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Study Case of the Experimental Rats after Splenectomy

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Abstract **The aim** of the study was to evaluate the degree of functional impairment after splenectomy by conducting experimental researches on the study of clinical and laboratory parameters. **Introduction.** In the structure of abdominal traumatism, spleen injuries occupy one of the leading places, the frequency of which is 20-25% of victims with abdominal trauma. Splenectomy is performed most often at spleen injuries. The tactics of preserving the spleen at its injuries has been gaining more and more supporters for the last few years. This is evidenced by the growing number of scientific papers that substantiate the expediency of preserving the spleen, and if it is technically impossible to preserve it, heterotopic allotransplantation of the spleen is performed. **Material and method.** The studies were conducted on 16 white mongrel rats weighing 300-400 g. On the 5^{th}, 15^{th} and 30^{th} days before splenectomy a general blood test was performed and indicators of the blood coagulation system, cellular and humoral immunity were determined. **Results.** Splenectomy was accompanied by an increase in the number of platelets and their functional activity, a violation of the release reaction of platelet factors, and a decrease in the retraction index. There was a shortening of the activated partial thromboplastin time. The concentration of fibrinogen was increased. Cellular and humoral immunity was suppressed. **Conclusion.** The conducted studies have shown that in order to improve the treatment results after splenectomy, it is necessary to perform heterotopic autotransplantation of the spleen, which will prevent changes in hemostasis and the immune system, and thereby reduce the risk of specific postoperative complications development.

Keywords Splenectomy, Experiment, Spleen autotransplantation, Fibrinogen, Hemostasis, T-helper

1. Introduction

Currently, more than 55% of the world's population lives in urban areas. An increase of the urban population (urbanization) can have a beneficial effect on the country's economy, but this trend also has some negative aspects for the health of the population. First of all, it is connected with an increase in injuries due to the expansion of construction work in urban areas, the growth in the number of motor transport, the appearance of high-speed bicycles, electric scooters, etc. [1].

Traumatism is one of the main causes of primary disability and mortality among the most able-bodied adults and children. According to the International Labor Organization, depending on the territorial circumstances, the structure of injuries is dominated by domestic injuries (more than 50%), the proportion of street and child injuries is high (up to 20%), sports injuries are often found (up to 10%), less often – industrial (up to 1.5%) and traffic injuries (up to 2%) [2].

There are economic costs although the social and emotional problems associated with injuries and fatalities due to accidents are beyond control. It would seem that the share of injuries as a result of accidents is low, but experts have estimated that the economic loss from injuries as a result of road accidents in Uzbekistan is 3.8% of the national gross domestic product. This fact has a negative impact on the development of the state [3].

Spleen injuries occupy one of the leading places in the structure of abdominal traumatism, the frequency of which is 20-25% of victims with abdominal trauma [4].

Splenectomy is performed most often at spleen injuries. The spleen performs many functions in the human body,: it takes part in hematopoiesis, it plays a leading role in the hemostasis system and the immune system of the body. All these facts can contribute to the development of postoperative complications after splenectomy, which sometimes lead to fatal outcomes. The tactics of preserving the spleen at its injuries has been gaining more and more supporters for the last few years. This is evidenced by the growing number of scientific papers that substantiate the expediency of preserving the spleen, and if it is technically impossible to preserve it, heterotopic allotransplantation of the spleen is performed (HATS) [5].

The spleen is an unpaired parenchymal organ of the immune system. The main functions of the spleen are lymphopoiesis, destruction of erythrocytes and platelets, blood deposition. To date, the functions of the spleen have not been definitively studied. However, the absence of the spleen as an organ is associated with many negative aspects that have an impact on the functional state of the body. One of the most common reasons for performing splenectomy are

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spleen injuries.

The issues of changing the laboratory status after splenectomy are debatable. Thus, a number of authors show that there is a change in the main indicators of the vascular-platelet link of hemostasis in the postoperative period: an increase in the number of platelets and their functional activity; a violation of the platelet factor release reaction, as well as a decrease in the retraction index. Changes can also be traced in the coagulation link of the hemostasis system: there is a shortening of the activated partial thromboplastin time, an increase in the concentration of fibrinogen and inhibition of fibrinolysis. There is a decrease in the concentration of IgG and M, the total amount of complement and its C3 fraction, the number of mature T-lymphocytes, T-helpers and T-killers in the immune system. At the same time, there is an increase in the number of T-cytotoxic and B-lymphocytes, as well as the concentration of Ig E and C4 complement fraction [6-8].

But, a number of researchers point to the fact that splenectomy does not affect the functional parameters of the body due to the full compensation of lost functions by other organs and systems [9,10].

The aim of the study was to evaluate the degree of functional impairment after splenectomy by conducting experimental researches on the study of clinical and laboratory parameters.

2. Material and Methods

The studies were conducted on 16 white mongrel rats weighing 300-400 grams at the age of 3 to 4 months in compliance with the requirements of the European Convention for the Protection of Vertebrates Used for Experimental and Other Scientific Purposes (Strasburg, 1986). On the 5th, 15th and 30th days before splenectomy a general blood test was performed and indicators of the blood coagulation system, cellular and humoral immunity were determined. After each period of blood sampling for tests, 4 animals were removed from the experiment.

A complete blood analysis was carried out on a «Mindray» hematological analyzer using standard reagents. Immunoglobulins were determined by the enzyme immunoassay using reagents from the company "Hema" (Russia) on the RT-2100C "Raito" enzyme immunoassay analyzer. The prothrombin index (PTI) and Activated partial thromboplastin time (APTT) were determined using “HUMAN” reagents on the “Huma Clot Junior” analyzer. The remaining parameters of the coagulation system were determined by the method of M.A. Kotovshchikova (2012). Indicators of cellular immunity were carried out by the method of immunofluorescence analysis. Statistical processing of the material was carried out by calculating the average value (M), the average deviation (m) and the Student's reliability criterion. Differences were considered significant at t-Student equal to or greater than 2.0.

3. Results

The general condition of the experimental animals was satisfactory before splenectomy. The animals were active, took food, drank water. The movements in the cage were active. The coat was smooth, without changes. Animals responded well to external stimuli.

On the 1st-2nd day after splenectomy, the general condition of the experimental animals did not change much. Only moderate restriction of active movements was noted. There were no signs of inflammation in the area of the postoperative wound. The animals continued to actively eat and drink water. No significant changes in the general status of experimental animals were observed in the subsequent observation periods.

Table 1. Indices of the general blood test after splenectomy

Index	Unit of measurement	Initial level	After splenectomy		
			The 5th day	The 15th day	The 30th day
Hemoglobin	g/l	88.4±11.3	82.8±6.5	82.4±2.0	84.8±2.8
t-Student			0.430	0.523	0.309
Red blood cells	$*10^{12}$/l	3.4±0.4	3.0±0.2	3.2±0.2	3.2±0.1
t- Student			0.894	0.447	0.485
Leukocytes	$*10^{9}$/l	6.9±0.8	11.6±1.0	11.3±0.7	10.8±0.7
t- Student			3.670	4.139	3.669
Platelets	$*10^{9}$/l	355.8±19.1	453.1±24.6	483.1±26.1	458.3±23.8
t- Student			3.124	3.936	3.359
Reticulocytes	%	23.1±2.3	29.5±1.4	28.9±1.4	29.0±0.5
t- Student			2.377	2.154	2.507
Lymphocytes	%	58.6±3.3	63.3±2.1	60.6±1.6	61.5±1.0
t- Student			1.202	0.545	0.841
Monocytes	%	4.4±0.3	4.4±0.2	4.3±0.3	4.3±0.2
t- Student			0.000	0.236	0.277

Table 2. Blood coagulation parameters after splenectomy

Index	Unit of measurement	Initial level	After splenectomy		
			The 5th day	The 15th day	The 30th day
Fibrinogen	g/l	2.8±0.3	3.8±0.4	3.8±0.3	3.8±0.3
t-Student			2.000	2.357	2.357
PTI	%	71.4±4.1	86.1±2.9	86.5±3.6	86.5±1.5
t-Student			2.927	2.767	3.459
Thrombotest	Level	3.3±0.6	4.1±0.5	4.1±0.4	4.0±0.5
t-Student			1,024	1,109	0,896
APTT	Sec	19.7±1.4	15.9±1.2	14.8±0.8	14.5±0.5
t-Student			2.061	3.039	3.498
INR		1.1±0.1	0.8±0.1	0.7±0.1	0.8±0.1
t-Student			2.121	2.828	2.121

Table 3. Cellular and humoral immunity parameters after splenectomy

Index	Unit of measurement	Initial level	After splenectomy		
			The 5th day	The 15th day	The 30th day
CD 3+ (T-lymphocytes)	%	41.8±2.0	32.7±1.2	32.5±2.6	32.8±0.8
t-Student			3.902	2.835	4.178
CD 4+ (T-helpers)	%	31.4±1.4	22.3±1.2	21.6±1.9	22.0±1.0
t-Student			4.935	4.152	5.464
CD 16+ (T-suppressors)	%	14.4±2.0	7.3±1.2	7.5±0.9	7.3±0.4
t-Student			3.044	3.146	3.481
CD 20+ (B-lymphocytes)	%	8.4±1.1	12.1±0.8	13.8±1.6	13.3±1.3
t-Student			2.720	2.781	2.877
IgA	g/l	1.1±0.1	1.3±0.2	1.3±0.1	1.5±0.1
t-Student			0.894	1.414	2.828
IgM	g/l	1.6±0.2	1.3±0.1	1.1±0.1	1.1±0.1
t-Student			1.342	2.236	2.236
IgG	g/l	7.3±0.5	4.3±0.7	4.3±0.3	4.2±0.4
t-Student			3.487	5.145	4.841

In the postoperative period laboratory blood tests were performed. In the general blood test in the early period after splenectomy, there was a decrease in the hemoglobin index from 88.4 ±11.3 g/l to 82.8± 6.5 g/l, followed by an increase of its level to the initial value in the follow-up period of up to 30 days. There was no statistically significant difference from the baseline at any time ($t<2.000$). A similar trend was noted with the indicators of red blood cells. In terms of leukocytes at an initial value of 6.9± 0.8*109/l, after splenectomy on the 5^{th}-15^{th} day, a statistically significant increase to 11.3±0.7*109/l ($t=4.139$) followed by a slow decrease was noted. However, by the 30^{th} day, a significant difference from the initial level remained ($10.8±0.7*10^9$/l; $t=3.669$). A similar trend was noted with the indicators of platelets, reticulocytes, lymphocytes. In contrast to the indices of leukocytes, platelets and reticulocytes, no statistically significant differences were observed in the indices of lymphocytes in any period of observation. The indices of monocytes did not undergo any special changes at different times after splenectomy (Tab. 1.).

Significant shifts were observed in various parts of hemostasis after splenectomy. In particular, there was an increase in the number of platelets and their functional activity, including adhesive ability, the release reaction of platelet factors was disrupted, the retraction index was reduced. Changes were also observed in the coagulation link of the hemostasis system: there was a shortening of the activated partial thromboplastin time (APTT), the concentration of fibrinogen was increased, fibrinolysis was inhibited. We noted a significant increase in fibrinogen levels from 2.8±0.3 g/l to 3.8±0.4 g/l on the 5^{th} day ($t=2.000$). This dynamics persisted on the next day of the experiment, on the 30^{th} day the fibrinogen index remained high (3.8±0.3 g/l; $t=2.357$). The level of PTI varied according to the

fibrinogen indexes. The thrombotest index was increased, but unlike the level of fibrinogen and PTI, there were no significant differences from the initial level. On the 5th day after splenectomy, the APTT statistically significantly decreased from 19.7=1.4 seconds to 15.9=1.2 (t=2.061). The downward trend of this indicator was also noted in the following days of observation, by the 30th day APTT was 14.5±0.5 seconds (t=3.498). Similarly to the APTT indicator, the index of the international normalized ratio (INR) was also changed (Tab. 2.).

Splenectomy promoted a decrease in the content of CD3+, CD4+, CD16+ cells, while the serum concentration of CD20+, IL-2, IL-6 and tumor necrosis factor increased. Changes in humoral immunity consisted in lowering the level of immunoglobulins of all classes (IgG, IgA, IgM). In general, surgical intervention has a multifaceted effect on the body's immune system. There was a statistically significant decrease in the level of CD3+ (T-lymphocytes) from 41.8=2.0% to 32.7= 1.2% (t=3.902) by the 5th day after splenectomy. The achieved level by this time has not changed much in the following days of observation. Similarly, the level of CD4+ (T-helpers) and CD 16+ (T-suppressors) was changed. Unlike CD3+ (T-lymphocytes) and CD4+ (T-helpers), the level of CD16+ (T-suppressors) was decreased almost 2 times from the initial level. Indicators of CD20+ (B-lymphocytes) had significantly increased from 8.4=1.1% to 12.1= 0.8% (t=2.720) by the 5th day. In terms of humoral immunity, there was a slight increase in IgA and a decrease in IgM, IgG. There was a significant decrease in the level of Ig G in the immediate period after splenectomy. This indicator had already decreased by the 5th day from 7.3± 0.5 g/l to 4.3± 0.7 (t=3.487) with the retention of the achieved level in the following days of observation. IgM and IdA levels changed at a slower pace, but their indicators had also significantly differed from the initial value by the 30th day of the experiment (Tab. 3.).

4. Discussion

The spleen performs a number of vital functions in humans' and animals' bodies. The main function of the spleen is to maintain the immune resistance of the body. The number of antibodies produced in the spleen is 60 times higher than that in the lymph nodes, while the fact that it is impossible to produce a full number of antibodies by other organs in the absence of the spleen has been proven [11].

Some authors have conducted narrowly focused studies aimed at studying the immune competence of the body after undergoing splenectomy. During the study of this issue, it was revealed that the removal of the spleen entailed a decrease in the production of immunoglobulins, a change in the function of phagocytes, a decrease in the number of opsonins, tafsin and complement, and a violation of the quantitative ratio of immunocompetent cells, which together led to the formation of pronounced violations of the specific immune response. At the same time, in most cases, it led to a decrease in the body's resistance to various infectious agents, and in 10% it became a cause of fatal sepsis development [9,12-13].

In our studies, there was a decrease in the level of CD3+, CD4+, CD16+, a significant increase of CD20+. A slight increase of IgA, a decrease of IgM, IgG was notedIn the indicators of humoral immunity.

The function of the spleen in thrombocytopoiesis is great, which is proved by pronounced thrombocytosis recorded in patients who have undergone splenectomy, which in turn leads to significant and difficult-to-correct disorders in hemostasis [7,14].

Analysis of early and late complications after splenectomy showed that in almost half of the patients thromboembolic and purulent-septic complications of varying severity were developed, among which the most threatening were thromboembolism of the pulmonary artery and its branches, cerebral vessels, as well as portal vein thrombosis, the cause of which was a violation of hemostasis in the form of hypercoagulation [5,8,15].

Our experimental studies showed that there was a significant increase in the level of fibrinogen from 2.8 ± 0.3 g/l to 3.8± 0.4 g/l on the 5th day, the thrombotest index was increased. But unlike the level of fibrinogen and PTI, there were no significant differences from the initial level.

The literature presents data from an interesting study in which a comparative analysis of the incidence of purulent-septic complications in patients after various surgical interventions in groups with a spleen and after splenectomy was carried out. The analysis showed that purulent-septic complications with identical pathologies were 58 times more likely to develop in the group of patients with a history of splenectomy, and mortality in this group was 14 times higher [16].

The very fact that during splenectomy, the body loses an organ that is the receptacle of one third of the lymphoid tissue and reticuloendothelial cells suggests that this surgery in any case will entail a violation of the immunological status of the patient in varying degrees of severity. The above data prompted surgeons to think about the need to make attempts to preserve the spleen. For the most part, this is possible with traumatic injuries of the spleen, but the main problem in this case is the difficulty of ensuring final hemostasis during organ-preserving surgery on the spleen.

Thus, the conducted studies show that splenectomy is accompanied by pronounced changes in the blood coagulation system, cellular and humoral immunity indicators are changed, which justifies the expediency of performing organ-preserving surgeries or performing heterotopic allotransplantation of the spleen (HATS).

5. Conclusions

The main functions of the spleen are to maintain the immune resistance of the body, correction of hemostasis

disorders, which dictates the expediency of performing organ-preserving surgeries at pathologies of the spleen.

Splenectomy is accompanied by significant shifts in various parts of hemostasis: there is an increase in the number of platelets and their functional activity; the release reaction of platelet factors is disrupted; the retraction index is decreased; activated partial thromboplastin time is shortened; the concentration of fibrinogen is increased; fibrinolysis is inhibited.

The postoperative period after splenectomy is accompanied by changes in cellular immunity (the content of CD3+, CD4+, CD16+ cells is reduced, the concentration of CD20+ is increased). Changes in humoral immunity consist in lowering the level of immunoglobulins of all classes (IgG, IgA, IgM).

To improve the results of treatment after splenectomy, it is necessary to perform heterotopic autotransplantation of the spleen, which allows to prevent changes in hemostasis and the immune system, and thereby reduce the risk of developing specific postoperative complications.

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