Abstract

Objectives: Liver transplant is the cornerstone line of treatment for chronic liver diseases; however, the long list of complications and obstacles stand against this operation. Searching for new modalities for treatment of chronic liver illness is a must. In the present research, we aimed to compare the effects of transplant of undifferentiated human mesenchymal stem cells, in vitro differentiated mesenchymal stem cells, and adult hepatocytes in an experimental model of chronic liver failure.

Materials and Methods: Undifferentiated human cord blood mesenchymal stem cells were isolated, propagated, and characterized by morphology, gene expression analysis, and flow cytometry of surface markers and in vitro differentiated into hepatocyte-like cells. Rat hepatocytes were isolated by double perfusion technique. An animal model of chronic liver failure was developed, and undifferentiated human cord blood mesenchymal stem cells, in vitro hepatogenically differentiated mesenchymal stem cells, or freshly isolated rat hepatocytes were transplanted into a CCL4 cirrhotic experimental model. Animals were killed 3 months after transplant, and liver functions and histopathology were assessed.

Results: Compared with the cirrhotic control group, the 3 cell-treated groups showed improved alanine aminotransferase, aspartate aminotransferase, albumin, and bilirubin levels, with best results shown in the hepatocyte-treated group. Histopathologic examination of the treated groups showed improved fibrosis, with best results obtained in the undifferentiated mesenchymal stem cell-treated group.

Conclusions: Both adult hepatocytes and cord blood mesenchymal stem cells proved to be promising candidates for cell-based therapy in liver regeneration on an experimental level. Improved liver function was evident in the hepatocyte-treated group, and fibrosis control was more evident in the undifferentiated mesenchymal stem cell-treated group.

Key words: Cell-mediated therapy, End-stage liver disease

Introduction

Orthotopic liver transplant is an established treatment for patients with end-stage liver disease. The shortage of donor organs continues to be the rate-limiting factor for liver transplant throughout the world. Hepatocyte transplant is a promising alternative to orthotopic liver transplant to treat chronic liver diseases and can also be used as a “bridge” to liver transplant in cases of liver failure. Hepatocyte transplant has been proposed as a method to support liver function in acute or chronic hepatic insufficiency and as a “cell therapy” for metabolic diseases in the liver. Transplant of hepatocytes has been shown to significantly improve or correct prothrombin time, serum albumin and bilirubin levels, and survival in a rat model of terminal end-stage cirrhosis. In addition, metabolic defects in bilirubin metabolism, albumin secretion, copper excretion, familial intrahepatic cholestasis, and tyrosinemia have been corrected by hepatocyte transplant.

Stem cell therapy is attractive for its potential to support tissue regeneration, as it requires minimally

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invasive procedures and shows few complications in liver diseases.\textsuperscript{10,11} Stem cells are capable of differentiating into specialized cell types, including hepatocytes; utilization of these hepatocytes can allow the damaged liver to be regenerated.\textsuperscript{12} Stem cells originating from various intra- and extrahepatic sources have been investigated for the treatment of hepatic diseases.\textsuperscript{13,14} There are several potential advantages of using adult stem cells to regenerate tissues, including few ethical concerns, good known biologic behavior, easy accessibility, and low costs.\textsuperscript{10} One of the promising types of stem cells is mesenchymal stem cells (MSCs), which have been discussed in this context.\textsuperscript{15} In this study, we compared hepatocytes, undifferentiated MSCs, and in vitro hepatocyte-differentiated MSCs in the treatment of experimentally induced CCl\textsubscript{4} liver cirrhosis.

**Materials and Methods**

**Animal model**

Six-month-old male Lewis rats weighing ~150 g were used as the donor animal model, and 3-month-old BALB/c mice weighing ~40 g were used as the recipient animal model. The animals were given access to rodent chow and water and were maintained in 12:12-h light/dark cycles. All experiments were carried out in accordance with the rules and regulations of the Theodor Bilharz Research Institute Ethical Committee for handling of laboratory animals. Mice were injected intraperitoneally with CCl\textsubscript{4}/corn oil (1:4) emulsion at a dose of 0.5 mL/kg body weight twice weekly for 12 weeks to induce liver cirrhosis.

**Cord blood sample collection and isolation of mononuclear cells from cord blood**

We followed the procedures outlined previously.\textsuperscript{16} Human cord blood samples were collected from full-term donor mothers who had cesarean sections. Signed informed written consent was obtained from all donors. Samples were tested for hepatitis C and B virus and human immunodeficiency virus, and mononuclear cells were separated by the density gradient cell separation technique using Ficoll solution. Cells were suspended in Dulbecco’s modified Eagle medium (DMEM) and counted using a hemocytometer, and viability was determined by mixing equal volumes of cell suspension with 0.4% trypan blue stain (Invitrogen, Carlsbad, CA, USA).

**Culture, propagation, and characterization of mesenchymal stem cells**

Mononuclear cells were seeded in sterile tissue culture flasks at a density of 1×10\textsuperscript{5} cells/cm\textsuperscript{2} in DMEM supplemented with 30% fetal bovine serum (FBS; Invitrogen), 1% penicillin/streptomycin (Biochrom, Cambridge, UK), 1% L-glutamine (Lonza, Basel, Switzerland), and\textsuperscript{10-17} M dexamethasone (Sigma-Aldrich, Hamburg, Germany). When cells reached a confluency of 80% to 90%, they were trypsinized according to Litwin and associates.\textsuperscript{17} Cells were characterized by morphologic changes, flow cytometry analysis of surface markers (mesenchymal markers CD105/FITC, CD44/FITC, CD90/PE; exclusion markers CD45/FITC and CD34/FITC), and gene expression by real-time polymerase chain reaction for Oct4 and Sox2 using QuantiTect SYBR green polymerase chain reaction (Qiagen, Valencia, CA, USA). For further characterization, MSCs were allowed to differentiate into osteocytes by culturing them in DMEM supplemented with mesenchymal cell growth supplement (Lanza), L-glutamine, penicillin, dexamethasone, ascorbate, and \(\beta\)-glycerophosphate for 2 weeks, with results confirmed by alizarin red dye at pH 4.2 (Sigma, St. Louis, MO, USA). Cells were also allowed to differentiate into adipocytes by culturing them in adipogenic induction medium (supplemented with recombinant human insulin, L-glutamine, rat MSC FBS, dexamethasone, indomethacin, 3-isobuty-1-methyl-xanthine, and GA-1000) for 3 days followed by adipogenic maintenance medium (supplemented with recombinant human insulin, L-glutamine, rat MSC FBS, and GA-1000) for 3 days. Three cycles of induction and maintenance were carried out for optimal adipogenic differentiation, and cells were stained with Oil Red O stain.

**Hepatogenic differentiation and characterization of differentiated mesenchymal stem cells**

Cells were cultured in hepatogenic differentiation medium I, which consisted of DMEM supplemented with hepatocyte growth factor (HGF) (1 \(\mu\)L/mL), fibroblast growth factor-4 (2 \(\mu\)L/mL), endothelial growth factor (2.5 \(\mu\)L/60 mL), 1% penicillin/-streptomycin, 1% L-glutamine, and 1% FBS. Medium was changed twice weekly for 2 weeks. Hepatogenic differentiation medium I was replaced after 2 weeks by hepatogenic differentiation medium II, which consisted of DMEM supplemented with HGF (1 \(\mu\)L/mL), fibroblast growth factor-4 (2 \(\mu\)L/mL),
oncostatin M (24 μL/60 mL), insulin-transferrin-selenium 4 (5 μL/1 mL), dexamethasone (4 μL/mL), 1% penicillin/streptomycin, 1% L-glutamine, and 1% FBS. Medium was changed twice weekly for 2 weeks. Cells were characterized by morphologic changes throughout the period of differentiation (28 days). Synthetic function of the cells was assessed by measuring albumin concentration in culture media of the differentiated cells on days 0, 7, 10, 14, 21, 24, and 28 using the albumin human enzyme-linked immunosorbent assay kit (Abcam, Cambridge, UK). Excretory function of the cells was assessed using indocyanine green (ICG) stain and cytopathologic analysis. The latter was performed by staining cells with hematoxylin and eosin and periodic acid-Schiff for histopathologic assessment. Immunoperoxidase staining was also performed using an avidin-biotin complex immunoperoxidase technique 18 with antihuman primary antibodies against albumin and alpha-fetoprotein (AFP) for identification.

Isolation of hepatocytes
Hepatocytes were isolated from Lewis rat livers by double perfusion technique according to Wang and associates. 19 Cell count was determined using a hemocytometer, and cell viability was checked by trypan blue stain (Invitrogen). 20 Cells having viability >75% were used for transplant, taking into consideration that the isolation procedure was carried out on the same day of transplant.

Animal transplant
Mice were divided into 3 test groups and one pathologic control group. Normal mice served as normal control. Group 1 was injected intrahepatically with 1 × 10⁶ freshly isolated rat hepatocytes in 0.1 mL phosphate-buffered saline (PBS), mice in group 2 were injected intrahepatically with 1 × 10⁶ undifferentiated MSCs in 0.1 mL PBS, and mice in group 3 were injected intrahepatically with 1 × 10⁶ hepatogenically differentiated MSCs in 0.1 mL PBS. Twelve weeks after transplant, mice were killed, blood samples were collected, and livers were dissected.

Histopathologic and immunoperoxidase staining of liver specimens
Hepatic specimens were cut into 5-μm-thick sections using a microtome and stained with hematoxylin and eosin for histopathologic assessment. For assessment of fibrosis, sections were stained with Masson stain and Sirius red stain. Automatic Computer Image Analysis System (Zeiss, Oberkochen, Germany) was used for the quantitation of the collagen content. Image analysis was performed using the computer software program Axiovision 4.8 (Zeiss). The sectional area of the red-stained fibrous tissue of examined specimens was measured in squared micrometers in 5 consecutive microscopic fields, at ×5 magnification to yield the fibrotic area (μm²), and fibrotic area percent was then calculated relative to the total area examined (fibrotic index).

Immunohistochemistry was performed using an avidin-biotin complex immunoperoxidase technique 18 with antirat primary antibodies against AFP and Prox1 and antihuman primary antibodies against albumin and AFP. Positive and negative control slides for each marker were included in each session. As a negative control, a liver tissue section was processed as described but with the primary antibody omitted.

Biochemical analyses
Blood samples were obtained after animal death and centrifuged to separate sera. Serum levels of aspartate aminotransferase (AST), alanine transaminase (ALT), albumin, and bilirubin were measured and compared with samples obtained from the pathologic control group.

Results
Animal model preparation
Liver cirrhosis was reached 12 weeks after CCl4 injection, with fibrosis level of A4F4 according to the METAVIR scoring system. Microscopic examination of liver sections showed loss of hepatic lobular architecture with severe hydropic degeneration of the hepatocytes. Portal tracts were thickened and extended with chronic inflammatory cells and fibrotic tissue. Liver specimens of the deceased mice also showed formation of small and large regenerating nodules (Figure 1A). Thirty-six mice were divided into 4 groups: 1 pathologic control and 3 recipient groups. Eight healthy mice served as normal control.

Culture of mesenchymal stem cells from cord blood
One sample was collected and found negative for hepatitis C and B virus and human immuno-deficiency virus. Mononuclear cell count was 90 × 10⁶
Mesenchymal stem cell culture was initiated with a cell concentration of $1 \times 10^6$ mononuclear cells/cm$^2$. Classical MSC morphology of adherent fibroblastoid spindle-shaped cells growing in a monolayer was shown after 32 days of initial culture. Flow cytometry analysis of the isolated MSCs revealed positive expression levels of CD90, CD44, and CD105 in 98.2%, 63.4%, and 84.2% of cell populations, whereas positive expression levels of CD34, CD45, and HLA-DR were only shown in 0.06%, 1.72%, and 6.5% of cell populations. Mesenchymal stem cells also showed positive expression for Oct4 and Sox2. Mesenchymal stem cells were able to differentiate into osteocytes as confirmed by alizarin red dye at pH 4.2 (Sigma), which visualized red calcium phosphate precipitates and adipocytes, and as confirmed by Oil Red O staining, which visualized accumulations of neutral lipid vacuoles in the cytoplasm of the cells.

**Hepatogenic differentiation and characterization of differentiated mesenchymal stem cells**

With the double phase technique, MSCs were transformed into hepatocyte-like cells in 28 days, as confirmed by morphologic changes, histopathologic studies, immunoperoxidase staining, and function assessment. Significantly increased albumin levels in culture media of the differentiated cells from day 0 (2.3 ng/dL) to day 28 (30.0 ng/dL) were noticed. Functionality of differentiated cells was confirmed by the ICG staining test. Successful hepatogenic differentiation was also evidenced by hematoxylin and eosin staining, which showed characteristic morphology changes into hepatocyte lineage. Glycogen granules were identified by periodic acid-Schiff stain. Successful differentiation was confirmed by positive expression of both AFP and albumin on harvested differentiated cells stained with antihuman antibodies against both markers.

**Transplant results**

Mice from the 3 groups and the pathologic control group survived for 3 months after transplant before they were sacrificed.

**Transplant with hepatocytes: histopathologic examination of liver specimens**

The hepatocyte-treated group (8 mice) showed regained hepatic lobular architecture with mild to moderate hydropic degeneration (Figure 1B).

![Figure 1. Histopathologic Analysis of Liver Specimens From Different Groups](image)

(A) Liver section from pathological control subjected to CCl4, showing micro- and macrocrotic nodules (arrows) with severe ballooning and hydropic degeneration (hematoxylin and eosin [H&E], ×100). (B) Liver section from hepatocyte-treated mouse, showing intact hepatic architecture with mild hydropic degeneration (H&E, ×200). (C) Liver section from undifferentiated mesenchymal stem cell (MSC)-treated mouse, showing intact hepatic architecture with minimal hydropic degeneration (H&E, ×200). (D) Liver section from differentiated MSC-treated mouse, showing intact hepatic architecture with moderate hydropic degeneration and chronic inflammatory cells (arrow) (H&E, ×200).

![Figure 2. Immunoperoxidase Staining of Liver Specimens From Different Groups Using Anti-Alpha-Fetoprotein Antibody](image)

(A) Liver section from normal control showing no brownish cytoplasmic staining, denoting negative expression of antihuman alpha-fetoprotein (AFP) (3,3′-diaminobenzidine [DAB], ×200). (B) Liver section from hepatocyte-treated mouse, showing clusters of cells with brownish cytoplasmic staining denoting positive expression of antirat AFP (DAB, ×200). (C) Liver section from undifferentiated mesenchymal stem cell (MSC)-treated mouse showing sheets of cells with brownish cytoplasmic staining denoting positive expression of antihuman AFP (DAB, ×200). (D) Liver section from differentiated MSC-treated mouse showing few cell collections around central vein with brownish cytoplasmic staining denoting positive expression of antihuman AFP (DAB, ×200).
Transplant with hepatocytes: immunoperoxidase staining of liver specimens
Successful engraftment was confirmed by positive expression of rat AFP and Prox1 in 32.5±12.8% and 53.75±13.02% of cells in liver specimens stained with immunoperoxidase staining (Figures 2B and 3B).

Figure 3. Immunoperoxidase Staining of Liver Specimens From Hepatocyte-Treated Group Using Anti-Prox1 Antibody

(A) Liver section from positive control showing no brownish staining, denoting negative expression of antirat Prox1 (3,3′-diaminobenzidine, ×200). (B) Liver section from hepatocyte-treated mouse showing sheets of cells with brownish nuclear staining denoting positive expression of antirat Prox1 (3,3′-diaminobenzidine, ×200).

Transplant with hepatocytes: liver fibrosis assessment
The hepatocyte-treated group showed high significant improvement of fibrotic index (2.13±1.2) compared with the pathologic control group (P < .01) (Figures 4B and 5B).

Transplant with hepatocytes: biochemical analysis
Hepatocyte transplant significantly improved ALT, AST, bilirubin, and albumin levels compared with the pathologic control, undifferentiated MSC-treated, and differentiated MSC-treated groups (P < .05). Results for each parameter are shown in Table 1.

Transplant with undifferentiated mesenchymal stem cells: histopathologic examination of liver specimens
Histopathologic studies of liver sections of the undifferentiated MSC-treated group showed regained hepatic lobular architecture with ballooning and mild hydropic degeneration (Figure 1C).

Transplant with undifferentiated mesenchymal stem cells: immunoperoxidase staining of liver specimens
Liver specimens of the undifferentiated MSC-treated group stained with anti-human AFP and albumin immunoperoxidase showed successful engraftment and in vivo differentiation into hepatocyte-like cells. This was confirmed by positive expression of AFP and albumin in 47.5±13.88% and 23.75±13.05% of cells (Figures 2C and 6B).

Transplant with undifferentiated mesenchymal stem cells: liver fibrosis assessment
The undifferentiated MSC-treated group showed highly significant improvement of fibrotic index (1.90±0.6) versus the pathologic control group (P < .01) (Figures 4C and 5C).

| Table 1. Mean Values of Aspartate Aminotransferase, Alanine Transaminase, Albumin, and Bilirubin in Sera of Different Groups After Treatment |
|---------------------------------|----------------|----------------|----------------|
| Parameter                       | Serum AST, U/L | Serum ALT, U/L | Serum Albumin, g/dL | Serum bilirubin, mg/dL |
| Normal control                  | 97.75 ± 6.81   | 3312 ± 4.015   | 4.21 ± 0.42       | 0.38 ± 0.50 |
| Pathologic control              | 34462 ± 41.21  | 6088 ± 15.14   | 1.66 ± 0.16       | 0.60 ± 0.07 |
| Hepatocyte-treated group        | 269.25 ± 40.43 | 39.00 ± 5.30   | 3.02 ± 0.15       | 0.44 ± 0.50 |
| Undifferentiated MSC-treated group | 278 ± 18.84    | 40.37 ± 18.04  | 2.96 ± 0.20       | 0.48 ± 0.08 |
| Differentiated MSC-treated group | 279.62 ± 61.75 | 46.87 ± 7.69   | 2.71 ± 0.26       | 0.52 ± 0.09 |

Abbreviations: ALT, alanine transaminase; AST, aspartate aminotransferase; MSC, mesenchymal stem cells
Transplant with undifferentiated mesenchymal stem cells: biochemical analysis

Treatment with undifferentiated MSCs improved ALT, AST, bilirubin, and albumin levels with high significance compared with that shown in the pathologic control and differentiated MSC-treated groups \((P < .01)\). Results for each parameter are shown in Table 1.

Transplant with differentiated mesenchymal stem cells: histopathologic examination of liver specimens

Mice from the differentiated MSC-treated group showed intact hepatic lobular architecture with spotty necrosis and mild to moderate hydropic degeneration (Figure 1D).

Transplant with differentiated mesenchymal stem cells: immunoperoxidase staining of liver specimens

Positive expression of human AFP and albumin was observed in 20.62 ± 13.21% and 10.62 ± 8.21% of cells of liver specimens in the differentiated MSC-treated group stained with immunoperoxidase staining. These findings indicate successful engraftment and in vitro differentiation into hepatocyte-like cells (Figures 2D and 6C).

Transplant with differentiated mesenchymal stem cells: liver fibrosis assessment

The differentiated MSC-treated group showed highly significant improvement of fibrotic index (3.1±0.68) versus the pathologic control group \((P < .01)\) (Figures 4D and 5D).

Transplant with differentiated mesenchymal stem cells: biochemical analysis

Mice treated with differentiated cells showed improvements of AST, ALT, albumin, and bilirubin levels compared with the pathologic control group. Results for each parameter are shown in Table 1.

Discussion

Chronic liver injury often causes liver fibrosis, cirrhosis, and cancer. Liver cirrhosis has been considered an irreversible disease because it is nearly impossible to reverse the fibrotic changes with conventional treatments. However, research has witnessed a marked change in this perspective. Although liver transplant has become standard therapy in the treatment of patients with liver failure, several problems should be considered in their treatment. Other approaches have been proposed, in particular cellular-based procedures. Adult hepatocytes and stem cells may be used instead of whole...
organ transplant or integrated within the bioartificial devices to replace the missing synthetic and metabolic liver functions.

The aim of this study was to compare 3 cell-based modalities (hepatocytes, undifferentiated MSCs, and in vitro hepatocyte-differentiated MSCs) in the treatment of experimentally induced CCl4 liver cirrhosis. First, Balb/c mice were injected with CCl4 for 12 weeks to develop liver cirrhosis, which was followed by the preparation of different cell suspensions. Rat hepatocytes were isolated by the double perfusion technique of Seglen, where liver is perfused with isotonic solution and then with collagenase buffer to dissociate cells from live architecture. After cell viability was tested, cells were injected on the same day of isolation. Mesenchymal stem cells were isolated from human cord blood and showed full characterization parameters of MSCs, as also similarly identified previously. Moreover, gene expression analyses of isolated MSCs revealed the presence of specific pluripotent markers, Oct4 and Sox2, similar to the work of Panepucci and associates, Baharvand and associates, and Hua and associates. Cod blood MSCs were successfully differentiated into hepatocyte-like cells, as shown by their morphologic changes, increasing levels of albumin in culture medium, and positive ICG and periodic acid-Schiff staining tests, as well as through detection of positive hepatocyte markers (AFP and albumin) by immunoperoxidase staining. Similar to our findings, other researchers succeeded in the in vitro differentiation of MSCs to hepatocytes using similar differentiation protocols.

Cirrhotic livers of mice were injected with each type of cell individually and analyzed for liver function, architecture, and fibrotic changes 12 weeks later. All 3 lines of cell therapy improved liver function, as shown by increased serum albumin level and decreased AST, ALT, and bilirubin levels. In addition, fibrosis was ameliorated compared with that shown in the pathologic control group. Similar results were reported previously for hepatocyte transplant, and undifferentiated MSC engraftment, and hepatocyte-differentiated MSC engraftment. The hepatocyte-treated group showed the best results regarding improved liver function, followed by the undifferentiated MSC-treated group and finally the in vitro predifferentiated hepatocyte-like cell-treated group. Mou and associates reported similar results with injection of undifferentiated and in vitro differentiated menstrual MSCs into splenic pulps of mice subjected to partial hepatectomy. Our histopathologic results showed the therapeutic effects of undifferentiated MSCs in improving fibrosis and restoring normal liver architecture. Our results are in agreement with studies that focused on investigating antifibrotic effects on the injured liver in animal models of liver fibrosis.

Several mechanisms have been suggested for MSC-mediated improvements in liver function and cirrhosis severity. Mesenchymal stem cells have been shown to be able to produce matrix metalloproteinase, an enzyme capable of degrading the extracellular matrix, which alleviates hepatic cirrhosis directly. Moreover, it was demonstrated that MSCs were able to produce HGF, which in turn could promote the MSCs to undergo the process of transdifferentiation into parenchymal hepatocyte and suppress hepatic stellate cells and transforming growth factor β, the main players in hepatic fibrosis. In addition, MSCs might attenuate hepatic fibrosis through suppression of hepatic stellate cells by the secretion of interleukins 6 and 10.

In our study, both the hepatocyte-treated and hepatocyte-differentiated MSC-treated groups succeeded in the amelioration of CCl4-induced fibrosis. In agreement with this observation, Badrawy and associates and Nagata and associates, using injected rat hepatocytes, found improved liver fibrosis by formation of hepatocyte sheets in a mouse CCl4 liver-injured model. In addition, Piryaei and associates reported reductions in pathologic insult of the liver in chronic liver disease using in vitro hepatocyte-differentiated MSCs.

The antifibrotic effect of undifferentiated MSCs could be attributed to the fact that a large number of healthy parenchymal hepatocytes (2-3 × 1010) are needed to maintain normal function of an adult liver. In severe fibrotic or cirrhotic livers, the number of hepatocytes is significantly reduced, and supplying cirrhotic livers with adequate numbers of healthy hepatocytes, from the undifferentiated MSCs with their high proliferation ability, is more likely to restore normal liver function.

Conclusions and recommendations
Human cord blood MSCs were successfully isolated by the density gradient cell separation technique, propagated, and fully characterized by molecular
studies, flow cytometry, and functional analysis by chemical techniques. We were also able to differentiate along the hepatogenic lineage. We found that the double perfusion technique is successful in isolation of adult rat hepatocytes with high viability percentage. We also succeeded to develop a model of CCL4-induced liver cirrhosis in mice that were transplanted with either type of cells to study their therapeutic potential on liver cirrhosis to improve liver function and restore normal hepatic architecture.

Although there were similarities in the individual group results between our study and previous ones, a comparative study allowed us to highlight the effects of undifferentiated MSCs in treating chronic liver diseases when subjected to the same conditions as the other 2 cellular modalities for therapy. Both adult hepatocytes and cord blood MSCs proved to be promising candidates for cell-based therapy in liver regeneration on an experimental level. Improved liver function was evident in the hepatocyte-treated group, and fibrosis control was more evident in the undifferentiated MSC-treated group. Further studies are recommended on higher animal models for longer periods. Moreover, the use of dual therapeutic cell types (hepatocytes and undifferentiated MSCs), in further experimental trials, are encouraged to allow a simultaneous improvement in both fibrosis and functional activity parameters.

References