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Activation of reparative liver regeneration following the combined transplantation of multipotent mesenchymal stromal cells and hepatic stellate cells

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Abstract

Aim. To study the effect of combined transplantation of multipotent mesenchymal stromal and hepatic stellate cells on the reparative liver regeneration.

Methods. Laboratory mice were given intravenous administration of multipotent mesenchymal stromal and hepatic stellate cells after partial hepatectomy. The mice were divided into four groups: control, experimental 1 (injection of multipotent mesenchymal stromal cells), experimental 2 (co-transplantation of multipotent mesenchymal stromal cells and hepatic stellate cells), the comparison group. Comparison of the experimental groups with the control group and the comparison group was carried out. Each group consisted of 14 animals. The control and experimental groups underwent partial hepatectomy. The experimental mice were injected with the cells into the lateral tail vein 1 hour after the operation. Multipotent mesenchymal stromal cells were administered at a dose of 4 million cells/kg (120 thousand cells/mouse), hepatic stellate cells — in the amount of 9 million cells/kg (270 thousand cells/mouse), suspended in 0.2 ml 0.9% NaCl solution. The control group animals were injected with 0.2 ml 0.9% NaCl solution into the lateral tail vein. The comparison group consisted of mice without partial hepatectomy, injected with 0.2 ml 0.9% NaCl solution. To assess reparative regeneration of the liver, morphometric parameters of the liver, blood biochemical parameters on the 3rd and 7th days after partial hepatectomy were studied. The severity of apoptosis was assessed by the immunohistochemical method, the activity of deoxyribonucleic acid (DNA) repair enzymes of the poly (ADP-ribose) polymerases was determined by flow cytometry. The number of micronucleated hepatocytes was also determined. The hepatocyte growth factor (HGF) content was measured by using an enzyme-linked immunosorbent assay in serum. The significance of differences in the compared samples was determined by using the Student's t-test. Statistical data processing was performed by using the SPSS Statistics software version 17.0.

Results. It was found that the combined transplantation of multipotent mesenchymal stromal and stellate liver cells causes restoration of the activity of alanine aminotransferase (a decrease of 30.3%, p=0.016), aspartate aminotransferase (a decrease of 27.7%, p=0.021), alkaline phosphatase (a decrease of 21.1%, p=0.036), an increase in the protein synthetic function of the liver (increase in albumin level by 36.6%, p=0.009), an increase in hepatocyte growth factor level by 74.3%. These changes were accompanied by the restoration of liver morphometric parameters: there was an increase in the mitotic activity of hepatocytes by 28.7% (p=0.008), the nuclear area of hepatocytes by 26.7% (p=0.006), the number of binucleated hepatocytes by 26.1% (p=0.004), which led to the restoration of liver mass. There was a decrease in the level of apoptosis by 28.8% (p=0.006) and a decrease in the number of micronucleated hepatocytes by 22.7% (p=0.001) compared with the control group, which may be related to an increase in the activity of Poly (ADP-ribose) polymerase repair enzymes detected in the study. The deviations were presented as a difference relative to the indicators of the control group (operated animals that were injected with 0.9% NaCl solution).

Conclusion. Combined transplantation of multipotent mesenchymal stromal and hepatic stellate cells activates reparative liver regeneration after partial hepatectomy.

Keywords: multipotent mesenchymal stromal cells, MSC, hepatic stellate cells, HSC, liver regeneration, partial hepatectomy.

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Background. The liver is regenerated at a physiologically low rate. Normally, in the liver, only 0.0012–0.01% of hepatocytes undergo mitosis. In the pathological liver, the rate of hepatocyte turnover increases by several orders of magnitude [1]. Cell proliferation begins in the periportal region. The resulting hepatocytes migrate along the hepatic trabeculae toward the central veins. Following resection, the remaining liver tissue undergoes hypertrophy [2, 3]. Two types of cells (hepatocytes and progenitor cells) are involved in restoring the mass of the liver segments remaining after surgery. Hepatic stellate cells (HSC; Ito's hepatic cells, perisinusoidal liver cells) are considered as one of the candidate hepatocyte progenitor cells [4].

HSCs are nonparenchymal cells that deposit vitamin A, provide remodeling of the extracellular matrix, participate in the regulation of the sinusoidal microvasculature, and synthesize growth factors (hepatocyte growth factor, vascular endothelial growth factor, epidermal growth factor, and transforming growth factor) [5]. Some authors emphasize the possibility of HSC differentiation into hepatocytes and cholangiocytes, which gives reason to consider them as liver stem cells [4, 6].

Considering the biological properties of HSCs, their use seems promising to activate liver regeneration after partial hepatectomy. Allogeneic transplantation of these cells may be accompanied by the development of immunological conflicts [7]. This can be avoided by combined HSC transplantation together with multipotent mesenchymal stromal cells (MMSC), which have immunosuppressive properties [8, 9]. In addition, the ability of MMSCs to activate reparative liver regeneration through the paracrine mechanism, through the formation of intercellular contacts, through fusion with hepatocytes, has been proven [10, 11].

This study aimed to analyze the effect of combined transplantation of MMSC and HSC on the activation of reparative liver regeneration after partial hepatectomy.

Materials and methods of research. The experiments were performed on 56 white outbred male mice aged 7–8 months, weighing 25–27 g. The experiments, care, and maintenance of animals were performed in accordance with Directive No. 63 of September 22, 2010 of the Presidium and Parliament of Europe "On the protection of animals used for scientific research" and the order of the Ministry of Health of the Russian Federation No. 267 of June 19, 2003 "On approval of the rules of laboratory practice."

The research was approved by the local ethics committee of the Ural State Medical University of the Ministry of Health of Russia, protocol No. 8 dated 10/20/2017.

The source of MMSC was the placental chorion of five female mice aged 3–4 months, with a gestation period of 18 days. The mononuclear cell fraction was obtained by sequential mechanical and enzymatic (accutase solution; Millipore, USA) processing of placental tissue. HSCs were isolated by collagenase-pronase perfusion of the liver with subsequent separation of cells in a histodense density gradient. MMSC were cultured in a CO_2 incubator (Thermo Scientific, USA) at a temperature of 37°C with a carbon dioxide level of 5% and a humidity of 90%. For transplantation into laboratory animals, MMSCs of the third passage were used. HSC were administered immediately after cell isolation [12].

Immunophenotyping of the MMSC suspension was performed by flow cytometry using monoclonal antibodies conjugated to fluorochromes (Becton Dickinson, USA). In the fraction of transplanted cells, the level of MMSCs with immunophenotypes positive for CD105 (Rat IgG_{2A} Anti-Mouse Endoglin/CD105-Fluorescein Clone 209701, RTU), CD29 (Rat IgG_{2A} Anti-Mouse Integrin beta 1/CD29-PE Clone 265917), Sca-1 (Rat IgG_{2A} Anti-Mouse Sca-1-APC Clone 177228) and negative for CD45 (Rat IgG_{2B} Anti-Mouse CD45-PerCP Clone 30-F11; Becton Dickinson, USA) on a Beckman Coulter Navios flow cytometer was assessed using the Mouse Mesenchymal Stem Cell Multi-Color Flow Cytometry Kit (Bio-Techne, USA). The count of viable cells with the $CD45^-CD105^+Sca1^+CD29^+$ phenotype was 93%.

The functional properties of MMSCs were assessed according to their capacity to differentiate into the adipocytic and osteogenic lineages. The composition of the medium that induced osteogenic differentiation was MesenCult™ Osteogenic Stimulatory Supplement (StemCell Technologies, Canada); that of adipocyte differentiation medium was MesenCult™ Adipogenic Stimulatory Supplement (StemCell Technologies, Canada) and MesenCult™ MSC Basal Medium (Mouse) (StemCell Technologies, Canada) in a ratio of 1:4, and 2 mmol of L-glutamine solution (StemCell Technologies, Canada).

Osteogenic differentiation was confirmed by a histochemical method to increase the expression of alkaline phosphatase (ALP), as well as by von Kossa staining, which revealed the presence of mineralized calcium phosphate. Differentiation in the adipocyte aspect was confirmed by histochemical staining of lipid vacuoles with Oil Red O.

HSCs were identified by flow cytometry by evaluating endogenous retinoid fluorescence. Cell viability before transplantation was determined

Table 1. Distribution of animals by group

Time of sacrifice	Control group	Experimental group 1 (MMSC)	Experimental group 2 (MMSC + HSC)	Comparison group
Day 3	7 mice	7 mice	7 mice	7 mice
Day 7	7 mice	7 mice	7 mice	7 mice

Note: MMSC — multipotent mesenchymal stromal cells; HSC — hepatic stellate cells.

using 7-AAD (7-aminoactinomycin D) dye; it was 95–97%.

The mice were divided into four groups, namely control, experimental 1 (administration of MMSC), experimental 2 (cotransplantation of MMSC and HSC), and a comparison group (mice without partial hepatectomy, which were injected with 0.2 ml of 0.9% NaCl solution).

Each group consisted of 14 animals. The control and experimental groups underwent partial hepatectomy by C. Mitchell and H. Willenbring. Zoletil 10 mg/kg (Virbac, France) was used for anesthesia [13]. The animals were sacrificed on postoperative days 3 and 7 by cervical dislocation (Table 1).

The cells were injected into the lateral tail veins of mice of the experimental groups, namely MMSC at a dose of 4 million cells/kg (120 thousand cells/mouse), and HSC in the amount of 9 million cells/kg (270 thousand cells/mouse), suspended in 0.2 ml of 0.9% NaCl solution. A 0.2 ml volume of 0.9% NaCl solution was injected into animals of the control group into the lateral tail vein. The comparison group consisted of mice without partial hepatectomy, which received 0.2 ml injections of 0.9% NaCl solution.

On the days 3 and 7 after the injection of cells, the liver morphometry and biochemical parameters of blood serum were examined. To assess the morphometric parameters of the liver, histological sections 3–5 μm thick were prepared and stained with hematoxylin and eosin. For morphometric data analysis, a computer program for image analysis (Biovision, Russia) was used. The count of hepatocytes per 1 mm^2, the area of hepatocytes, the area of the hepatocyte nucleus, the area of the hepatocyte cytoplasm, the nuclear-cytoplasmic index, the count of binucleated hepatocytes per 1 mm^2, and the mitotic index were analyzed.

To assess apoptosis, a set of primary [Caspase-3 Antibody (L-18) goat polyclonal IgG, 1:100; Santa Cruz Biotech, USA] and secondary (donkey anti-goat IgG-FITC, 1:100; Santa Cruz Biotech, USA) antibodies was used on histological sections to identify effector caspase-3. The amount of programmed death of hepatocytes was determined by calculating the apoptotic index.

The micronucleus test was performed after mechanical and enzymatic treatment [pronase E, type I collagenase, and DNase[1] (Sigma)] of liver cells and staining with 2.5% aceto-orcein with additional staining of the cell cytoplasm with 1% alcohol solution of light green [14].

To assess the severity of reparative processes in liver cells, the amount of poly-ADP-ribosapolymer[2], which is the product of the poly-ADP-ribosylation reaction, was analyzed, determining the primary [Anti-Poly (ADP-Ribose) Polymer antibodies, Abcam] and secondary [Rabbit Anti-Chicken IgY H&L (FITC)] antibodies on a flow cytometer. The average fluorescence intensity of the cell population was calculated, which characterizes the expression of antigens (receptor density) inside the cell [15].

Blood serum biochemical parameters [albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and ALP] were determined by kinetic methods using a Chem Well 2910 analyzer (Combi) and Olvex Diagnosticum reagents (Russia).

The HGF Mouse ELISA kit (Enzyme-Linked Immunosorbent Assay; Abcam) was used to measure quantitatively the hepatocyte growth factor in blood serum by enzyme immunoassay.

The initial data had a normal distribution. The Shapiro–Wilk test was used to test the normal distribution. The significance of differences in the compared samples was determined using the Student's *t*-test. The data were presented as arithmetic mean (M) and standard deviation. Statistical data processing was performed using the SPSS Statistics software package (version 17.0).

Results. When assessing the morphometric parameters of the liver on the day 3 after partial hepatectomy in animals after administration of MMSC and HSC, an increase in liver weight by 20.2% ($p = 0.005$) was revealed compared with the control group. The restoration of its mass was accompanied by an increase in the mitotic activity of hepatocytes by 23.8% ($p = 0.016$), a decrease in programmed cell death by 27.7% ($p = 0.022$), as well as an increase in the count of binuclear hepatocytes by 26.8% ($p = 0.008$), an increase in the area of the nucleus of hepatocytes by 25.5%

[1] DNA — deoxyribonucleic acid.

[2] ADP — adenosine diphosphate.

Table 2. Morphometric characteristics of reparative processes in the liver of mice on the day 3 after partial hepatectomy

Indicators	NaCl (comparison group)	NaCl + partial hepatectomy (control group)	MMSC + partial hepatectomy (experimental group 1)	MMSC + HSC + partial hepatectomy (experimental group 2)
Liver weight, g	1.81±0.15	1.04±0.09[1]	1.11±0.10[1]	1.25±0.10[1,2]
Apoptotic index, ‰	0.43±0.04	2.13±0.20[1]	1.96±0.19[1]	1.54±0.15[1,2]
Count of hepatocytes with micronuclei, ‰	2.21±0.18	3.37±0.26[1]	3.07±0.23[1]	2.97±0.20[1]
Mitotic index, ‰	0.74±0.06	8.1±0.60[1]	7.91±0.58[1]	10.03±0.75[1,2]
Count of hepatocytes, per 1 μm^2	1525.57±101.06	1206.72±91.96[1]	1167.86±93.55[1]	1160.0±113.14[1]
Hepatocyte area, μm^2	267.53±6.39	331.81±24.02[1]	343.71±22.61[1]	333.43±18.20[1]
Hepatocyte cytoplasm area, μm^2	219.14±7.12	243.64±19.25	237.63±15.94	249.14±9.84
Hepatocyte nucleus area, μm^2	48.40±3.57	67.13±7.01[1]	67.89±6.9[1]	84.29±8.61[1,2]
Nuclear-cytoplasmic index	0.22±0.02	0.27±0.01[1]	0.29±0.01[1]	0.34±0.02[1,2]
Count of binucleated hepatocytes, per 1 mm^2	234.43±9.92	380.97±10.15 [1]	386.71±21.39[1]	484.0±35.71[1,2]

Note: [1]$p < 0.05$ with comparison group; [2]$p < 0.05$ with a control group; MMSC — multipotent mesenchymal stromal cells; HSC — hepatic stellate cells.

($p = 0.011$) and the nuclear-cytoplasmic index by 24.9% ($p = 0.003$).

On the day 3 after MMSC transplantation in animals with partial hepatectomy, no changes in the morphometric parameters of the liver were revealed relative to the control group (Table 2).

On the day 7 after the administration of MMSC in mice with partial hepatectomy, the liver mass increased, which was due to an increase in the mitotic activity of hepatocytes, inhibition of apoptosis, an increase in the count of binuclear hepatocytes, and an increase in the area of the nucleus.

At the same time, with cotransplantation of MMSC and HSC, on day 7 of follow-up, the morphometric parameters of mice in the experimental group retained the changes detected on day 3, namely a decrease in programmed cell death of hepatocytes by 28.8% ($p = 0.014$), an increase in the count of binuclear hepatocytes by 26.1% ($p = 0.006$), an increase in the size of the nucleus by 26.7% ($p = 0.001$), and an increase in the nuclear-cytoplasmic index by 31.0% ($p = 0.001$). The increase in the count of binucleated hepatocytes can be explained by the fact that in the early stages of reparative regeneration, a significant proportion of mitoses is acytokinetic.

Also, on day 7 after partial hepatectomy, increased mitotic activity was retained, contributing to the restoration of liver mass. An increase in the activity of DNA repair enzymes was noted, which resulted in a decrease in the count of hepatocytes with micronuclei in experimental groups 1 and 2 (Table 3).

Partial hepatectomy was accompanied by a significant increase in the level of hepatocyte growth factor in the blood serum on postoperative day 3. The administration of MMSC did not lead to a significant change ($p = 0.723$) this indicator relative to the control group, while cotransplantation of MMSC and HSC caused an even greater increase in the level of hepatocyte growth factor (by 32% compared with the indicator of the control group; $p = 0.001$).

On day 7 after partial hepatectomy, the effect of the injected cells was also noted. The level of hepatocyte growth factor was 35% in experimental group 1 ($p = 0.002$), and in experimental group 2, it was 57% higher than in the control group ($p = 0.001$; Table 4).

When the biochemical parameters of blood on day 3 in experimental group 1 were analyzed, the activity of enzymes characterizing the cytolysis of hepatocytes (AST, ALT) decreased. In the group of mice injected with MMSC and HSC, a decrease in the activity of the enzymes of hepatocyte cytolysis and cholestasis (ALP) with a simultaneous increase in the concentration of urea was revealed (Table 5).

On day 7 after partial hepatectomy in experimental group 2, the levels of total protein and albumin increased, while the levels of total protein and albumin reached the values of the comparison group. An increase in the level of urea, a decrease in the level of cytolysis enzymes (AST, ALT) and cholestasis (ALP) were also recorded, and the levels of enzymes were similar to those of the comparison group. An increase in glucose and

Table 3. Morphofunctional characteristics of reparative processes in the liver of mice on day 7 after partial hepatectomy

Indicators	NaCl (comparison group)	NaCl + partial hepatectomy (control group)	MMSC + partial hepatectomy (experimental group 1)	MMSC + HSC + partial hepatectomy (experimental group 2)
Liver weight, g	1.76±0.13	1.15±0.09[1]	1.53±0.12[2]	1.48±0.09[2]
Apoptotic index, ‰	0.39±0.03	1.25±0.09[1]	0.94±0.07[1.2]	0.89±0.08[1.2]
Count of hepatocytes with micronuclei, ‰	2.18±0.11	2.77±0.23[1]	2.21±0.16[2]	2.14±0.18[2]
Mitotic index, ‰	0.73±0.06	4.51±0.47[1]	5.76±0.49[1.2]	5.80±0.37[1.2]
Activity of enzymes of the PARP family in liver cells, MFI	45.2±4.1	59.3±5.21[1]	80.6±7.5[1.2]	86.3±8.06[1.2]
Count of hepatocytes, per 1 μm^2	1538.14±103.59	1427.71±116.98	1485.14±116.20	1354.0±138.0
Hepatocyte area, μm^2	264.66±5.87	286.41±22.44	275.14±24.16	292.57±20.94
Hepatocyte cytoplasm area, μm^2	214.21±8.10	223.03±17.97	204.37±22.80	212.21±13.88
Hepatocyte nucleus area, μm^2	50.46±3.29	63.39±5.12 [1]	76.63±4.92[1.2]	80.36±7.08[1.2]
Nuclear-cytoplasmic index	0.24±0.02	0.29±0.02[1]	0.38±0.02[1.2]	0.38±0.02[1.2]
Count of binucleated hepatocytes, per 1 mm^2	237.29±8.24	320.77±10.64[1]	393.90±23.23[1.2]	404.71±27.47[1.2]

Note: [1]$p < 0.05$ with comparison group; [2]$p < 0.05$ with a control group; MMSC — multipotent mesenchymal stromal cells; HSC — hepatic stellate cells; MFI — mean fluorescence intensity of the cell population.

Table 4. Level of hepatocyte growth factor in the blood serum

Day after surgery	NaCl (comparison group)	NaCl + partial hepatectomy (control group)	MMSC + partial hepatectomy (experimental group 1)	MMSC + HSC + partial hepatectomy (experimental group 2)
Day 3	4.22 ± 0.35	18.21 ± 1.76[1]	20.02 ± 2.0[1]	24.12 ± 2.36[1,2,3]
Day 7	4.49 ± 0.39	9.58 ± 0.88[1]	13.0 ± 1.02[1,2]	16.7 ± 2.62[1,2,3]

Note: [1]different from the comparison group at $p < 0.05$; [2]different from the control group at $p < 0.05$; [3]different from the experimental group at $p < 0.05$; MMSC — multipotent mesenchymal stromal cells; HSC — hepatic stellate cells.

Table 5. Biochemical parameters of the blood of mice on day 3 after partial hepatectomy

Indicators	NaCl (comparison group)	NaCl + partial hepatectomy (control group)	MMSC + partial hepatectomy (experimental group 1)	MMSC + HSC + partial hepatectomy (experimental group 2)
Total protein, g/l	68.31±3.93	50.03±4.82[1]	55.61±4.24[1]	53.67±4.34[1]
Albumin, g/l	30.84±4.25	19.80±2.51[1]	21.60±3.14[1]	22.01±2.04[1]
Urea, mmol/l	6.21±0.87	4.37±0.33[1]	4.59±0.36[1]	5.26±0.29[1.2]
Aspartate aminotransferase, U/L	97.26±8.47	209.53±13.85[1]	158.99±14.38[1.2]	156.97±13.35[1.2]
Alanine aminotransferase, U/L	81.13±8.66	155.24±9.38[1]	116.73±12.51[1.2]	114.16±13.53[1.2]
Alkaline phosphatase, U/L	66.34±5.24	106.67±10.45[1]	82.0±7.26[2]	83.47±8.40[1.2]
Glucose, mmol/l	5.73±0.69	3.66±0.29[1]	4.20±0.46[1]	3.87±0.32[1]
Total bilirubin, μmol/l	8.90±1.14	21.99±5.47[1]	21.29±2.36[1]	20.13±1.61[1]
Fibrinogen, g/l	3.27±0.18	2.27±0.26[1]	2.11±0.18[1]	2.67±0.22[1]

Note: [1]$p < 0.05$ with comparison group; [2]$p < 0.05$ with a control group; MMSC — multipotent mesenchymal stromal cells; HSC — hepatic stellate cells.

Table 6. Biochemical parameters of the blood of mice on day 7 after partial hepatectomy

Indicators	NaCl (comparison group)	NaCl + partial hepatectomy (control group)	MMSC + partial hepatectomy (experimental group 1)	MMSC + HSC + partial hepatectomy (experimental group 2)
Total protein, g/l	66.46±4.36	44.27±3.62[1]	49.63±2.80[1]	60.27±5.09[2,3]
Albumin, g/l	31.41±3.38	20.59±1.90[1]	23.13±2.32[1]	28.06±2.16[2]
Urea, mmol/l	6.11±0.61	4.57±0.46[1]	5.57±0.48[2]	5.63±0.35[2]
Aspartate aminotransferase, U/L	104.56±9.07	153.86±16.96[1]	103.57±12.42[2]	111.21±10.01[2]
Alanine aminotransferase, U/L	89.23±4.43	137.10±16.29[1]	86.34±7.52[2]	95.50±8.57[2]
Alkaline phosphatase, U/L	63.30±4.00	83.11±5.93[1]	64.23±6.00[2]	65.61±4.36[2]
Glucose, mmol/l	6.44±0.62	4.30±0.29[1]	4.91±0.51[1]	5.20±0.34[1,2]
Total bilirubin, μmol/l	9.37±0.65	15.41±2.76[1]	14.81±2.02[1]	13.70±0.83[1]
Fibrinogen, g/l	3.20±0.23	2.20±0.23[1]	2.83±0.20[2]	2.87±0.24[2]

Note: [1]$p < 0.05$ with comparison group; [2]$p < 0.05$ with a control group; [3]$p < 0.05$ with experimental group 1; MMSC — multipotent mesenchymal stromal cells; HSC — hepatic stellate cells.

fibrinogen levels was also revealed. The administration of MMSC was not accompanied by restoration of the protein-synthetic function of the liver (Table 6). In addition, an increase in the level of total protein in experimental group 2 was detected relative to that in experimental group 1 (Table 6).

Thus, the studies enable the establishment of a positive effect of combined transplantation of MMSC and HSC on the morphofunctional state of the liver after partial hepatectomy.

Discussion. In this work, we studied the effect of MMSC and combined transplantation of MMSC and HSC on the restoration of hepatic morphofunctional parameters after partial hepatectomy. The data obtained indicate the advantage of combined transplantation of these types of cells in partial hepatectomy. It was revealed that combined transplantation of MMSC and HSC provides an increase in liver weight already on day 3, and on day 7 it is restored to the values of the comparison group due to an increase in the mitotic activity of hepatocytes and a decrease in programmed cell death.

The literature provides the data indicating the efficiency of HSC transplantation in case of partial hepatectomy. At the same time, the mechanisms of the participation of these cells in the activation of reparative regeneration have been established. The authors demonstrated that these cells have an effect through the production of biologically active substances, as well as through differentiation into hepatocytes [4].

The positive effect of the combined cell transplantation obtained in this study can be explained by the ability of HSC to produce hepatocyte growth factor, which serves as a powerful mitogen for hepatocytes, promoting the activation of cellular and intracellular regeneration. The revealed change in blood biochemical parameters [normalization of the level of cytolysis enzymes (AST, ALT) and cholestasis (ALP)] can be due to the ability of MMSC to produce anti-inflammatory factors [16, 17].

The studies also enable the establishment of a mechanism for reducing the count of hepatocytes with micronuclei, which are known to reflect the level of pathological mitoses. In turn, the decrease in cells with micronuclei may be due to the revealed activation of the DNA repair system. DNA repair enzymes of the PARP family, correcting damage in the DNA structure, cause a decrease in programmed cell death and decrease the amount of pathological mitoses.

CONCLUSIONS

1. Allogeneic combined transplantation of MMSC isolated from the placental chorion and HSC promotes the activation of liver regeneration. This effect is manifested in the activation of the mechanisms of cell regeneration by increasing the mitotic activity of hepatocytes and inhibition of programmed cell death.

2. The cotransplantation of these types of cells is accompanied by a decrease in the amount of pathological mitoses and an increase in the count of binuclear hepatocytes.

3. The studies indicate the ability of combined transplantation of multipotent mesenchymal stromal and HSC to ensure restoration of the morphofunctional state of the liver after partial hepatectomy and provide a basis for pilot clinical trials.

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