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REACTION OF RAT HEPATOCYTES DURING CORRECTION OF ACUTE EXPERIMENTAL ASEPTIC INFLAMMATION OF THE PERITONEUM BY THE ADMINISTRATION OF CRYOPRESERVED PLACENTA

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The study of the morphometric parameters of rat hepatocytes during the correction of acute experimental aseptic inflammation of the peritoneum was performed by the injection of cryopreserved placenta. It was found that starting from the 10th day of the experiment, the mean values of hepatocytes' large and small diameters, as well as the cell area, tended to increase. The value of the nuclear-cytoplasmic index from the 2nd to the 14th day of the experiment was higher than in the intact group. In the study of the size of hepatocytes' nuclei from the 3rd–5th day of the experiment, a gradual increase in their large and small diameters was noted, which reached maximum values on the 7th and 10th days. Analysis of morphometric parameters proves the efficacy of cryopreserved placenta in the treatment of inflammatory processes.

Key words: experiment, diameters of hepatocytes, morphometric study.

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РЕАКЦІЯ ГЕПАТОЦИТІВ ЩУРІВ ПРИ КОРЕКЦІЇ ГОСТРОГО ЕКСПЕРИМЕНТАЛЬНОГО АСЕПТИЧНОГО ЗАПАЛЕННЯ ОЧЕРЕВИНИ ШЛЯХОМ ВВЕДЕННЯ КРІОКОНСЕРВОВАНОЇ ПЛАЦЕНТИ

Проведено дослідження морфометричних показників гепатоцитів щурів при корекції гострого експериментального асептичного запалення очеревини шляхом введення кріоконсервованої плаценти. Встановлено, що з 10-ї доби експерименту середні показники великого діаметру гепатоцитів, а також показники малого діаметру клітин та їх площа мали тенденцію к збільшенню. Величина ядерно-цитоплазматичного індексу з 2-ої по 14-у доби експерименту була більше показників інтактної групи. При дослідженні розмірів ядер гепатоцитів з 3–5-ої доби експерименту повільно збільшувався великий та малий діаметр, на 7-у, 10-у доби величини мали найбільший показник. На 30-у добу експерименту всі показники досягали величини показників інтактної групи. Аналіз морфометричних показників доводить ефективність застосування кріоконсервованої плаценти в практиці лікуванні запальних процесів.

Ключові слова: експеримент, діаметри гепатоцитів, морфометричні дослідження.

The study is a fragment of the research project "Experimental and morphological study of the effect of cryopreserved preparations of cord blood and embryofetoplacental complex (EFPC), diferelin, ethanolol and 1 % ester of methacrylic acid on the morphofunctional state of a number of internal organs", state registration No. 0119U102925.

Today, the problem of treating hepatitis is even more significant and relevant, due to the fact that the disease in Ukraine is growing rapidly. At the current stage, the development of industry is increased and leads to an increase in the number of foreign, toxic and other pathogenic substances, which attack the important organs of the human body, particularly the liver [2, 6]. Detoxification of toxic substances, which may be endogenous and exogenous, occurs in the liver, to which a barrier (detoxifying) function is inherent. It is necessary to take into account that neutralizing factors of any genesis have negative effect on the cells of the liver, causing changes in the structure of hepatocytes, and as a result, lead to the impairment of their main functions [2, 6, 8, 14].

There are a lot of literary sources, dedicated to the development of severe experimental aseptic inflammation, as a negative impact on the morphological structure of the liver cells, namely hepatocytes. The state of hepatocytes is corrected by methods, aimed to restore the cell structure by hepatoprotective drug. There is also evidence of a positive effect of drugs during the correction of hepatocytes, but most authors do not specify the terms for the cells restoration in the case of using various drugs [8, 9]. Therefore, it is relevant and necessary to find and study the impact on metabolic disorders by new factors that should in the shortest possible time to restore both the structure and functional state of hepatocytes. In this regard, a great attention is drawn to tissue therapy, the use of which in the treatment of various pathologies is growing from year to year, so the results of this study are considered modern and effective means of restoring the organs under pathological conditions in many fields of medicine [4, 10, 13].

According to the literature, placental tissues contain biologically active substances in large quantities, which determines the use of placental tissue in modern medicine. In this case, the patient receives a number of balanced complexes and biologically active substances of natural origin that affect various parts of the body's metabolism as a whole, increase its nonspecific resistance and ability to resist adverse environmental factors and stressful situations, stimulate repair processes [4, 10 , 12, 13].

Based on in-depth research, the positive effect of cryopreserved placenta on the course of inflammatory processes has been proven [4, 10, 12, 13].

However, the large amount of available experimental and clinical data still does not fully reveal the mechanisms and timing of tissue therapy in the dynamics of the disease, therefore some issues remain unresolved, which proves the need for further studies in this field.

The purpose of the study was to determine the morphometric and statistical parameters of hepatocytes with the introduction of the cryopreserved placenta in rats against the background of acute experimental aseptic peritonitis.

Materials and methods. The object of the study was 50 sexually mature rats weighing 180-200 g, which were kept in normal conditions of the PDMU vivarium. Experimental studies were performed in accordance with the "General Principles of Animal Experiments", approved by the V National Congress on Bioethics (Kyiv, 2013) and in accordance with the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986), according to the Law of Ukraine No. 3447-IV of 21.02.2006 "On protection of animals from cruel treatment" and the Helsinki Declaration of the World Medical Association "Ethical principles of medical research with human participation as an object of study" (2010).

The experimental animals were divided into two groups: 1 – intact animals (5), 2 – animals (45), which underwent a single subcutaneous application of a fragment of cryopreserved placenta [4] against the background of acute experimental aseptic peritoneal inflammation, which was simulated by intraperitoneal administration of 5 mg – carrageenan (Sigma, USA), diluted in 1 ml of isotonic sodium chloride solution per 1 animal.

Terms of withdrawal of animals from the experiment: 1st, 2nd, 3rd, 5th, 7th, 10th, 14th, 21st and 30th days. Paraffin sections of rat liver were made according to generally accepted methods and used to measure hepatocyte metrics. [1, 3, 5].

For the analysis of morphometric parameters of hepatocytes, common statistical methods were used by means of Microsoft Office Excel 2007 [7, 11].

The probability of differences in quantitative results was determined using Student's t-test, they were considered statistically significant in the generally accepted in medical and biological examinations, the probability of error $p<0.05$.

Results of the study and their discussion. The performed morphometric studies permitted to establish that from the 1st to the 10th day of the experiment the large diameter of hepatocytes had mean values, which were smaller compared to the intact group and amounted to 23.128±0.488 μm (at $p<0.001$, $p1>0.05$). Their magnitude increased from the 10th to the 30th day of the experiment, but did not reach the intact index – 24.143±0.488 μm (at $p<0.001$, $p1>0.05$). The numerical index of the small diameter of hepatocytes was equal to the size of the intact group, but from the 10th day of the experiment began to increase and was 17.583±0.281 μm (at $p<0.001$), and by the 10th day exceeded intact – 17.538±0.197 μm (at $p<0.001$, $p1>0.05$).

In the study of the mean area of hepatocytes it was found that in the first days of the experiment it is lower than in animals of intact group In the study of the average area of hepatocytes it was found that in the first days of the experiment it is lower than in animals of the intact group – 1118.923±41.040 μm (at $p>0.05$, $p1>0.05$), from the 10th day it began to increase and on the 30th day of the experiment reached the intact – 1320.944±41.076 μm (at $p>0.05$, $p1>0.05$).

The nuclear-plasma index was calculated, which from the 2nd to the 14th day of the experiment was greater than the intact – 0.244±41.007 μm (at $p <0.001$, $p1 <0.001$), and on the 21st and 30th day reached the intact group – 0.195±0.006 μm (at $p>0.05$, $p1<0.001$) (Table 1).

When measuring the large diameter of hepatocyte nuclei, it was found that its index, starting from the 3rd day of the experiment, compared to the intact group, slowly increased – 10.198±0.104 μm (at $p>0.05$, $p1<0.001$). On the 7th and 10th days, its largest value was observed – 11.223±0.115 μm (at $p<0.001$, $p1<0.02$). Concerning the value of the intact group, this index reached on the 30th day of the experiment – 9.976±0.094 μm.

Studies of the small diameter of hepatocyte nuclei in comparison with the intact group revealed that its value began to increase from the 5th day – 9.594 ± 0.083 μm (at $p<0.001$, $p1<0.001$) and almost reached the intact by days 21–30 of the experiment.

Table 1

Mean indices of hepatocyte size

Days of experiment	Index			
	Large diameter (D)	Small diameter (d)	Area (S)	Nuclear cytoplasmic index (IG_S)
Intact	26.412±0.324	16.355±0.325	1364.225±35.982	0.196±0.006
1	23.128±0.488 p<0.001 p_1>0.05	16.222±0.291 p>0.05 p_1>0.05	1118.923±41.040 p>0.05 p_1>0.05	0.194±0.006 p>0.05 p_1<0.001
2	23.949±0.288 p<0.001 p_1>0.05	16.797±0.195 p>0.05 p_1<0.005	1266.038±22.603 p>0.05 p_1<0.05	0.208±0.004 p<0.001 p_1<0.001
3	23.611±0.301 p<0.001 p_1>0.05	16.230±0.271 p>0.05 p_1<0.002	1211.896±28.455 p<0.001 p_1<0.02	0.244±0.007 p<0.001 p_1<0.001
5	23.590±0.300 p<0.001 p_1>0.04	16.234±0.271 p>0.05 p_1<0.002	1212.896±28.450 p<0.001 p_1<0.02	0.243±0.007 p<0.001 p_1<0.001
7	23.612±0.301 p<0.001 p_1>0.03	16.312±0.271 p>0.05 p_1<0.002	1223.74±28.270 p<0.001 p_1<0.02	0.289±0.007 p<0.001 p_1<0.001
10	25.409±0.309 p>0.05	17.583±0.281 p<0.001	1407.001±24.080 p<0.001	0.236±0.008 p<0.001
14	25.272±0.288 p<0.02	17.552±0.156 p<0.002	1396.393±22.072 p>0.05	0.207±0.007 p>0.05
21	25.094±0.477 p<0.001	17.479±0.247 p<0.001	1387.491±38.039 p>0.05	0.158±0.006 p<0.001
30	24.143±0.488 p<0.001 p_1>0.05	17.237±0.291 p>0.05 p_1>0.05	1320.944±41.076 p>0.05 p_1>0.05	0.195±0.006 p>0.05 p_1<0.001

Note: p is an index of the statistical difference significance between the indices of the intact group; p1 – is an index of the statistical difference significance between the indices of the group with acute aseptic peritonitis in the appropriate period.

Numerical values of the mean area of the nuclei, starting from the 2nd day of the experiment, slowly increased and reached on the 5th, 7th and 10th day the value by 1.3 times greater than that of the intact – 322,848±6,275 μm (at p<0.001, p1<0.001). On the 30th day of the experiment, this figure became equal to the size of the intact group (Table 2).

Table 2

Mean hepatocyte nuclei size (M ± m, μm)

Days of experiment	Index		
	Large diameter (D)	Small diameter (d)	Area (S)
Intact	9.976±0.094	7.881±0.134	249.304±5.861
1	9.262±0.094 p<0.001 p_1<0.001	8.071±0.089 p>0.05 p_1<0.001	236.435±4.577 p>0.05 p_1<0.001
2	9.691±0.068 p<0.02 p_1<0.001	8.380±0.066 p<0.002 p_1<0.001	255.496±3.053 p>0.05 p_1<0.001
3	10.198±0.104 p>0.05 p_1<0.001	8.733±0.089 p<0.001 p_1<0.001	280.007±4.165 p<0.001 p_1<0.001
5	10.874±0.089 p<0.001 p_1<0.001	9.594±0.083 p<0.001 p_1<0.001	328.737±4.664 p<0.001 p_1<0.001
7	11.080±0.094 p<0.001 p_1<0.001	9.218±0.131 p<0.001 p_1<0.001	322.848±6.275 p<0.001 p_1<0.001
10	11.223±0.115 p<0.001 p_1<0.02	8.972±0.162 p<0.001 p_1<0.001	319.653±7.699 p<0.001 p_1<0.001
14	10.106±0.150 p<0.001 p_1<0.001	8.739±0.145 p<0.001 p_1<0.001	282.693±8.152 p<0.001 p_1<0.001
21	8.941±0.110 p<0.001 p_1<0.001	7.210±0.105 p<0.001 p_1<0.001	204.407±4.793 p<0.001 p_1<0.001
30	9.278±0.094 p<0.001 p_1<0.001	8.087±0.089 p>0.05 p_1<0.001	237.282±4.585 p>0.05 p_1<0.001

Note: p is an index of the statistical difference significance between the indices of the intact group; p1 – is an index of the statistical difference significance between the indices of the group with acute aseptic peritonitis in the appropriate period.

In this work, we investigated the quantitative characteristics of mononuclear and multinuclear hepatocytes. It was found that from the 3rd day to the 10th day of the experiment the number of mononuclear cells began to increase and reached 92.31±1.02 μm (at p<0.05). The number of multinuclear hepatocytes was increased from the 2nd to the 10th day of the experiment, compared to the intact group of animals – 9.84±1.3 μm (at p<0.05). It should be noted that on the 10th day of the experiment, hepatocytes and cell nuclei had all the indices with the highest values.

The study performed a correlation analysis of the large diameter of hepatocytes with the area of hepatocytes (r1), which revealed a direct correlation: the larger the diameter is, the larger is the area. After correction of acute experimental aseptic peritonitis with the introduction of cryopreserved placenta, starting from the 14th day of the experiment, this correlation increased compared to the intact group and its largest value was recorded on the 30th day of the experiment (r=0.82 at p< 0.001). Correlation analysis was not performed with a smaller diameter, as these data were not very informative.

We also studied the correlation analysis of the hepatocytes' mean area with a large diameter of the nucleus (r2), which showed that the correlation was direct and clearly traced from the 10th day of the experiment: the results increased compared to the intact (r=0.92 at p<0.001) and reached its value on the 30th day of the experiment (Table 3).

Table 3

Correlation between area and large hepatocyte diameter (r1) and hepatocyte area and large nucleus diameter (r2)

Indices	Intact	Days of experiment								
		1	2	3	5	7	10	14	21	30
r1	0.53	0.83	0.73	0.72	0.72	0.71	0.66	0.80	0.72	0.82
r2	0.71	0.76	0.69	0.81	0.73	0.66	0.92	0.85	0.85	0.86
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Analysis of the results of morphometric study revealed that from the 10th day of the experiment, the mean values of large diameter hepatocytes, as well as indices of small cell diameter and their area tended to increase. Compared to the intact, the value of the nuclear cytoplasmic index from the 2nd to the 14th day of the experiment was greater than that of the intact group.

In the study of the size of hepatocytes' nuclei a slow increase in large and small diameters was found from the 3–5th day of the experiment, on the 7th, 10th day the values were the largest.

From the 2–3rd day the number of mononuclear and multinuclear hepatocytes began to increase, on the 10th day of the experiment the index reached the maximumvalues.

There was a direct correlation between large diameter with hepatocyte area and hepatocyte area with large cell nucleus diameter compared to the intact group.

Starting from the 1st day of the experiment (r=0.83 p<0.001) and up to the 30th day, a direct correlation was found, which was established by studying the large diameter and area of the hepatocyte with the introduction of cryopreserved placenta to correct acute experimental aseptic inflammation of the peritoneum.

When comparing the area with a large diameter of the hepatocyte nucleus (r=0.86 p<0.001), the correlation was also defined as direct. Regarding the size of the small diameter of hepatocytes and nuclei – in the study, they were not taken into account because their results were less informative.

Thus, morphometric and statistical studies of hepatocytes' response to the introduction ofthe cryopreserved placenta in acute experimental aseptic inflammation of the peritoneum in rats permit to determine the time of liver cells' recovery, while most authors do not specify this when using different drugs [9, 11]. The use of cryopreserved placenta permits to restore both the structure and, probably, the functional state of hepatocytes in the shortest possible time [5, 12, 14, 15]

Conclusion

Analyzing the results of the experimental study, it should be noted that the method of treatment of acute experimental aseptic peritonitis, which we proposed, can be attributed to the effectiveness and, in terms of performance, easily accessible, which is practically uncomplicated.

The morphometric study revealed that the mean large diameter of hepatocytes, small cell diameter and their area tended to increase from the 10th day of the experiment. From the 3–5th day of the experiment, the large and small diameters of the hepatocyte nuclei slowly increased, and on the 7th and 10th days, the values were the largest. The value of the nuclear-cytoplasmic index from the 2nd to the 14th day of the experiment was greater than that of the intact group. On the 30th day of the experiment, all indices were equal to the values of the intact group.

It is proved that the use of a complex of biologically active substances contained in cryopreserved placenta in acute experimental aseptic peritoneal inflammation leads to the normalization of the morphological state of hepatocytes' structural components. When using cryopreserved placenta, the anti-inflammatory effect of biologically active substances is manifested by the restriction of alternative and increased reparative phenomena.

Thus, based on this study, we can assume that the introduction of cryopreserved placenta not only implements its multiple properties, such as immunomodulatory and immunostimulatory, desensitizing, antitoxic, hepatoprotective, stimulation of regeneration but also affects its influence and reduces recovery time.

Prospects for further research are the possibility of clinical use of cryopreserved placenta for the correction of inflammatory processes from the early stages of the disease in parallel with drugs that are part of treatment protocols in practical medicine.

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