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EFFECT OF DIFFERENT MATERIALS USED FOR DURAPLASTY ON CHANGES OF THE PERIPHERAL BLOOD VALUES

Abstract. *We are doing this research study to find the changes in peripheral blood indices in experimental animals 2 weeks, 2 and 6 months after duraplasty. We used autologous fascia lata, a collagen based material and a chitin-chitosan film for duraplasty. Using allografts and materials of biological origin leads to a change of peripheral blood in the early postoperative period, indicating the development of general nonspecific reaction. 2 months after the operation all blood indices returned to the control ones. Using chitin-chitosan films does not lead to a significant decrease in hemoglobin and red blood cells and exhibits less pronounced inflammatory reactions.*
Key words. *Dura mater, duraplasty, blood indices, chitin-chitosan film.*

Introduction. As for medical, social and economic losses TBI leads in the structure of morbidity and mortality from injuries and ranks third in the structure of injuries. 1,5 million people die and 2.4 million become disabled worldwide every year due to TBI [7]. About 30% of patients with TBI of varying severity die in the prehospital phase, from 20 to 30% among those hospitalized die in hospital, and another 10-20% become disabled. 50-60% of discharged patients recover completely or have minor neurological complications, sustained abnormal function occurs in 7.1% of patients with TBI [3, 9].

In penetrating TBI the integrity of dura mater of brain and arachnoid of brain gets broken. This group of injuries include penetrating injuries of the skull, open depressed fractures of the cranial vault and skull base fractures involving leakage of cerebrospinal fluid (CSF). Replacement of DMB defects is made using materials of biological and non-biological origin, organic and inorganic nature [2].

The classic method for duraplasty uses the patient's fascia lata of thigh. The advantage of this technique is the absence of an immune response to the material, an ease of operation and lack of the financial burden on the patient or clinic. However, an additional trauma and a likelihood of postoperative complications restrict the use of this technique [4]. Today numerous medical tools used for duraplasty and based on natural (collagen) and synthetic materials have been developed [1, 5].

Chitosan and its derivatives is one of the materials which can be the base for the development of biocompatible implants [6]. Chitosan is a natural polysaccharide, which is derived from the chiton. Chitosan has no allergenic properties, it is biocompatible, biodegradable, has antibacterial properties and is able to stimulate regeneration. An important advantage of this material is its relatively low cost. Unfortunately, there are only rare data on the development of materials for duraplasty based on chitosan. In our previous work we proved the benefits of using a material based on chitin and chitosan, which are in the lack of toxicity, positive impact on the regeneration and the absence of inflammation [8]. We also studied blood indices during the experiment, which can be used to assess the safety of using the material.

Objective: to trace the changes of peripheral blood indices in the early and late postoperative periods after duraplasty with experimental chitin / chitosan film, with a piece of fascia lata of thigh and an agent based on collagen.

Materials and methods. The material for duraplasty was obtained from 3% solution of chitosan (mol. mass 200 kDa, degree of deacetylation is 80-90%). To this effect, 10 ml of 3% solution of chitosan in 1% acetic acid was poured onto Teflon circular substrate (substrate diameter 8 cm, height of the solution layer was

5 mm), the solvent evaporated at room temperature for 48-72 hours. To improve the mechanical properties and to reduce the degradation time of the film, chitin particles (1-2 mm) were added in the solution of chitosan. The ratio of chitosan and chitin was 50:50 and 80:20. Shaking homogeneously, we distributed particles in a viscous solution of chitosan to obtain a homogeneous suspension. The resulting film was treated with 5% NaOH solution for 2 hours, repeatedly washed with distilled water and treated with glycerin for 30 minutes to give it elasticity and softness.

Design of the experiment and surgery.

In order to compare the effectiveness of innovative implant we conducted a an experiment on 90 rabbits aged 3-4 months, which were divided into three series:

Series I (18 animals) – duraplasty was performed with the use of an autograft - the fascia lata.

Series II (36 animals) – duraplasty was performed with the use of collagen based material. The animals in this series were divided into 2 groups:

1st – plasty without fixing the material,

2nd – plasty with fixing the material using atraumatic suture.

Series III (36 animals) – duraplasty was performed with the use of membranes based on chitosan reinforced with chitin. The animals in this series were divided into 2 groups:

1st – plasty without fixing the material,

2nd – plasty with fixing the material using atraumatic suture.

After general anesthesia and peripheral vein catheterization, we shaved the head from the supraocular edge of the skull to the base of the ears. After treating the surgical field with a solution C-4, we performed a T-shaped incision, first in the frontal plane between the ears edges drawn before their foundation and then perpendicular to it in the sagittal plane from the line of the first cut on the middle line almost to the bridge of the nose. Triangular patches of skin were separated in different directions. The temporal muscle was disjointed by an arcuate cut and separated by means of a raspatory with the periosteum in the lateral direction but not more than required by future access for trepanation. Using a trepan and a sharp cutter 0.5 cm in diameter, two holes at 0.5-0.7 cm apart were made. The only trepanation access and aligning of sharp edges was made by the diagonal pliers. The wound was cleaned from chips and debris and, if necessary, hemostasis with the diploe was carried out. The DMB was dissected crosswise.

The fascia lata was fixed to the DMB using noninvasive suture material. The collagen-based material and chitosan-based implants were fixed due to their adhesive properties. In order to compare the effectiveness of fixing the membranes in the second subgroup of series II and III, we used an additional fixation of the materials by means of atraumatic suture . The implant was pre-formed larger than the defect, it was placed between the intact DMB and the inner surface of the bone (Figure).

After the plasty, without covering the bone defect, we performed myorrhaphy and passed to the other side of the head where we performed duraplasty with the same stages. The operation was completed by stitching the skin and applying aseptic dressings.

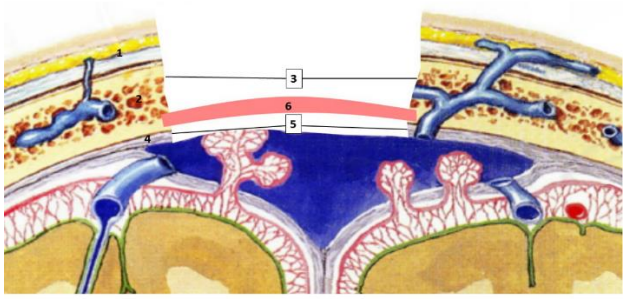

Figure. Scheme of duraplasty by using chitin-chitosan films: 1 – integument; 2 – skull; 3 – formed bone defect; 4 – dura mater; 5 – defect of the dura mater; 6 – material for plasty.

In order to assess the overall response of animals to the implant material the animals were taken 2 mL of venous blood before the surgery (control) and 2 weeks, 2 and 6 months after the plasty. We also tested the blood for hemoglobin, red blood cells, white blood cells and their populations and platelets.

All figures were recorded as mean value ± error of the mean value (M ± m). The reliability of the difference between the figures was

calculated using Student t-test using the program SPSS-Statistic 21.0 (trial-version).

Results and discussions. In intact animals the content of blood cells and hemoglobin corresponds to the literature on blood parameters of rabbits. Duraplasty with the fascia lata leads to a slight decrease in hemoglobin and erythrocyte count 2 weeks after the surgery, which may be the result of blood loss during the operation. (Table. 1).

Table 1

Hemoglobin and blood corpuscles at different time after duraplasty with a piece of the fascia lata.

	Hemoglobin	erythrocytes	leukocytes	Platelets
Control	103,6±7,3	4,32±0,67	6,86±1,3	256±34,5
2 weeks	91,6±3,5	3,98±0,39	9,66±0,85	297,6±51,3
2 months	100,2±1,8	4±1,2	7,9±0,65	279,2±25,8
6 months	105,6±4,7	4,3±0,92	6,96±1,03	286±40,3

However, after 2 and 6 months of observation the indices returned to the control level. The content of white blood cells increased after 2 weeks by 40.81% compared with the control and by15,16% (r≤0,05) after 2 months and only after 6 months of observation they returned to the control level. The periods of 2 weeks and 2 months after the surgery are also characterized by an increased proportion of neutrophils, basophils and eosinophils (Table. 2). The percentage of lymphocytes, in contrast, decreased by 64.92% (r≤0,05) 2 weeks and by 36.85% (r≤0,05) – 2 months after the plasty. The level of platelets and monocyte percentage remained reliably constant throughout the observation period.

Using a collagen-based material leads to similar changes in the peripheral blood but there is a difference due to the method of fixing the material. For instance, the level of hemoglobin and red blood cells reduced significantly after 2 weeks when using suture material for fixing artificial materials (Table. 3). With that, its recovery can only be observed at the last observation period, as opposed to a group of animals without fixation, in which hemoglobin and red blood cells indices return to that of the control as early as 2 months after the surgery.

The number of leukocytes increased by 36.44% 2 weeks after the surgery and does not depend on the method of fixing the material for duraplasty. Platelet count does not change during the experiment.

Table 2

Wbc blood at different time after duraplasty using a piece of the fascia lata.

	neutrophils	basophils	eosinophils	lymphocytes	monocytes
Control	44,2±2,3	1±0,2	1,2±0,3	45,6±2,9	8,4±1,2
2 weeks	70,2±3,9	3,2±0,5	1,6±0,5	16±1,7	8,8±0,6
2 months	61,6±1,6	2,6±0,4	1,4±0,4	28,8±3,3	5,4±0,9
6 months	43,8±3,3	0,8±0,3	1,2±0,2	50±4,2	6,6±0,5

Table 3

Hemoglobin and blood corpuscle at different time after duraplasty based on collagen.

	Hemoglobin	Erythrocytes	Leukocytes	Platelets
control	103,6±5,8	4,32±1,1	6,86±0,4	256±34,5
2 weeks (without fixation)	95,6±4,8	3,96±0,7	9,08±1,1	246±84,6
2 weeks (with fixation)	90±9,2	3,74±0,5	9,36±0,8	290±44,3
2 months (without fixation)	105±3,7	4,28±0,6	7,22±0,92	274,2±29,5
2 months (with fixation)	95±5,1	3,94±0,2	7,48±0,5	348±60,0
6 months (without fixation)	103,2±4,3	4,3±0,8	6,66±1,4	290±45,2
6 months (with fixation)	105,6±8,2	4,22±0,5	6,32±0,7	278±33,7

Changes in the leukogram in case synthetic materials are used are less significant compared to the plasty with a piece of the fascia lata. For example, the percentage of neutrophils increased from 47.05% (r≤0,05) after 2 weeks in the absence of fixing material to 64.25% (r≤0,05) with the suture material. However, unlike the previous series, the parameters of leukogram do not differ from the controls after 2 months of observation (Table. 4).

Table 4

Wbc blood at different times after duraplasty using a collagen based material

	neutrophils	basophils	eosinophils	lymphocytes	monocytes
Control	44,2±2,3	1±0,3	1,2±0,5	45,6±3,2	8,4±0,9
2 weeks (without fixation)	65±5,2	0,6±0,2	3,2±0,3	23,8±1,8	5,4±0,4
2 weeks (with fixation)	72,6±3,1	1±0,4	3,8±0,0,4	19,6±1,3	5,2±0,7
2 months (without fixation)	45±4,0	1,2±0,5	5,4±0,2	35,8±2,7	8,6±0,5
2 months (with fixation)	49,6±2,7	1,6±0,5	3,8±0,6	33,8±4,1	10,6±1,3
6 months (without fixation)	41,4±1,2	1,2±0,3	1,4±0,2	48±3,3	8,4±0,9
6 months (with fixation)	45,4±4,6	0,6±0,2	1,2±0,4	41,4±3,8	11,4±0,6

Using chitin-chitosan films is accompanied by a slight decrease in hemoglobin and erythrocyte count 2 weeks after the surgery, perhaps due to hemostatic properties of chitosan, as evidenced by many authors [6]. 2 and 6 months after the operation the blood parameters returned to their control levels. The number of leukocytes grew by18.2% (r≤0,05) after 2 weeks, whereas there was no significant difference between the group without the use of suture material, and a group of graft fixation. Chitosan is known to have antibacterial properties and anti-inflammatory action, so a slight increase in inflammatory cells may be indicative of the monomer action of chitin-chitosan film during its degradation. Fluctuations of platelets level during the experiment was unreliable (table. 5).

The leukogram indices show a slight growth

Table 5

Hemoglobin and blood corpuscle at different time after duraplasty with chitin-chitosan film.

	Hemoglobin	Erythrocytes	Leukocytes	Platelets
control	103,6±7,4	4,32±0,4	6,86±0,2	256±33,2
2 weeks (without fixation)	98,4±4,2	3,92±0,5	8,18±0,25	274±41,4
2 weeks (with fixation)	98,2±2,9	3,84±0,2	7,98±0,3	293±53,4
2 months (without fixation)	105±5,4	4,38±0,67	6,46±0,7	280±27,8
2 months (with fixation)	103,8±4,6	4,26±0,7	6,14±0,3	189±21,9
6 months (without fixation)	104±6,2	4,26±0,4	6,54±0,5	272±56,2
6 months (with fixation)	102,6±5,9	4,38±0,6	6,68±0,7	254±41,1

in overall reaction, especially in comparison with previous experimental animal groups. Neutrophil percentage increased by 28.05% (r≤0,05) and 37.1% (r≤0,05) after 2 weeks in animals without using suture material and those with using it respectively. The percentage of eosinophils increased a little, lymphocytes decreased with a maximum of 28.1% (r≤0,05). After 2 months, all leukogram indices returned to those of controls.

Conclusions. Thus, using the allografts and biological transplants causes changes in peripheral blood indices in the early postoperative period, indicating the development of nonspecific general reactions. 2 months after the plasty all the studied parameters correspond to the control ones. Applying chitin-chitosan film as a transplant

Table 6

Wbc blood at different time after duraplasty with a collagen based material

	neutrophils	basophils	eosinophils	lymphocytes	monocytes
Control	44,2±1,3	1±0,2	1,2±0,3	45,6±2,33	8,4±1,1
2 weeks (without fixation)	56,6±2,3	1±0,5	1,8±0,4	32,8±2,8	7,8±0,6
2 weeks (with fixation)	60,6±1,9	1±0,1	1,4±0,2	30±1,8	7±0,3
2 months (without fixation)	49,6±2,8	0,6±0,2	0,8±0,3	40,2±3,1	8,8±0,7
2 months (with fixation)	43,2±1,9	0,8±0,3	0,8±0,1	46,6±2,4	8,6±0,5
6 months (without fixation)	48±3,2	1,2±0,2	1±0,4	41,4±1,8	8,4±1,4
6 months (with fixation)	42,4±4,1	1,2±0,4	0,8±0,4	47,6±2,5	8±0,7

does not lead to a significant decrease in hemoglobin and red blood cells and demonstrates a less pronounced inflammatory response.

Prospects for further research. We are going to study the mechanisms of regeneration the defect of the dura mater using chitin-chitosan implants.

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