**
Alanine aminotransferase, U/L	614.17	978.10	**0.000000**
Prothrombin index,%	62,80	39,60	**0.000000**
APTT, second	53,60	56.55	**0.045296**
Fibrinogen, g/l	1.66	1.39	**0.000245**

Table 6

The results of comparison of the mean values of the indicators in the second and third groups of liver transplants in rabbits after 8 hours of conservation

Index	The average value of the index in a group	The level of significance of the differences (p)

	2	3	
Bilirubin total, µmol/L	5.44	6.31	**0.000000**
Aspartate aminotransferase, U/L	322.82	1040.15	**0.000000**
Alanine aminotransferase, U/L	277,60	978.10	**0.000000**
Prothrombin index,%	74.70	39,60	**0.000000**
APTT, second	45.35	56.55	**0.000000**
Fibrinogen, g/l	2.32	1.39	**0.000000**

Using analysis of variance with repeated measurements, we compared average values in different groups and different measurements. The main results of variance analysis upon assessing the mean total bilirubin are shown in Fig. 1.

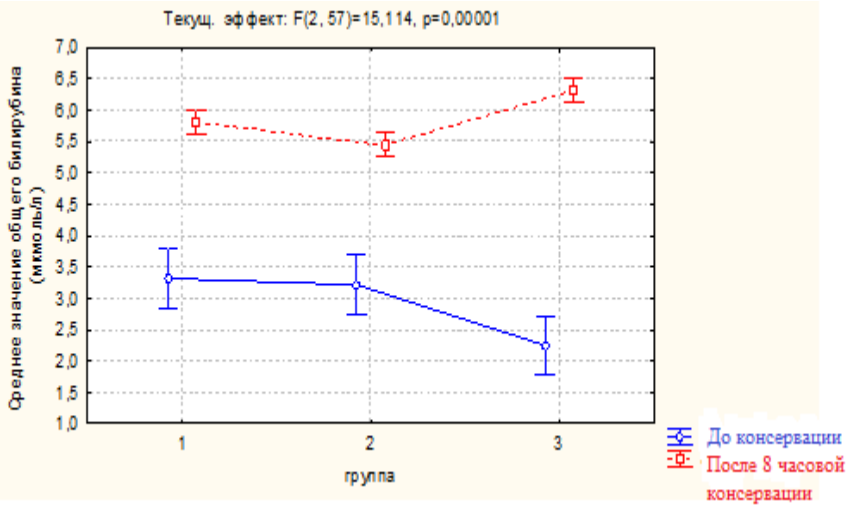

Fig. 1. Graph of mean values of total bilirubin (µmol/l) in three groups before and after preservation.

Variance analysis showed that there were statistically significant changes in the mean parameters in the different groups before and after preservation of liver transplants rabbits ($p = 0.00001$). And if before the preservation of the graft in the third group had the lowest levels of bilirubin, after 8 hours preservation of liver transplants in rabbits, the lowest level observed in the second group, while the highest level of bilirubin in the liver grafts third group of rabbits. The main results of analysis of variance in the assessment of the average value of aspartate aminotransferase (AST) before and after the 8-hour preservation are shown in Fig. 2.

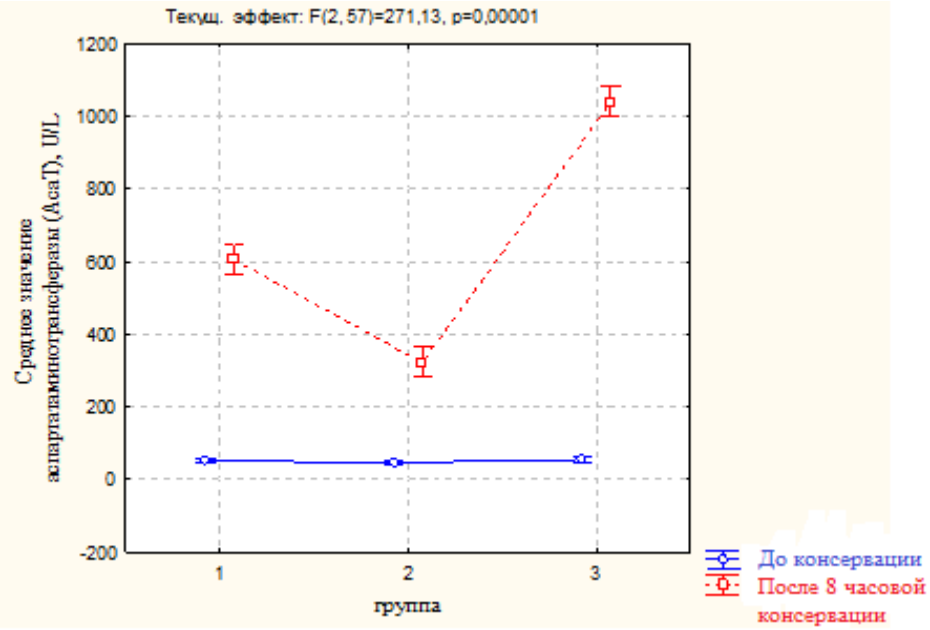

Fig. 2. Graph of mean values of AST, U/L in three groups before and after 8-hour preservation.

Analysis of variance showed that the studied parameters affected the relationship factors (p = 0.00001): there were statistically significant differences in average values before and after the 8-hour preservation of liver transplants in rabbits according to the group. So if averages to conservation AST in three groups differed insignificantly, after preservation have a statistically significant difference: AST lowest level observed in the second group, while in the first and third groups AST level was significantly higher. The main results of variance analysis upon assessing the mean alanine aminotransferase (ALT) in the groups before and after 8-hour preservation are shown in Fig. 3.

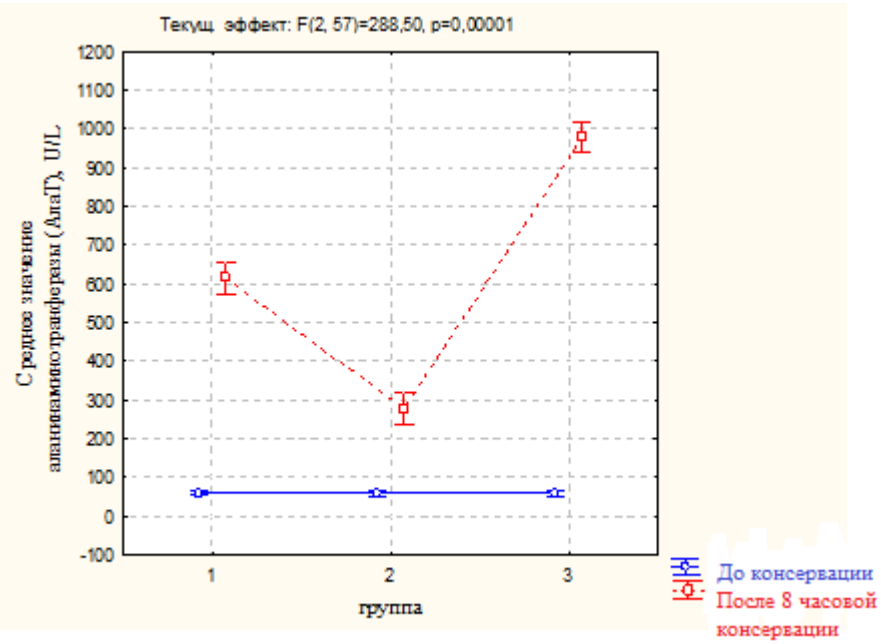

Fig. 3. Graph of mean values of ALT in three groups before and after 8-hour preservation.

Analysis of variance showed that the identified statistically significant changes in the average values of the indicator which occurred before and after 8 hours of conservation in the different groups (p = 0.00001). And if before the preservation average ALT liver grafts of rabbits in three groups differed insignificantly, after 8 hours of conservation transplants the lowest level observed in the second group, and the highest in the third group.

Discussion of results. Studies with laboratory animals showed that all indicators didn't have statistically significant differences in mean values of parameters in the two groups before conservation. After preservation of liver transplants rabbits observed a statistically significant difference in the groups depending on the preservative solution.

When compared using the Mann-Whitney test in a group with a combined preservative after the 8-hour preservation of rabbit liver transplants in comparison with the first and third groups of significantly lower levels of total bilirubin (p1-2 = 0,002086, p2-3 = 0,000000) and the highest level of bilirubin is noted in the third group. A similar situation is with the level of the free and conjugated bilirubin.

When compared using the Mann-Whitney test in the second group (combined preservative) after 8-hour liver transplant preservation rabbit compared to the first and third groups was significantly lower than the level of ALT and AST — cytolysis syndrome (AST p1-2 = 0,000000, p2-3 = 0,000000; ALT p1-2 = 0,000000, p2-3 = 0,000000), and the highest level of AST and ALT observed in the third group.

When compared using the Mann-Whitney test in the second group after 8 hours rabbit liver transplant preservation as compared with the first and third groups is statistically significant higher level of hemostasis (fibrinogen, PB aPTT), i.e. closest to those parameters normally (fibrinogen p1- 2 = 0,000006, p2-3 = 0,000000; PTI p1-2 = 0,000000, p2-3 = 0,000000, aPTT p1-2 = 0,000000, p2-3 = 0,000000), and hypocoagulation most severe manifestations are observed in the third group.

According to research, the use of an emulsion as an independent perfluororganic preservative is not possible for reasons: not prevents cell swelling (osmolarity — 280-310 mOsm/l; pH — 7.2-7.8); perfluoroorganic emulsion does not affect the sodium-potassium pump, due to the concentration of sodium and potassium ions close to the plasma; inability to maintain homeostasis and the pH of the cell, due to the absence in the composition of the buffer system. Perfluoroorganic emulsion does not affect the intracellular metabolism and thus do not support intracellular homeostasis during cold ischemia. Use of the perfluororganic emulsion is an acceptance of reactive oxygen species and lack of effect on cell metabolism in ischemia [4].

Application of NTC solution allows hypothermia inactivate cell body function by removal of extracellular sodium and calcium, as well as intensive buffering extracellular space by histidine to extend the period of cold ischemia.

NTC solution composition similar to the extracellular fluid with high and low buffer system as compared with the plasma concentrations of sodium and potassium ions, which prevents cell swelling and degradation of the delay cell, serves as a buffer to maintain the proper homeostasis. At the same time preservation solution NTC body limited in time due to the formation of reactive oxygen species and cell wall injury [12, 14, 15].

The main advantage of non-oxygenized perfluorocarbon emulsion is its intactness in tissues and organs, i.e. the drug is not metabolized in the cells (hepatocytes), unlike other antihypoxants. Unique tropism of perfluororganic emulsion to oxygen served as the basis for the application of the combined preservative with the purpose of acceptance and neutralization of reactive oxygen species, and therefore reduction of free radical oxidation and the disturbance in the tissues and organs [2, 4].

Application of the combined preservative prevents disturbance in the hepatic graft through absorption of ROS molecules by perfluororganic emulsion, thereby preventing damage to organs and tissues. In our study, we have shown that the combined preservative significantly reduces the disturbance of hepatic transplant, with a significant decrease in the level of ALT and AST compared with other groups. Thus, in the group with the combined preservative we observed a statistically significant reduction of PRI after 8 hours of rabbit hepatic graft preservation.

Conclusions

The modern idea of PRI liver transplant remains the cornerstone of clinical practice. Nevertheless, therapeutic approaches such as pharmacological intervention can help prevent or limit the disturbance. The use of combined preservative, a new therapeutic concept for the prevention of disturbance of liver transplant, provides a better quality of graft due to inactivation of ROS and reduction of lipid peroxidation.

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This article is dedicated to one of the problems of liver transplantation and how to solve it. The incidence of complications after liver transplantation depends on ischemia-reperfusion injury of the graft. The global shortage of donor organs causes usage of different techniques of graft conservation and functioning. This article describes the basic links of pathogenesis and offers a new technique for the prevention of ischemia-reperfusion injury of the liver.