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INFLUENCE OF PLATELET-ENRICHED PLASMA ON THE MORPHO-FUNCTIONAL STATE OF LIVER IN RATS WITH INDUCED NON-ALCOHOLIC STEATOHEPATITIS AND DYSLIPIDEMIA

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Abstract

Non-alcoholic steatohepatitis (NASH) is a consequence of the progression of non-alcoholic fatty liver disease (NAFLD), which can lead to cirrhosis or hepatocellular insufficiency. NAFLD is a predictor of cardiovascular diseases (CVD) development. The important factor of the latter development is atherogenic dyslipidemia, and this is a type of 20-80 % NASH patients. The objective: to study the effectiveness of platelet-enriched plasma (PRP) influence on the morpho-functional state of liver in rats with non-alcoholic steatohepatitis. Materials and methods. The study was carried out on 80 adult male Wistar rats. Animals were divided into three groups: I group - animals that received an atherogenic diet for 90 days (n=10); II group - animals with simulated NASH and DL which received a normal diet for 30 days (n=30); III group - animals with simulated NASH and DL and PRP
correction \( n=30 \); group of intact animals \( n=10 \). NASH was simulated by introducing an atherogenic diet, which consisted of lard and 50 g / kg of butter for 90 days. PRP was punctured in the tissue of liver, twice at 0.05 ml with 7 days interval. The animals were taken out of the experiment after 90 days of the atherogenic diet and on the 30th day after its cessation or PRP injection. The indicators of body mass, liver mass index (LMI), lipogram, hepatic transaminases were determined, and the further pathological examination of the liver tissue was done. **Results and discussion.** On the 90th day of the atherogenic diet, the GH level was 3.19 ± 0.56 mmol/l, ALT activity was 118 ± 6.12 U/l, AST 86 ± 4.52 U/l, morphological signs of NASH were detected. On the 30th day, the lipid profile of group II rats did not have statistical differences from group I, in rats after correction of NASH with the use of PRP the level of LLD decreased by 51%, ALT activity - by 54%, AST - by 51% compared to control group \( p <0.05 \), morphologically revealed I - II degree steatosis, focal protein dystrophy. **Conclusions.** On the 90th day of an atherogenic diet NASH was formed in experimental animals. They had dyslipidemia, which progressed for at least 30 days while maintaining a usual diet. After correction with PRP the level of atherogenic lipoproteins was significantly decreased in rats with NASH and DL; the activity of liver enzymes was lower compared to the group with simulated NASH and DL and the group with progression of NASH and DL for 30 days, morphologically the decrease in liver steatosis and severity of protein dystrophy comparing with a group with simulated NASH and DL and a group with progressive NASH and DL was observed for 30 days.

**Key words:** experimental nonalcoholic steatohepatitis; dyslipidemia; platelet-rich plasma.

**Introduction.** Non-alcoholic fatty liver disease (NAFLD) is the most common diffuse liver disease in developed countries, where the number of patients is constantly growing [1,2]. In the near future, it is expected that the number of patients with cirrhosis caused by NAFLD will prevail over the share of cirrhosis of viral etiology and will be the leading cause of liver transplantation [3, 4].

It is known that NASH is a progressive form of NAFLD and occurs due to inflammation and apoptosis of hepatocytes [5]. With a long course of NASH may aggravate the pathological process with the subsequent development of complications such as liver cirrhosis, liver failure, or hepatocellular carcinoma [6].

With prolonged dyslipidemia (DL) there is an accumulation and overload of hepatocytes with lipids with subsequent disruption of the structural organization of cells and
the development of an inflammatory reaction [4-6]. The development of atherogenic dyslipidemia, including, leads to a decrease in the level of high-density lipoprotein (HDL) less than 1.0 mmol/l and the accumulation of low-density lipoprotein (LDL), which transport the bulk of plasma cholesterol esters directly to cells and have a high degree of ]. The accumulation of lipid inclusions leads to the release of free fatty acids, activation of lipid peroxidation, proinflammatory cytokines and the development of chronic inflammation of hepatocytes, which is mediated by the release of proinflammatory cytokines [4 - 6]. Thus, DL and NASH are interrelated processes [1, 2].

According to current recommendations of the American Society of Cardiology, first-line drugs for the treatment of DL are statins [8]. Statin therapy is a long-term one, so it often leads to side effects, in particular, of hepatobiliary system, which can be manifested by the development of cytolytic syndrome with increased liver enzymes, hepatitis, type 2 diabetes and, in severe cases, rhabdomyolysis [9, 10]. The combined course of NASH and DL is associated with an increased risk of side effects [9, 10], so the search for new, effective and safe methods of lipid-lowering therapy is an urgent issue.

In recent years much attention has been paid to the study of the effects of cell therapy in various fields of medicine [11]. In particular, platelet-enriched plasma (PLP) significantly reduces the destructive effects of hepatotoxic substances [12, 13, 14]. The positive effect of PLP on regeneration processes is due to the action of many bioactive substances, including hepatocyte growth factor, vascular endothelial growth factor, epithelial growth factor, transforming growth factor and insulin-like growth factor contained in platelet granules [11].

**The aim of the study**: to investigate the effectiveness of platelet-enriched plasma on the morpho-functional state of the liver in induced non-alcoholic steatohepatitis in rats.

**Materials and methods.** The study was conducted on 80 adult male rats, Wistar line, aged 3 - 4 months, weighting 130 - 150 gr. Animals were divided into groups: Group I consisted of animals kept on an atherogenic diet for 90 days (n = 10); Group II included animals with simulated NASH and DL, which received a normal diet for 30 days (n = 30); Group III consisted of animals with simulated NASH and DL and correction of PLP (n = 30); intact group (n = 10).

NASH simulation was performed by replacing the standard vivarium feed with an atherogenic diet with the addition of lard and butter at a rate of 50 g/kg for 90 days.

PLP was obtained by a series of centrifugation of peripheral blood of rats using SmartPrep (Harverst Corp.). PLP obtained was punctured into the right hypochondral area of liver tissue (as the most accessible area), twice by 0.05 ml with a 7 day interval, following the
rules of asepsis and antiseptics. The first day of PLP injection was considered the first day of the experiment. Animals were taken out of the experiment on the 90th day of pathology simulation and on the 30th day of PLP correction. For this dislocation of the first cervical vertebra under ether anesthesia was done.

All experimental animals were subjected to anthropometric measurements, including body weight and calculation of liver mass index (LMI). Laboratory tests, namely, general blood test (GBT), lipidograms - the level of total cholesterol (GC), triglycerides (TG), very low density lipoproteins (VLDL), low density lipoproteins (LDL), high density lipoproteins (HDL), calculation of atherogenic index (AI) and determination of hepatic transaminases activity: alanine aminotransferase (ALT), aspartate aminotransferase (AST) and de Ritis index (AST / ALT), determination of blood glucose levels. The hepatic tissue obtained was fixed in a buffered solution of formalin at a concentration of 10%, followed by pouring into paraffin "Histomix" and obtaining sections of 3 - 5 μm on a rotary microtome Leica RM2125, stained with hematoxylin - eosin (HE), according to Van Gison, Sudan III according to the standard methods of CLSI GP28-A [ISBN 1-56238-563-1] of the US Institute of Clinical and Laboratory Research.

Statistical data processing was performed using Microsoft Excel, Statistica 7.0. Estimation of the probability of difference of mean values was performed using a pair of Student's t-test. The results were considered statistically significant at a value of p <0.05.

All procedures with animals were carried out in accordance with international biotic rules and regulations (European Communities Council Directives of 24 November 1986, 86/609 / EEC) during the period of the year when the daylight exceeded 10 hours. Preparation of animals for the experiment, all invasive interventions, anesthesia and taking out of the experiment were carried out in accordance with the Law of Ukraine "On protection of animals from cruel treatment" № 27, Article 230 of 2006, as amended by the Law № 1759-VI (1759-17) dated 15.12.2009, Vidomosty Verhovnoyi Rady, 2010, № 9, p.76).

**Results and discussion.** On the 90th day of pathology simulation an increase in the level of GC by 68% (p = 0.03), VLDL - by 126% (p = 0.038), LDL - 2 times (p = 0.042). The level of HDL decreased almost thrice (p = 0.000005), AI was 5.25 ± 0.36 and increased almost 5 times (p = 0) compared with the intact group; ALT activity increased almost twice (p = 0), AST - 1.5 times compared with the intact group (p = 0), glucose level increased by 130% (p = 0000002) (Table 1). LMI was 6.3 ± 0.81, which is 57.5% (p = 0.000006) more than the intact group (Table 2).
Table 1 - Dynamics of biochemical parameters of blood serum of the studied groups

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Intact group, n=10</th>
<th>Group I, 90th day, n=10</th>
<th>Group II, 30th day, n=30</th>
<th>Group III, 30th day, n=30</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT, u / l</td>
<td>42 ± 3.05</td>
<td>118±6.12*</td>
<td>74.5±6.24*</td>
<td>54±6.31&amp;*</td>
</tr>
<tr>
<td>AST, u / l</td>
<td>35 ± 2.07</td>
<td>86 ± 4.52 *</td>
<td>78 ± 7.13 *</td>
<td>42 ± 2.64 &amp; #</td>
</tr>
<tr>
<td>de Ritis index</td>
<td>0.84 ± 0.1</td>
<td>0.66 ± 0.06</td>
<td>0.66 ± 0.01</td>
<td>0.79 ± 0.04</td>
</tr>
<tr>
<td>GC, mmol/l</td>
<td>1.9 ± 0.11</td>
<td>3.19 ± 0.53 *</td>
<td>2.98 ± 0.32 *</td>
<td>2.05 ± 0.16 &amp; #</td>
</tr>
<tr>
<td>LDL, mmol/l</td>
<td>0.57 ± 0.07</td>
<td>1.81 ± 0.37 *</td>
<td>1.62 ± 0.24 *</td>
<td>0.88 ± 0.12 &amp; #</td>
</tr>
<tr>
<td>VLDL, mmol/l</td>
<td>0.42 ± 0.05</td>
<td>0.95 ± 0.15 *</td>
<td>0.92 ± 0.17 *</td>
<td>0.40 ± 0.12 &amp; #</td>
</tr>
<tr>
<td>HDL, mmol/l</td>
<td>0.91 ± 0.07</td>
<td>0.31 ± 0.06 *</td>
<td>0.53 ± 0.07 *</td>
<td>1.32 ± 0.09 &amp; #</td>
</tr>
<tr>
<td>TG, mmol/l</td>
<td>0.9 ± 0.06</td>
<td>1.44 ± 0.12 *</td>
<td>1.39 ± 0.18 *</td>
<td>0.95 ± 0.12 &amp; #</td>
</tr>
<tr>
<td>AI, c.u.</td>
<td>1.1 ± 0.02</td>
<td>5.25 ± 0.36 *</td>
<td>4.21 ± 0.35 *</td>
<td>26 ± 0.17 &amp; #</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>4.08 ± 0.69</td>
<td>9.4 ± 0.63 *</td>
<td>8.2 ± 0.56 *</td>
<td>.21 ± 0.31 &amp; #</td>
</tr>
</tbody>
</table>

*Note: the indicators are presented in the form of M ± m, when M is the arithmetic mean, m is the deviation from the mean; * - p <0.05 compared with the intact group; & - p <0.05 in comparison with Group I; # - p <0.05 compared with Group II.

Table 2 - Body weight and liver mass index in experimental animals

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Intact group, n=10</th>
<th>Group I, 90th day, n=10</th>
<th>Group II, 30th day, n=30</th>
<th>Group III, 30th day, n=30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight</td>
<td>131 ± 10.21</td>
<td>250 ± 12.5 *</td>
<td>236 ± 11.61 *</td>
<td>205 ± 10.22 * &amp; #</td>
</tr>
<tr>
<td>LMI</td>
<td>4.0 ± 0.18</td>
<td>6.3 ± 0.31 *</td>
<td>6.1 ± 0.22 *</td>
<td>4.8 ± 0.13 * &amp; #</td>
</tr>
</tbody>
</table>

*Note: the indicators are presented in the form of M ± m, when M is the arithmetic mean, m is the deviation from the mean; * - p <0.05 in comparison with the intact group; & - p <0.05 in comparison with group I; # - p <0.05 compared with group II.

There was a tendency to leukocytosis and an increase in ESR in all group of animals, but the indicators were not statistically significant.

Macroscopically the liver was enlarged, pale yellow, dense. Microscopically there was pronounced protein dystrophy, signs of hydropic dystrophy, single areas with bridging necrosis with perivascular lymphocytic infiltration, centrolobular intracellular cholestasis and severe vascular plethora, single areas of centrilobular fibrosis, mainly centrolobular micro-, macrovesicular fatty degeneration, steatosis of III-IV degree. The changes detected correspond to the formed NASH (Fig. 1).

On the 30th day of the experiment the II group animals demonstrated a slight improvement in lipid profile and decrease of hepatic transaminases compared with the initial data, while but the indicators were not statistically significant (Table 1). Compared with the intact group, GC increased by 57% (p = 0.029), VLDL - by 119% (p = 0.008), LDL - by 184% (p = 0.002), HDL decreased by 42% (p = 0.005), AI increased almost 4 times and was 4.21 ± 0.35 (p = 0), glucose level increased by 101% (p = 0.00043), ALT activity was higher.
by 77% (p = 0.00039), AST - by 123% (p = 0.000001) (Table 1). The LMI was higher by 52.5% compared with the intact group and was 6.1 ± 0.22 (p = 0) (Table 2).

Fig. 1. Liver tissue of rat group I (90 days of atherogenic diet).

Signs of steatosis III-IV degree: Expressed diffuse macro- and microvesicular fatty infiltration of hepatocytes. Sudan III staining. Ampl. x200

Macroscopic examination of the II group animals liver showed liver enlargement in size, and pale yellow coloration. Histologically: diffuse protein dystrophy with foci of hydropic dystrophy, blood vessels plethora, bilirubinostasis, single areas of centrilobular fibrosis, steatosis of III – IV grade (Fig.2).

On the 30th day in the III group animals the data of blood serum biochemical examination in comparison with group II showed the following: GC was lower by 31% (p = 0.01), VLDL - by 56.5% (p = 0.015), LDL - by 45% (p = 0.08). HDL indicators increased twice and a half (p = 0), AI decreased by 70% and amounted to 1.26 ± 0.17 (p = 0), glucose level decreased by 36 % (p = 0.0000019). There was a decrease of hepatic transaminases, in particular ALT decreased by 27.5% (p = 0.02), AST - by 46% (p = 0.000015) (Table 1). The LMI was lower by 21% compared with group II and was 4.8 ± 0.71 (p = 0.000004) (Table 2). However, compared with the intact group, GC increased by 8%, LDL - by 54%, VLDL decreased by 5%, HDL increased by 45%, AI increased by 15%, glucose increased by 27%; ALT activity was higher by 29%, AST - by 20% but the data were not statistically significant (p> 0.05) (Table 1). Regarding LMI, it was higher by 20% (p = 0.0007) (Table 2). Compared with group I, the level of GC decreased by 36% (p = 0.047), vLDL - by 58% (p = 0.007),
LDL - by 51% (p = 0.02), HDL increased by more than thrice (p = 0); ALT activity decreased by 54% (p = 0), AST - by 51% (p = 0) (Table 1). LMI decreased by 24% in comparison with Group I (p = 0.00007) (Table 2).

Fig. 2. Liver tissue of rat group II (30 days of NASH progression).

Signs of steatosis III-IV degree: Expressed diffuse macro- and microvesicular fatty infiltration of hepatocytes.

Sudan III staining, Ampl. x200.

Pathomorphological examination revealed: liver’s enlargement, pale yellow coloration. Microscopically: foci of protein dystrophy, vascular plethora, lymphocytic infiltration (Fig. 3), microvesicular fatty dystrophy, grade I-II steatosis (Fig. 3).

In animals of the intact group on the 30th day of the experiment, biochemical parameters did not exceed the average values in male Wistar rats (Table 1). Macroscopically the liver was normal in size, dark red in color, moderate density. Microscopically histoarchitectonics was preserved, beam structure of tissue was clearly expressed, inflammatory and dystrophic changes were not observed (Fig. 4).

In studies about PLP effect on fibrosis affected hepatic tissue due to hepatotoxic substances action, PLP protective effect on hepatocytes was noted, as well as reduction of inflammatory phenomena, destructive effects of toxins, and manifestations of fibrosis, restoration of liver detoxification function [12, 13, 14]. The data given coincide with our results when correcting the simulated NASH and DL.
Fig. 3. Liver tissue of rat III group (30 days of the experiment correction of ZTP). Signs of steatosis I-II degree: Focal macrovesicular fatty infiltration of hepatocytes. Sudan III staining, Ampl. x200.

Fig. 4. Liver tissue of the rat intact group. Sudan III staining, ampl. x200.

Therefore, we can talk about the effectiveness of PLP as a promising method for the treatment of these hepatic pathological conditions.
**Conclusions:**

1. The use of atherogenic diet with additional oral administration of lard and butter at the rate of 50 g / kg for 90 days leads to the formation of non-alcoholic steatohepatitis and dyslipidemia, which progressed for at least 30 days and manifested by increased AI, atherogenic imbalance of lipid content, increased liver trasaminases activity, III-IV degree steatosis, expressed by protein dystrophy, areas of hepatocytes necrosis.

2. On the 30th day after PLP use in non-alcoholic steatohepatitis and dyslipidemia animals there was a decrease in the manifestations of cytolysis syndrome, partial normalization of lipid metabolism, a significant reduction in the manifestations of steatosis (I - II degree), small focal protein dystrophy.

3. Thus, it can be argued that PLP use is a promising area in the experimental therapy of non-alcoholic steatohepatitis and dyslipidemia and requires further research.

**Prospects for further research.** Further investigations are needed to study the efficacy of the developed experimental therapy in combination with standard hypolipidemic therapy at a long-term use, as well as to assess the dynamics of changes in morpho-functional status of liver after therapy cessation.

**References:**


