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MONITORING OF MORPHOLOGICAL EFFECTS AUTOLOGICAL MESENCHIMAL STEM CELLS, TRANSPLANTED IN LIVER WITH VIRUS CYRROSIS (CLINICAL OBSERVATION)

Abstract

Introduction. The importance of the HCV-infection is determined by the wide spread, progressive course, the formation of liver cirrhosis (LC) and hepatocellular carcinoma. The mechanisms of the effect of the virus on hepatic cells, the processes of fibrogenesis and fibrolysis, mechanisms of the reverse development of the LC remain unexplored. There is no effective pathogenetic therapy. **The objective of the study:** to determine the effectiveness and safety of intrahepatic transplantation of mesenchymal stem cells (MSCs) in chronic HCV-infection at the stage of LC. **Materials and methods.** A patient with HCV-LC who has a secondary hemorrhagic vaculities who underwent autologous MSCs transplantation into the liver tissue. The liver biopsy specimens were studied in dynamics by light and electronic microscopy and by immunohistochemistry. **Results.** The transplantation and posttransplantation periods proceeded without complications. After the introduction of MSC the signs of micronodular LC remained. In some parts of the samples, the septa looked thin, sometimes perforated, indicating a resorption in this place of fibrous tissue. There was a decrease in the degree of transdifferentiation of stellate cells into myofibroblasts, a decrease in the number of fibrocytes and fibroblasts, there were no immune reactions in the form of deposition of amorphous and fibrous masses of moderate electron density along the sinusoidal capillaries that were significantly expressed in the primary biopsy. These changes were combined with the appearance of hepatocyte heterogeneity in the density of the cytoplasmic matrix, the state and quantity of organelles and inclusions, and the structural improvement of intracellular organelles. **Conclusion.** Autologous transplantation of mesenchymal bone marrow stem cells reduces the degree of destructive changes in hepatocytes, the severity of fibrosis and contributes to the improvement of the morpho-functional state of the liver, and therefore, it can be recommended as an important component of medical interventions.

Key words: *Cirrhosis, hepatitis C virus, liver damage, mesenchymal bone marrow stem cells*

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HCV — hepatitis C virus, GGT — gamma-glutamyl transpeptidase, HSC — hepatic stellate (Ito) cells, IHCR — immunohistochemical reaction, MSCs — mesenchymal stem cells, Mh — mitochondrion, LC — liver cirrhosis, ALP — alkaline phosphatase

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Introduction

The chronic infection caused by the hepatitis C virus (HCV) has been studied for more than 25 years since the discovery of the pathogen. Since then, the structure and life cycle of the virus as well as the natural course and epidemiology of the disease have been studied, the economic burden of the infection on society has been convincingly demonstrated, and antiviral therapy drugs have been developed. At the same time, the persisting significance of the disease has been determined by a number of components: data on the high prevalence and progressive course of the disease, including the development of liver cirrhosis (LC) and hepatocellular carcinoma as well as the possibility of viral replication not only in hepatocytes, but also in B lymphocytes [7, 11,17]. The mechanisms by which the virus affects hepatic cells, the regeneration of hepatocytes, the processes of fibrogenesis and fibrolysis, including those in patients with hepatic and extrahepatic viral replication, remain underexplored, and the mechanisms of already developed LC regression are poorly known. There is no effective pathogenetic therapy that would promote such a regression.

The objective of the study is to determine the effectiveness and safety of intrahepatic transplantation of mesenchymal stem cells (MSCs) in chronic HCV infection at the LC stage.

Materials and methods

In what follows we will present the result of autologous MSCs transplantation in the liver tissue of patient T. with HCV-LC and secondary (caused by HCV) hemorrhagic vasculitis.

The antibodies to HCV (anti-HCV+) were detected in the blood serum of the patient T. for the first time in 1998. She was followed-up by an infectious disease specialist at the community-based facility with a diagnosis of chronic hepatitis C (RNA HCV+) that was moderate in its degree of clinical and biochemical activity. She never received antiviral therapy. Follow-up laboratory blood tests revealed signs of active chronic hepatitis with an increase of the aminotransferases values that were 3–4 times in excess of the upper limit of the normal range, signs of kidney injury (erythrocyturia of up to 40 cells per field of view), and increased rheumatoid factor activity. No evidence of hematological abnormalities was revealed when the patient was examined by a hematologist. Vasculitis affecting the skin and kidneys was diagnosed. The patient started taking prednisolone at 40 mg/day in 2007. Periodic exacerbations of vasculitis were recorded when the drug dose was reduced. The patient refused treatment with antiviral therapies.

Since 2005, the following principal diagnosis was made on the basis of the presence of the process activity and the progressive course of the disease while taking into account the changes revealed in the examinations results: LC of viral (HCV) etiology (anti-HCV+, RNA HCV+, HCV genotype 1), Child-Pugh class A. Intrahepatic portal hypertension. Splenomegaly. Thrombocytopenia. Esophageal varices, grade 1. Vasculitis affecting the skin and kidneys (unconfirmed membranoproliferative glomerulonephritis). Secondary diagnosis: chronic pancreatitis, gallstones, chronic calculous cholecystitis. In 2009, the patient was invited to participate in an ongoing clinical study on transplantation of autologous MSCs from the bone marrow. The study design proposed an initial period of hospitalization in order to conduct a comprehensive examination and harvest MSCs from the bone marrow. Diagnostic tests and instrumental methods included a biochemical blood test with determination of total bilirubin, AST, ALT, alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGTP), creatinine, urea, and alpha-fetoprotein values; complete blood count and urinalysis; prothrombin time and INR tests; ultrasound examination of the abdominal organs; blood HCV RNA PCR test; and percutaneous liver biopsy with a complex morphological study of the biopsy specimen in order to establish possible regression of liver fibrosis. The above mentioned tests were subsequently used to monitor the patient's status in the post-transplant period. Rehospitalization was provided 1 month later for the purpose of transplanting MSCs into the liver tissue and to assess the safety and tolerability of the procedure. The purpose of the follow-up hospitalization 6 months after transplantation was to monitor the effectiveness and to assess the long-term effects of therapy.

In August 2009, the patient was admitted to the hospital of the Belarusian Research Center for Pediatric Oncology, Hematology and Immunology.

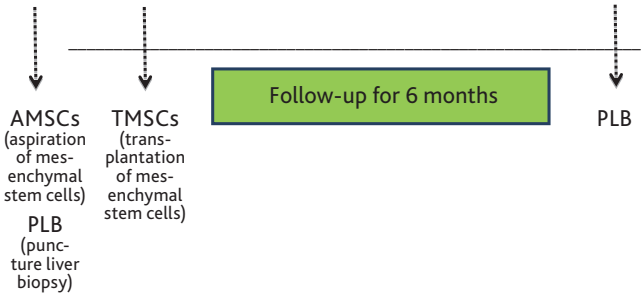

Figure 1. *Study design*

Note: AMSC — autologous of mesenchymal stem cells; PLB — puncture liver biopsy; TMSCs –transplantation of mesenchymal stem cells

Clinical examination confirmed an infection caused by the HCV genotype 1, with moderate biochemical disease activity at the stage of formed LC. The blood PCR test revealed the replication activity of the virus with HCV RNA value of 3.39×10^5 IU/ml. MELD score was 13. Signs of vasculitis were recorded: vascular purpura in the area of the shins, kidney injury (erythrocyturia), and arthralgias. Blood test for autoimmune diseases, including anti-mitochondrial M2 antibodies (AMA-M2), antinuclear antibodies (ANA), anti-liver/kidney microsomal antibodies type 1 (LKM-1), produced negative results. HIV infection and HBV infection were ruled out by the blood EIA test.

During the initial hospitalization, the bone marrow was harvested using several iliac bone punctures under local anesthesia in aseptic conditions. The MSC autograft was obtained from 35 ml of the patient's bone marrow; 242×10^6 MSC were isolated. Three passages were carried out afterwards to obtain a sufficient number of MSCs, and the duration of cell culture was 42 days. As a result of cell expansion *in vitro*, 113×10^6 MSCs were derived, which made it possible to obtain the MSC autograft at a mean dose of 1.8×10^6/kg of the patient's weight. The fact that it took 3 passages to derive this amount of cell mass indicates the high proliferative activity of the cells. The results of immunophenotypic analysis of expanded MSCs *in vitro* indicated that the CD90 antigen was present in 98 % of cells, the CD105 antigen was in 96 % of cells, and the CD44 antigen was in 98 % of cells. At the same time, the number of cells with antigens CD45 and CD34 on their surfaces, which are hemopoietic markers, was less than 1 %, and no cells expressing the CD14 antigen were identified. This indicated that the biotransplant for the infusion consisted of MSCs, and there was no contamination with myeloid cells. The viability of cells in the MSCs autograft was 99 %. A freshly prepared culture of MSCs was used for infusion; the preparation time for the transplant of MSCs was 2 hours prior to administration. Before the transplantation, the cells were tested for HCV RNA using the PCR method. The result was negative.

Morphological verification of the diagnosis was performed by percutaneous biopsy with the examination of the biopsy specimen concurrently with the bone marrow harvesting procedure. To exclude the limitations of a possible sampling error, a 20 mm tissue specimen containing 12 portal tracts was obtained with biopsy.

Initially, the biopsy specimen was assessed using light microscopy. There have already been discussions in the literature of specific features of the abnormalities in the liver tissue of patients with HCV-caused LC and extrahepatic manifestations of infection when using this method. In this study, we used a complex morphological approach, in which the immunohistochemical method was additionally introduced, that allowed assessment of the extent of the sinusoidal capillarization phenomenon (using CD34 expression) and the state of myofibroblast transdifferentiation (using expression of alpha smooth muscle actin), as well as electron microscopy together with the evaluation of non-parenchymal and parenchymal compartments of the liver.

The following symptoms were assessed in the biopsy specimens: fibrous septa, including their thickness and location; the presence and size of regenerative nodes; intralobular infiltrates; the nature and severity of liver cell damage. This allowed us to determine the depth of the structural changes that impact the disease course in a patient with systemic manifestations of HCV infection. The morphological changes revealed were subsequently used to specify the time-related changes of the disease 6 months after autologous transplantation of MSCs.

The results of a morphological study based on the light microscopy data obtained before the transplantation of MSCs showed the abnormalities typical of LC stage 4B according to the Laennec

fibrosis scoring system in the patient's liver specimens [2]. Damage of the hepatic lobular structure and formation of regenerative nodes were observed. Small regenerative nodes were observed in less than half of the tissue of the examined specimen. Growth of fibrous tissue presented as narrow and wide septa was discovered around regenerative nodes. The observed changes were indicative of an increased gradient of portal pressure and corresponded to the clinical signs of portal hypertension revealed in the patient. Inflammatory infiltrates were manifested irregularly with a predominance of lymphocytes penetrating deep into the lobules in some areas (Figures 2a, 2b).

The immunohistochemical study allowed us to detect moderate "capillarization" of sinusoids in the specimens along with significant transdifferentiation of Ito cells into myofibroblasts (Figures 3a and 3b.)

The data that were obtained in the study of the biopsy specimen using electron microscopy made a significant contribution to our understanding of the pathological process occurring in the liver. Advanced destructive abnormalities associated with destruction of the cytoplasmic membranes were observed in microvasculature. Aggressive lymphocytes were discovered. Strong immune reactions were revealed with deposition of material with increased electron density along the pathways of the sinusoidal capillaries, which confirmed and supplemented the data of the immunohistochemical study (Figures 4a and 4b).

Alteration of hepatocytes followed by their death through necrosis or apoptosis was discovered over the entire area of the biopsy specimen. As a rule, cell borders could not be detected. Fibrous structures (Figures 4c and 4d) formed in the cytoplasm of a large number of cells, probably representing fibrils

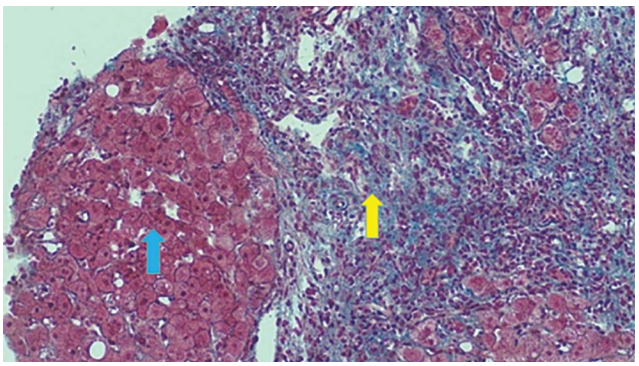

Figure 2a. *Formed micronodular cirrhosis. Regenerator node with proliferation of fibrous tissue (yellow arrow), false segment (blue arrow). Masson coloring. – ×126*

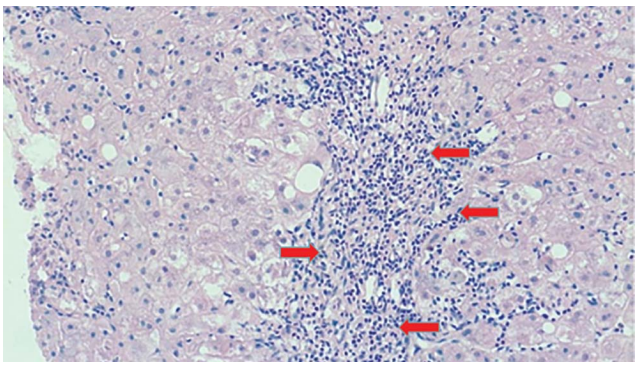

Figure 2в. *Inflammatory lympho-macrophage infiltration in fibrotic septa and periportal region (red arrows). Stained with hematoxylin-eosin. – ×63.*

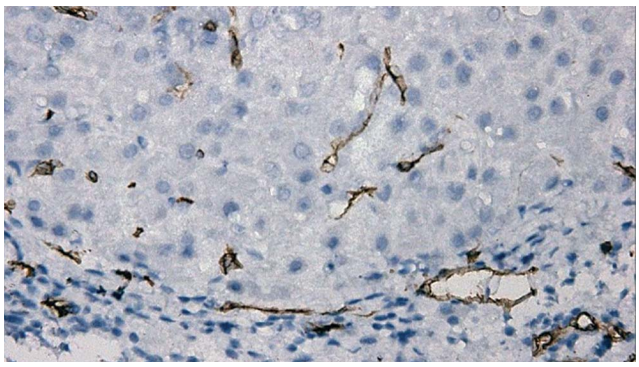

Figure 3a. *IHR (immunohistochemical reaction): CD34. × 400. Moderate capillarization of sinusoids*

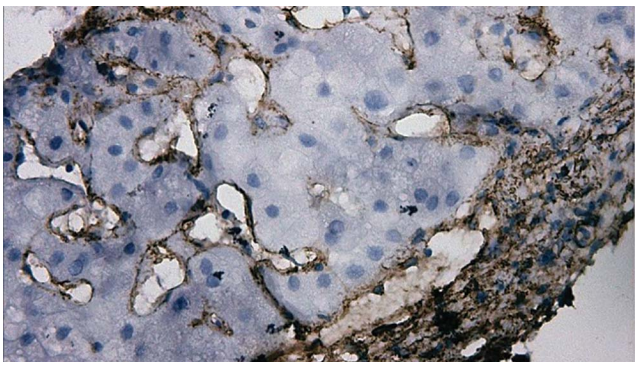

Figure 3в. *IHR (immunohistochemical reaction): α-SMA. × 400. The pronounced transdifferentiation of Ito cells into myofibroblasts*

of abnormal protein located in the cytoplasm of the hepatocytes without any surrounding membrane.

Significant destructive abnormalities were observed in the mitochondria (Mh), which was probably due to osmotic disequilibrium in the intermembrane space. Condensed and abnormally shaped mitochondria were discovered. Often, their condition was exacerbated by disintegration of the outer membrane and detachment of the inner membrane together with formation of bubbles and the destruction of the mitochondrial ultrastructure (Figure 4f). Expansion of intercristal spaces and fragmentation of Mh with the appearance of microclasmatosis were observed.

Previously, similar structural changes in the organelles that are associated with some pathological conditions have been described in the literature. According to a number of authors, the respiration and phosphorylation level drops as a result of these rearrangements, since the amount of endogenous ATP in condensed Mh is several times higher than in "typical" ones, and their ability to synthesize ATP for "export" is three times lower [1]. At the same time, swollen Mh with enlightened matrix (Figure 4g), which are characterized by a low level of energy supply, were found in the biopsy specimen. There were lysis of the cristae, homogenization of the matrix, and an increase in the number of large mitochondrial granules in these organelles. The latter is due to an impairment of the function responsible for the exchange of divalent cations, including Ca.

The agranular endoplasmic reticulum was characterized as sufficiently developed, and its profiles, typically, were significantly dilated. Isolated vacuolated membranes of rough endoplasmic reticulum (RER) were detected at some sites in the hepatocyte cytoplasm. Polymorphic residual bodies were discovered quite often. Bile-containing lysosomes were discovered in some hepatocytes, and local or total cytolysis was also recorded.
Numerous large transparent or fine-grained vacuoles were detected in the hepatocytes cytoplasm as well as in the lumens of sinusoidal capillaries. Large lipid inclusions were found in some hepatocytes.
Round nuclei in the surviving hepatocytes with fine-grained chromatin typically contained a compact nucleolus with a predominantly fibrillar component, which corresponded to their inactive state.

In October 2010, the patient underwent transplantation of MSCs into the liver tissue. The surgery and the postoperative period were uncomplicated. The patient was discharged from the hospital on the 8th day of the hospitalization. After that, the patient underwent outpatient monitoring.

The follow-up results demonstrated clinical improvement already during the 1st month after transplantation of MSCs: general weakness and manifestations of the dyspeptic syndrome disappeared, and appetite improved. There were no recent elements of the shin rash; brown spots were noted. They had developed due to hemosiderin deposition after the previously existing purpura had disappeared.

The patient was rehospitalized to evaluate the results of therapy 6 months after MSCs transplantation. The patient presented no complaints at the time of admission. Brown pigmentation was discovered on the skin of the lower limbs. The sclerae were icteric. Regional lymph nodes were not enlarged. Breathing sounds were vesicular. RR was 16 per minute. The heart sounds were regular and soft. HR was 80 bpm. BP was 130/80 mm Hg. The tongue was moist and white-coated at the tongue root. The abdomen was not enlarged, and it was soft and nontender. The liver protruded from the costal margin by 1 cm. The enlarged spleen was palpated. Stool was regular and had a normal color. Urination was comfort and painless, up to 5 times per day. Urine color was normal. There was a tendency for the platelet count to increase (from 38,000 to 49,000 units/μL) in the complete blood count. Other parameters of the complete blood count that did not change before the transplantation were within normal values throughout the entire post-transplant follow-up period. In the biochemical blood test, there was a tendency for the values of bilirubin (from 24 to 21.7 μmol/L), ALT (from 115 to 106 IU/mL), serum iron (from 32 to 17.9 μmol/L), and amylase (from 156.3 to 88 IU/mL) to decrease. Increased ALP levels persisted (276 U/L before and 280 U/L after transplantation). Albumin, cholesterol, urea, and creatinine values did not change

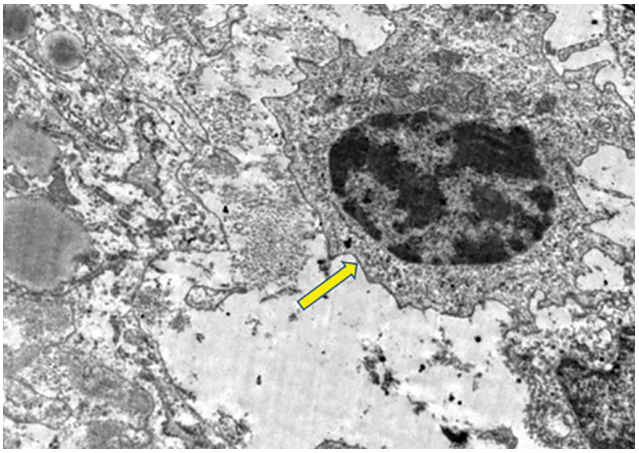

Figure 4a. *Aggressive lymphocyte in the lumen of the sinusoidal capillary (arrow). × 20 000*

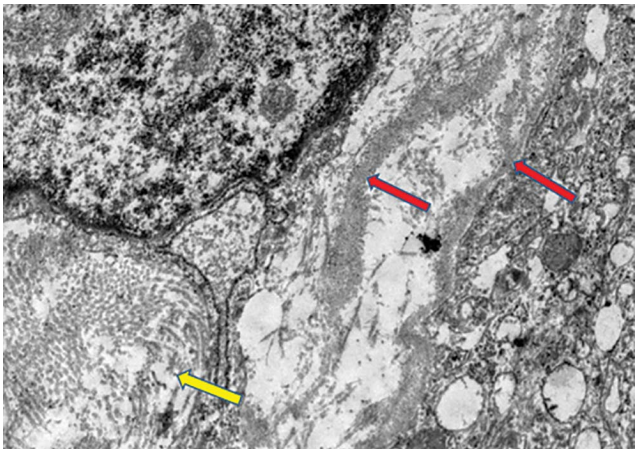

Figure 4в. *Immune reaction (red arrow) and a large bundle of collagen fiber fibrils in the pericapillary space (yellow arrow). – × 20 000*

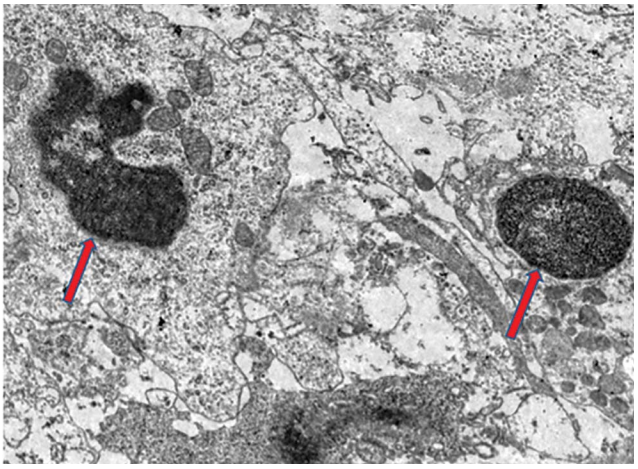

Figure 4c. *Apoptotic nuclei of hepatocytes (arrows). – × 15 000*

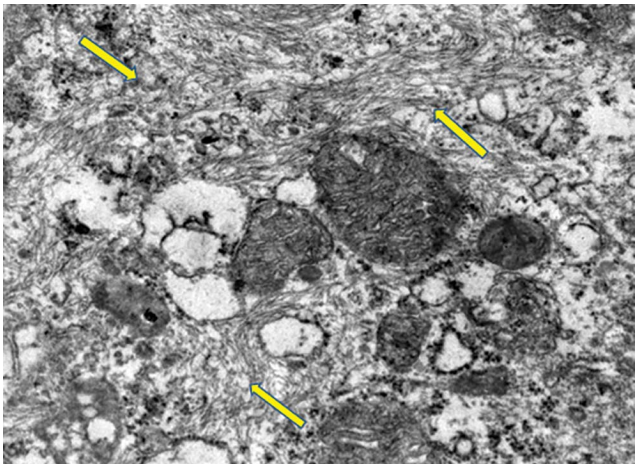

Figure 4d. *Fibrils of pathologically altered protein located in the cytoplasm of the hepatocyte without the membrane bounding them (yellow arrows). – × 20,000*

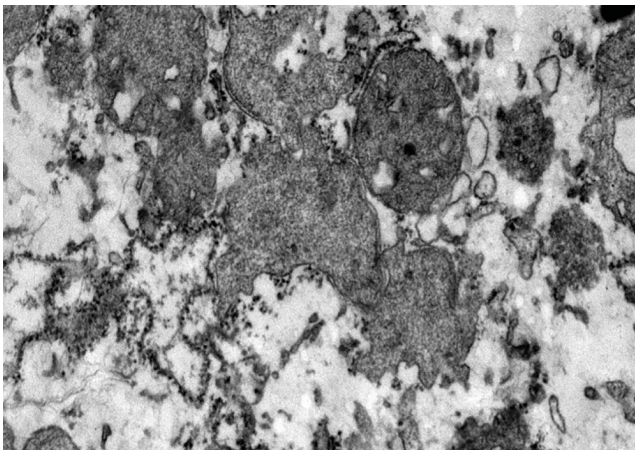

Figure 4f. *Destructively altered condensed mitochondria with loosening of the outer membrane and exfoliation of the inner membrane with the formation of bubbles. × 20 000*

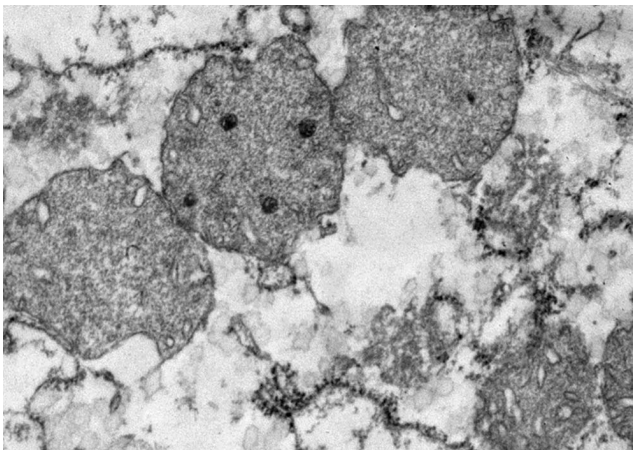

Figure 4g. *Swollen mitochondria with an enlightened matrix corresponding to a low-energy state. × 20 000*

and corresponded to normal values during the entire follow-up period. HCV RNA was detected in blood using the PCR method. Isolated erythrocytes were discovered in the urinalysis.

The signs of the formed micronodular LC with thin and thick septa persisted in the repeat percutaneous biopsy specimens of the liver that were assessed with light microscopy. However, in some parts of the examined specimens, the septa looked thinned, and sometimes perforated, indicating resorption of fibrous tissue at that site instead of its accumulation, which was previously described by a number of authors [23]. Populations of hepatocytes connecting at the sites of septal perforation in the biopsy specimen could be indirect evidence of this process. At the same time, the dilated sinusoids at the site of the connected regenerative nodes appeared to be parts of efferent microcirculation. Integrated parenchymal sites formed with thinning or perforation of the septa could contain several microcirculatory units, each of those being a former cirrhotic node.

Mild inflammatory lymph macrophage infiltration was observed in the fibrous septa and in periportal area (Figure 5a).

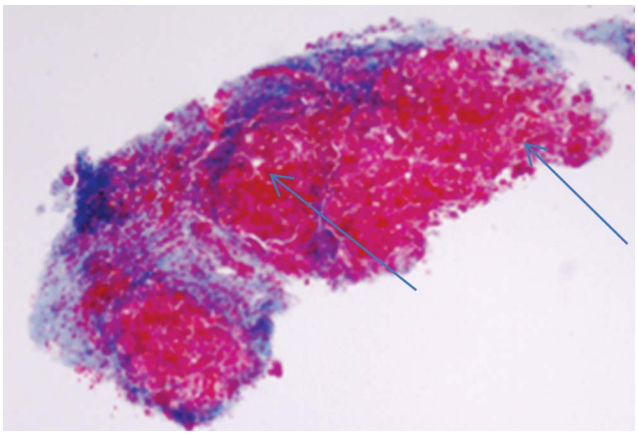

Figure 5a. *Formed micronodular cirrhosis. The cirrhotic node consists of several separate microcirculatory units, each of which, possibly, belonged to the former regenerative node. Painting according to the Mason.– ×32. Long arrows indicate the remnants of thin sept. The expanded sinusoids on both sides of the resorbed septum are parts of the efferent microcirculation*

An immunohistochemical study showed a decrease in intensity of transdifferentiation of stellate cells (HSC) into myofibroblasts while maintaining "capillarization" of sinusoids (Figures 6a and 6b).

Time-related changes of fibrotic abnormalities taking place in the liver tissue were also observed in the course of electron microscopic examination. Pericellular, pericapillary, and intracellular fibrous tissue was observed in the assessed biopsy specimens. Small areas of intralobular infiltration were discovered. Plasma cells, fibrocytes, and fibroblasts were detected occasionally.

A significant improvement was observed in the microvascular system after the transplantation of the MSCs. There were no immune reactions in the form of the deposition of amorphous and fibrous masses with moderate electron density along the

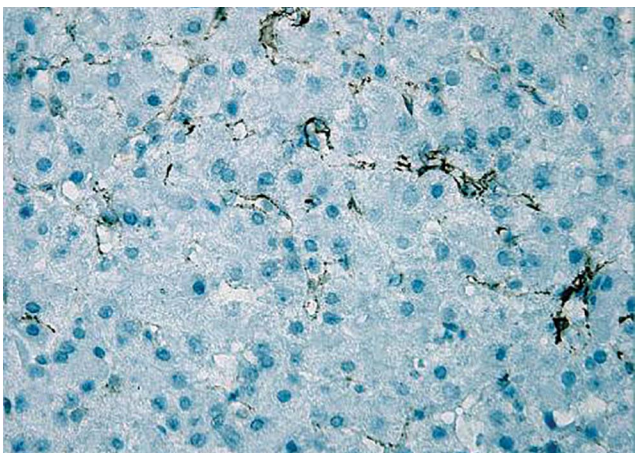

Figure 6a. *IHR (immunohistochemical reaction): α-SMA. × 400. Moderately expressed transdifferentiation of Ito cells into myofibroblasts*

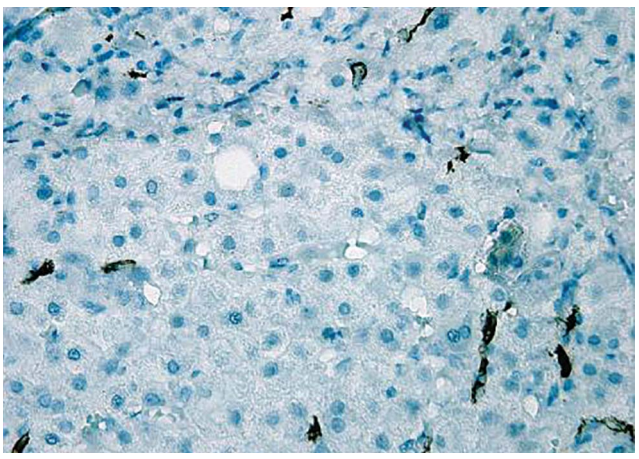

Figure 6в. *IHR (immunohistochemical reaction): CD34. × 400. Moderate capillarization of sinusoids*

sinusoidal capillaries ("capillarization of sinusoids"), which were significantly pronounced in the primary biopsy specimens. No large vacuoles occluding the lumen of the sinusoids were detected. This improvement was combined with the heterogeneity in the density of the cytoplasmic matrix of hepatocytes observed after transplantation, the state and quantity of organelles and inclusions (Figure 7a), and structural improvement of intracellular organelles. Thus, relative normalization of the mitochondrial ultrastructure was observed in the hepatocytes within most of the lobule. A large number of mitochondria were recorded in all hepatocytes. Numerous mitochondria did not have a matrix that was condensed, which would be indicative of a high-energy state (as observed in the primary biopsy), but rather had a moderate electron density, which is typical of an optimally energized status, with distinct cristae oriented predominantly across the long axis of the organelles in part of the cells (Figure 7b). Large intramitochondrial granules were detected in the matrix of the organelles, indicating a change in the exchange of divalent cations, mainly calcium. At the same time, there was no dilation or, especially, excessive dilation of the intercristal spaces with detachment of the inner membrane, as was observed in the primary biopsy. Mitochondria were mainly spread throughout the cells. Concentration of mitochondria around the nucleus was observed in some hepatocytes. Lysis of the cristae and homogenization of the matrix were discovered in the mitochondria of the hepatocytes within another part of the lobule, mainly in those where large lipid inclusions were detected. Mitochondria with a normal ultrastructure (to the left of the nucleus in the figure) and organelles with destructive abnormalities, up to the lysis of the cristae and homogenization of the matrix (to the right of the nucleus in the figure), were frequently discovered in one and the same hepatocyte (Figure 7c).

The morphometric analysis showed that the total mitochondrial area increased by 24 % in the repeated biopsy specimens, which correlated with an increase in the number of organelles by 20 % per slice area unit. However, the mean area of one mitochondria of the primary and repeated biopsy did not change (Table 1).

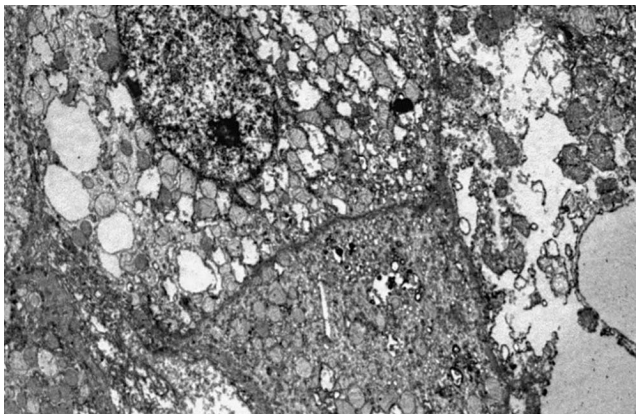

Figure 7a. *The expressed heterogeneity of hepatocytes in the number and degree of changes in the organelles and density of the cytoplasmic matrix. – × 5 000*

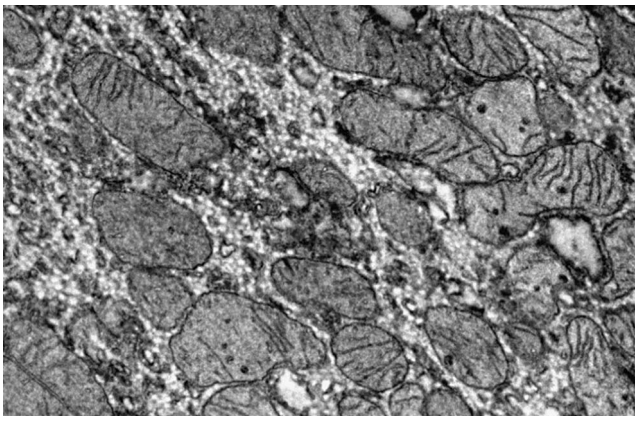

Figure 7в. *Numerous mitochondria characterized by polymorphism, a matrix of moderate electron density, distinct, crystals oriented predominantly across the long axis of the organelles, large intramitochondrial granules. – ×30 000*

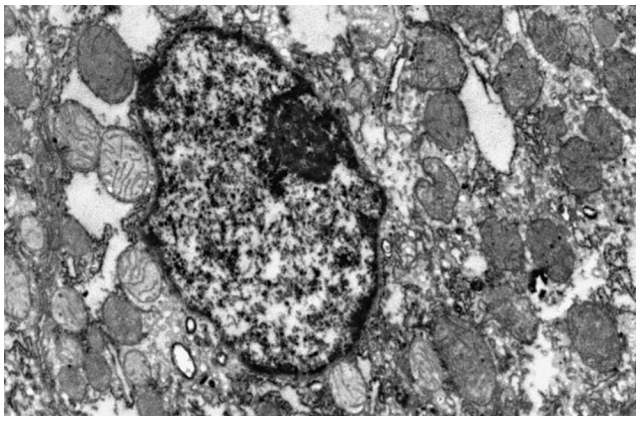

Figure 7c. *Heterogeneity of mitochondria in the hepatocyte. In the figure to the left of the nucleus is the mitochondria with normal ultrastructure, to the right – the lysis of the crista and the homogenization of the mitochondrial matrix. – ×12 000*

Table 1. *Comparative morphometric analysis of mitochondria of primary and repeated biopsy of patient T.*

Period	Area of mitochondria per 100 μm²	Amount of mitochondria per 100 μm²	The average area of one mitochondria μm²
Before transplantation	15,446	60,298	0,256
After transplantation	20,229	75,514	0,268

Single RER cisterns and numerous profiles of the SER being either polygonal or round in shape were identified between the mitochondria, which gave the cytoplasm a micro- or macrovacuolized appearance. The ultrastructural state of the nuclei indicated that they were in an inactive state.

No fibrous formations were detected in any hepatocyte in the repeated biopsy. However, they were detected in a large number of hepatocytes in the primary liver biopsy specimens. In addition, residual bodies were less common. There were no dying hepatocytes with cytoplasm replacement by fibrous structures, as was discovered in the primary biopsy specimens. There were no signs of occlusion of sinusoidal capillaries by large vacuoles, and no large vacuoles were detected in the hepatocytes cytoplasm as compared with the primary biopsy specimens.

Thus, the revealed morphological features indicated positive structural changes in the liver in response to the introduction of MSCs.

Our data are consistent with the commonly held opinion that dense fibrous tissue formed earlier in the process of restructuring in LC cannot undergo reverse development. At the same time, we demonstrated that the process of fibrogenesis is not static, and as a result of treatment of the liver tissue (in our case, with MSCs), the extracellular matrix may resolve and reduce the component of the sinusoids "capillarization" with simultaneous apoptosis of myofibroblasts, which play a key role in the process of fibrogenesis.

At the same time, we managed to demonstrate the positive effect of MSCs on the parenchymal compartment of the hepatic tissue. Structural changes were observed and interpreted as an improvement and even normalization of the mitochondrial ultrastructure, which changed the status of cells and resulted in their being placed in a more optimal energy and biosynthetic state. A decrease in regenerative processes as the result of MSCs was recorded in the hepatocytes, as indicated by the state of the nucleus and RER, which provided indirect evidence of the smaller scale of the damage caused by hepatocytes. At the same time, there were no cells that died with replacement of their cytoplasm by fibrous structures. The occlusion of the sinusoidal capillaries by large vacuoles was leveled.

Discussion

The process of fibrous tissue formation in the liver is a stereotyped response to damage, which is accompanied by the death of hepatocytes. The resulting changes are characterized by the interaction of many types of cells, including those that are resident and recruited into the liver (including bone marrow cells), which contributes to the development of inflammatory signaling pathways and, ultimately, leads to activation of normally resting HSC [6, 12, 14, 16, 21]. The latter are converted into myofibroblasts producing up to about 90 % of all extracellular matrix proteins in the liver [10, 12]. The interactions described above are dynamic and can contribute to both the development and regression of the fibrous tissue, controlling the activity of HSCs and content of the extracellular matrix in the liver tissue. Cell death, inflammation, and fibrosis are the key signs of the events that take place. Due to this observation, these have been proposed as the primary signals in the histological classification systems of Scheuer and Knodell [2, 8, 18]. In the case of acute liver damage, the described scenario of events subsequently leads to the restoration of the architectonics and functional status of the liver: (1) fibrosis ensures mechanical stability; (2) inflammatory cells contribute to the removal of cellular debris; (3) inflammatory signaling pathways play an important role in the development of liver regeneration [13, 19]. However, the same reactions become disadaptive under constant and prolonged exposure to the damaging factor, the continuing death of hepatocytes becomes uncontrolled and prolonged, which leads to chronic inflammation, the progression of the fibrosis, and the development of liver cirrhosis. Cirrhosis was considered irreversible for a long time. However, several

studies with morphological follow-up of biopsy specimens have demonstrated that fibrosis may decrease over time, even at the stage of cirrhosis [3, 9, 15].

We described a patient with LC and active HCV replication in the hepatic and extrahepatic sites, who underwent transplantation of MSCs into the liver tissue, and demonstrated partial improvement and regression of the pathological process in the parenchymal and non-parenchymal compartments. We selected several parameters for analysis, which were studied in more detail: sinusoidal fibrosis, septal fibrosis and the state of hepatocytes. Morphological study of the liver tissue using light microscopy demonstrated that the fibrotic septa separating the regeneration sites became thinner and fragmented six months after the transplantation of MSCs, and it was possible to recognize independently formed and topographically integrated microcirculation systems within the nodes located next to each other and separated by perforated septa. Regenerative nodes in cirrhosis are known to have a separate microcirculation system, and the changes we observed can indirectly indicate that the perforation of the septa followed the development of microcirculation. We also recorded partial resorption of fibrous tissue when studying the liver specimens through electron microscopy: there were no immune reactions in the form of deposition of amorphous and fibrous masses with moderate electron density along the sinusoidal capillaries ("capillarization of sinusoids"), which were significantly expressed in the primary biopsy specimens.

In this regard, it can be assumed that fibrolysis is more active in those parts of the liver where hepatocytes are not yet damaged or are only damaged slightly if the chronic inflammatory process in the liver persists together with LC. If hepatocytes die, the sinusoidal extracellular matrix appears to be able to combine with thin septa and then resolve [20]. At the same time, dense collagen septa that have formed in the course of cirrhosis and having undergone significant architectural restructuring cannot be resorbed completely and persist, which we observed in the case described here after transplantation.

On the contrary, the cirrhotic nodes in the course of fibrolysis can apparently grow larger due to their confluence at the time of lysis of thin septa (Figure 5a) and subsequently appear as large regeneration nodes surrounded by wide septa and areas where hepatic parenchyma have died. This hypothesis requires confirmation.

Another significant interference with complete reversion of cirrhosis is the presence of large regions of destroyed parenchyma [20]. In immunohistochemical studies of liver biopsy specimens, we observed the remaining foci of hepatocyte death, which were visualized as clearly restricted regions with sinusoids lined with CD34-positive endothelial cells (Figure 6b). These areas often contained arteries running separately and dilated sinusoids, which apparently indicated the presence of arteriovenous shunts. Sinusoids lined with CD34-positive endothelial cells may be equivalent to "capillarization", as described by Shaffner, Popper and others [18, 22], and persist as myofibroblast activity declines (Figure 6a).

At the same time, a number of authors described repopulation of damage foci as a result of activation of the so-called reparative complex consisting of proliferating ductules, hepatocytes surrounded with collagen, and CD34-positive sinusoids [20]. In the case of small damage foci, regeneration of hepatocytes occurs in the absence of an extracellular matrix in adjacent sinusoids, which is confirmed in our study by ultrastructural changes in the hepatocytes after transplantation of MSCs.

Our data are consistent with the common opinion that the dense fibrous tissue that formed previously cannot undergo reverse development in cirrhosis. At the same time, we demonstrated that the process of fibrogenesis is not static, and as a result of treatment of the liver tissue (in our case, with MSCs), the extracellular matrix may resolve and reduce the component of the sinusoids "capillarization" with simultaneous apoptosis of myofibroblasts, which play a key role in the process of fibrogenesis.

At the same time, we managed to demonstrate the positive effect of MSCs on the hepatocytes state. Structural changes were observed and interpreted as an improvement and even normalization of the mitochondrial ultrastructure, which changed the status of cells and resulted in their being placed in a more optimal energy state. A decline in

reparative processes under the influence of MSCs was recorded in the hepatocytes, as indicated by the state of the nucleus and RER, which provided indirect evidence that the hepatocytes were not as extensively damaged. At the same time, there were no cells that died with replacement of their cytoplasm by fibrous structures. The occlusion of the sinusoidal capillaries by large vacuoles was leveled.

Conclusions

1. The morphological characteristics of HCV-associated LC together with the time-related changes of fibrogenesis and fibrolysis processes do not depend on the predominant site of virus replication.
2. The transplantation of MSCs from the bone marrow is a promising method of compensating for chronic hepatocellular insufficiency, which contributes to the regression of fibrous tissue in the liver. The effectiveness of its impact depends on the initial severity of the disease and the reserve potential of the recipient liver.
3. The transplantation of MSCs reduces the degree of destructive abnormalities in the hepatocytes as well as the severity of the cirrhosis process, and it contributes to the improvement of the morphological and functional state of the liver. Therefore, it can be recommended as an important component of therapy.

Conflict of interests

The authors declare no conflict of interests.

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