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EXPERIMENTAL GROUNDS OF USING PLATELET-RICH PLASMA TO STIMULATE THE LIVER REGENERATION IN CASE OF CHRONIC HEPATITIS

Abstract. *The paper presents data on the possibilities of using platelet-rich plasma (PRP) in simulating CCl4- induced chronic hepatitis. It has been established that double PRP direct injection into the tissue of the organ leads to a rapid regeneration with normalization of biochemical parameters of the hepatic function, while the animals without PRP correction underwent active fibrosing and reduced protein synthesizing liver function. The experimental data give grounds to assert that the use of platelet-rich plasma is a promising method for stimulating liver regeneration in conditions of chronic hepatitis.*
Key words: *experiment; platelet-rich plasma; regeneration; liver.*

Introduction. A key problem of regeneration of an organ or tissue is forming an adequate blood supply through the formation of the vasculature. During the last decade, there have been many reports on applications of platelet-rich plasma (PRP) to correct the pathology of the musculoskeletal system components; for transplant engraftment and others. [1-3]. In this case there is an active process of neoangiogenesis that provides opportunities to recreate the morpho-functional state of the body not at the expense of the connective tissue, as happens the most frequently, but by reconstructing its parenchyma [4].

Objective: Considering the above, the purpose of our study was to ascertain the effectiveness of PRP in the liver regeneration in conditions of chronic hepatitis (CH).

Materials and methods. The study was conducted on mature male Wistar albino rats. The animals were divided into groups: Group I – rats, which were simulated CH by oral administration of oil solution of carbon tetrachloride (CCl4) at a concentration of 50% at a dose of 0.05 ml a day for 7 - 8 weeks [5]; The second group of rats with CH which were injected PRP into the right lobe of the liver (twice at intervals of 1 week). A separate group of animals served as an intact control.

Obtaining the platelet-rich plasma was carried out by its separation from the whole blood in a machine SmartPrep (manufacturer Harvester Corp, USA), certificate of state registration number 10179/2011 in accordance with the Order of State Inspection of Ukraine of 08 February 2011 r. Number 69.

The pathologic study of the liver was performed by a standard staining technique [6] using conventional and polarizing microscopy with a light microscope «Leica-DMLS». We measured the content of erythrocytes (E), leukocytes (L) and platelets (T) in all experimental animals; the content of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, total protein in the animals with simulated CH (the research was conducted at the CI "Center for Veterinary Medicine", Odessa).

The day after the termination of CCl4 effect or after the last injection of PRP was considered the first day of the experiment. Euthanasia of animals was carried out by shifting the cervical vertebrae under a light ether anesthesia on the 10th and 30th days of the experiment. Preparing the animals for the experiment, all invasive surgery, pain management and leaving the experiment were carried out in compliance with the relevant regulations (the Law of Ukraine "On protection of animals against cruel treatment» № 27 art.230 of 2006 as amended pursuant to Law number 1759- VI (1759-17) of 15.12.2009, VVR, 2010, № 9, art. 76 and general ethical

animal experimentation (National Congress on bioethics, 20.09.2001., Kyiv) and the ethic Code of scientists of Ukraine (NASU, 2009)).

Results and discussion. On the 10th day after cessation of SCl4 injection there was a sharp increase in the content of white blood cells and the concentration of cytolysis enzymes in the blood of the experimental animals (Table). At the same time there was a significant reduction in total protein and an increase of total bilirubin (Table).

Table

Hemogram values and liver function biochemical parameters in animals with chronic hepatitis and after correction with PRP

value (units)	Control	Group I (CH)		Group II (CH+PRP)	
		10th -day	30th day	10th day	30th day
E (10^{12}/l)	6,6±0,4	6,0±0,4*	5,3±0,2*“	6,7±0,8	6,9±1,3
L (10^9/л)	8,0±0,2	31,0±2,2*	27,4±1,8 * “	33,0±3,1*	24,4±2,0* “
Pl (10^9/l)	205±12,7	193,1±14,7	167,0±15,4 * “	218,0±11,4*	201,3±12,8 “
ALT (U / L)	57±3,5	78,0±6,3*	84,0±4,9 *	76,3±5,2*	61,0±4,9 “
AST (U / L)	184±4,9	245,0±7,2*	243,0±12,0*	237,0±14,2*	188,2±10,1 “
Total bilirubin (micromole /l)	2,4±0,1	4,6±1,1*	4,2±0,8*	4,3±0,7*	3,0±0,8 “
Total protein (g/l)	86,8±5,6	60,0±11,2	51,0±4,7* “	57,6±3,1*	77,3±5,2* “

*Note.: * - the difference is reliable relatively to the control (p<0,05);*
“ – the difference is reliable relatively to the previous term (p<0,05)

The pathological study revealed some foci of hepatocytes necrosis, areas of infiltration, vascular stasis, hepatocytes with signs of hydropic and fatty degeneration, perivenularly – an accumulation of the connective tissue, blood vessels hyalinosis (Figure 1).

On the 10th day after the last injection of PRP there was an increase of leukocytes in the blood of rats compared to the animals in the control group; the ALT content is reliably higher than in the control group and is not very different from this value in the first group, AST is reliably lower compared to the animals from the first group, but significantly higher than in the control group of animals; total bilirubin content is significantly higher than the control group; total protein content was not reliably different from the rate of animals from the first group and is significantly lower than that in the control group (Table 1).

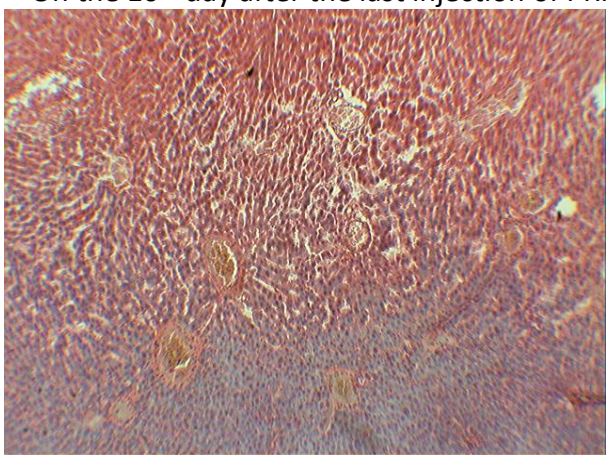

Figure 1. The structure of the liver of the animals in group I on the 10th day of the experiment (stained . hematoxylin-eosin, , magn. x200)

There are rare foci of necrosis in the hepatic tissue, blood vessels are dilated and filled, cells in a state of mitotic division are observed as well as a moderate perivascular fibrosis (Figure 2).

On the 30th day of the experiment the content of red blood cells and platelets in the blood of the first group animals continues to decrease, leukocytosis remains; the content of cytolysis enzymes remains significantly higher

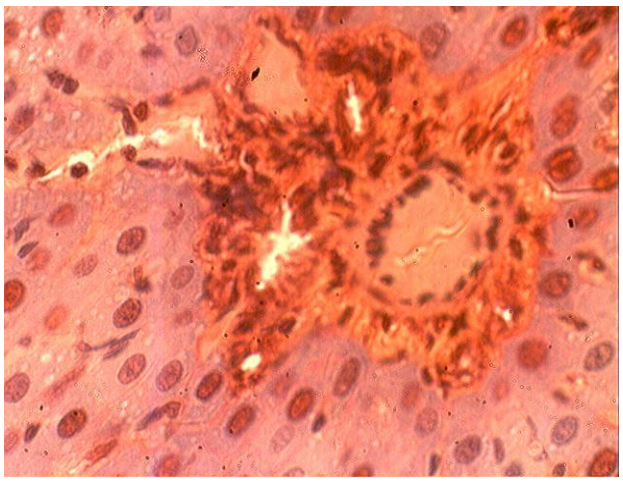

Figure 2. The structure of the liver of the animals in group II on the 10th day of the experiment (stained. hematoxylin-eosin, , magn. x400)

than that in the control group; total protein goes down both compared to the previous period, and to the control one, total bilirubin does not reliably change compared to the previous period, but remains significantly higher than the figure in the animals from the control group (Table 1).

There are disorders in the beam structure of the liver, necrosis foci, leukocyte infiltration, perivenular and perilobular fibrosis, fatty degeneration of hepatocytes (Figure 3).

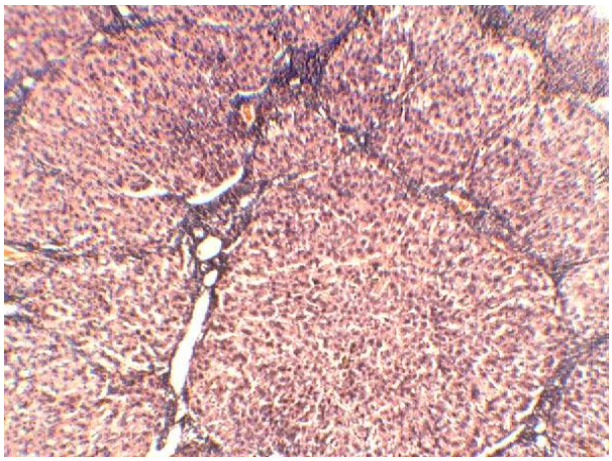

Figure 3. Hepatic tissue in the animals from the first group on the 30th day of the experiment (stained. hematoxylin-eosin, magn. x100)

In the second group of animals on the 30th day of the experiment, almost all hemogram values and cytolysis enzymes content were not reliably different from the control ones; leukocytosis remains, though with a noticeable tendency to decline; total bilirubin level is higher than in the control group, but this difference is not reliable; total protein is nearly identical to the control values (Table 1).

The beam structure in the liver is usual, the number of medium and small vessels increases, there are rare hepatocytes with signs of ballooning degeneration (Figure 4).

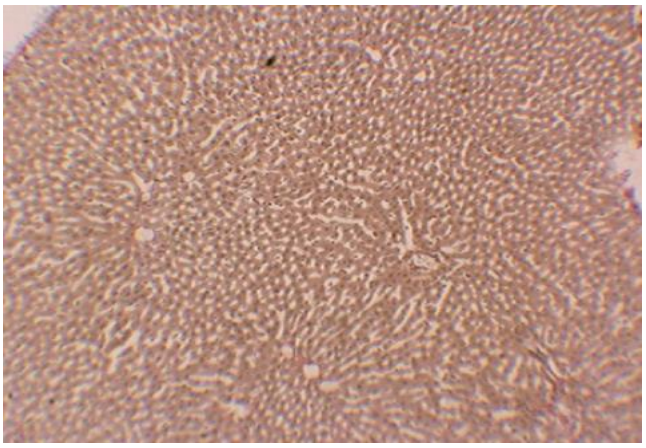

Figure 4. Hepatic tissue in the animals from the second group on the 30th day of the experiment (stained. hematoxylin-eosin, magn.x100).

The results show that in the group of animals with simulated pathological process distinct histological and functional changes, that are characteristic of active chronic hepatitis, occur. There were not any signs of regeneration in the liver up to the 30th day of observation after the cessation of toxic factor effect. The study that we had carried out earlier [7] showed a progressive proliferation of connective tissue, formation of false lobules, nodular transformation of the liver 6 weeks after the beginning of observation. However, there was a quite fast regeneration of the morpho-functional state of the liver in the group of animals which underwent correction of chronic hepatitis with platelet-rich plasma. This phenomenon can be explained by the influence of "cytokine cocktail" that comes as a part of PRP, namely: the vascular endothelial growth factor, platelet growth factor, endothelial growth factor and others. [8, 9]. The process of immune response to the damaging effect due to an activation of the immune system to minimize damage to cells for faster regeneration in a short term. [10] The powerful influence of high concentrations of biologically active substances that are involved in the processes of neoangiogenesis led to the growth of new blood

vessels that have created a unique framework for the diseased tissue, and progenitor cells came with the vessels. In addition, the progenitor cells in the PRP differentiate in the hepatic tissue into hepatocytes and stellate cells of the liver, preventing this way the formation of connective tissue components.

Conclusions. Considering the results of the experiment, it can be argued that the use of platelet-rich plasma is a promising method for stimulating liver regeneration in conditions of chronic hepatitis.

Prospects for further research. Further research should aim to clarify the duration of PRP effect, development of optimal doses and schemes of administration, identification of the basic biochemical processes occurring in the liver behind the correction with PRP. The question regarding the likelyhood of tumors at the areas where PRP is injected remains unanswered, we need a lnger experiment for that.

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