Effect of Transplanted Mesenchymal Stem Cells on Liver Functions in Experimental Liver Fibrosis in Rats

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Abstract

Liver failure is a potentially life-threatening condition for which organ transplantation is the only definitive therapy. The idea of stem cells suggests the probability of renewable sources of new tissues to treat many kinds of diseases, conditions, and disabilities. The present study aims to investigate the effect of transplanted mesenchymal stem cells (MSCs) on liver functions and degree of fibrosis in experimentally induced liver fibrosis in rats. 35 adult male albino rats were classified into 2 groups: Normal control group (10 rats) and CCl4-treated group (25 rats): Liver fibrosis was induced in rats by intraperitoneal injection of CCl4. To prove incidence of fibrosis, 5 animals livers was excised after 4 weeks for histopathology to confirm the occurrence of fibrosis. The remaining 20 animals were further subdivided into: Fibrosis non treated group (10 rats): Received CCl4 for 8wks, MSCs-treated group (10 rats): Received CCl4 for 8wks with single intravenous dose of MSCs at 4th week. MSCs were isolated from Wharton’s jelly. Serum ALT, AST, bilirubin and albumin were measured. MDA, SOD, and TGF-\textit{\beta}1 measured in liver tissue homogenate and histopathological and immunohistochemical studies for liver were assessed. Treatment with MSCs resulted in significant improvement in liver functions (ALT, AST, BIL and ALB) associated with significant reduction in tissue MDA with elevation in tissue SOD, Moreover, hepatic transforming growth factor beta 1 (TGF-\textit{\beta}1) was significantly down regulated in MSCs treated group. Histopathological examination of liver tissue matched the biochemical results.

In Conclusion: The present results spotlight on the good influence of the beneficial impact of MSCs in the treatment of experimental liver fibrosis and pave the way for the therapeutic application of MSCs on human liver fibrosis.

Key Words: Liver fibrosis – Mesenchymal stem cells – Wharton’s jelly.

Introduction

THE liver is the vital metabolic organ that regulates the body’s energy supply, secretes several essential compounds and clears substances by several methods, including recycling, inactivation and excretion [1]. According to the World Health Organization (WHO), chronic liver diseases are a serious worldwide press health problem [2]. Liver fibrosis and cirrhosis represent the final common pathway of virtually all chronic liver diseases and remain major causes of morbidity and mortality worldwide [3]. Advanced fibrosis is characterized by an accumulation of extracellular matrix rich in fibrillar collagens (predominantly collagen I and collagen III). It results in liver failure and portal hypertension and is associated with an increased risk of liver cancer [4].

Liver transplantation is a good and established treatment for liver failure resulting from several etiologies (viral, alcohol, metabolic insults) [5]. However, limited numbers of liver donors are available for the millions of patients who need them worldwide [6]. Furthermore, liver transplantation comes with considerable long-term side effects, such as chronic renal failure, post-transplantation lympho-proliferative disorder and cardiovascular complications. Therefore, strategies involving exogenous cell replacement must be considered [7].

Cell therapy can be defined as (the use of living cells to restore, maintain or enhance the function of tissues and organs) [8]. Stem or progenitor cells are defined by their capacity for cell renewal and asymmetric cell division, which leads to their differentiation and formation of one or more mature tissues and the preservation of the stem cell population [9].

Mesenchymal stem cells (MSCs) are defined as adherent cells which possess a proliferative potential and an ability to differentiate in vitro into...
chondrogenic, osteogenic, adipogenic and myogenic lineages. Under proper conditions, MSCs have been demonstrated to differentiate into hepatocyte-like [10] and neuron-like cells [11]. Apart from bone marrow, MSCs can be isolated from adipose tissue [12], umbilical cord blood [10] and various fetal tissues such as the placenta [13], amniotic fluid and amniotic membrane [14]. Many studies have shown that Wharton’s jelly in the human umbilical cord is also a rich source of primitive MSCs. Regardless of their sources; undifferentiated MSCs are adherent cells with a fibroblast-like morphology and are capable of self-replication through many passages. Therefore, they can potentially be expanded to sufficient numbers for tissue and organ regeneration [15].

The emerging field of stem cell therapy has raised great hope for improving the treatment of liver diseases, because it has the potential to promote liver repair and regeneration with fewer complications [1].

The present study aims to investigate the effect of transplanted mesenchymal stem cells on liver functions and degree of fibrosis in CCL4 induced liver fibrosis in rats.

Material and Methods

This experiment was approved by the Research Ethics Committee at Faculty of Medicine, Menoufia University. Umbilical cord samples were collected from full term pregnant women after taking consents, all animals received appropriate care according to the guidelines for Animal Experiments the Public Health Service Policy on Use of Laboratory Animals published by the National Institutes of Health. The practical part of the experiment was conducted at physiology, clinical pathology and pathology departments, Faculty of Medicine, Menoufia University at the period from December 2013 till March 2014.

Mesenchymal stem cells preparation:

The umbilical cord blood (UCB) was collected while the placenta was still in utero into sterile collection tubes, left at room temperature for clotting then centrifuged for 15 minutes at speed of 3000 r.p.m. then sera were collected in a single sterile falcon 50ml for heat inactivation by incubation at 56°C for 20 minutes then aliquoted and frozen.

Wharton’s jelly was cut into small pieces of about 1.5-2.5mm. Tissue culture plastic flasks 25cm² were prepared for culture by adding 5ml DMEM-LG with L-glutamine + 10% cord serum + 1% penicillin streptomycin, the flasks were incubated in a horizontal position in an incubator with saturated humidity containing 5% CO₂ at 37°C. At day 7, the tissue removed by changing the medium.

The flasks were washed extensively twice with warm media, and the adherent cells (MSCs) were kept in culture and were fed with fresh complete nutrient medium (about 1 weeks later). These cells were kept until the outgrowth of fibroblast-like cells (Fig. 1). Cells were harvested by trypsinization, the cells centrifuged at 3200 r.p.m. for 5 minutes. The supernatant was removed for counting the viable cells. The harvested MSCs at day 14 were identified by flow-cytometric analysis of CD29 and CD45.

Animals grouping:

Thirty five adult Wister male albino rats weighing 150-200 gm were housed in spacious wire mesh cages at room temperature and were kept with free access to standard rat chow diet and tap water. They were left for acclimatization for one week before the start of the study. Then they were divided into 2 main groups:

Group I: Normal control group (n= 10): Rats were subcutaneously injected with olive oil 0.5ml/kg twice a week for 8 weeks.

Group II: CCL4 treated group (n=25): Liver fibrosis induced by subcutaneous injection of 50% CCl4 solution (CCl4: oil= 1: 1) at a dose of 1 ml/kg twice a week, after 4 weeks 5 rats sacrificed the liver of each was excised for histopathology to confirm the occurrence of fibrosis. After insuring the success of the liver fibrosis model the other (20 rats) divided into:

Group II (A): Fibrosis non-treated group (n= 10): CCl4 injected for 8 weeks.

Group II (B): MSCs-treated group (n= 10): CCL4 injected for 8 weeks and a single dose of MSCs injected intravenously (2x10⁶ cells/rat) [16], intravenously in the rat tail [17], at the 4th week of induction of fibrosis.

Chemicals and reagents:

Carbon tetrachloride (Adwic, Egypt), Sterile cell culture low glucose-DMEM (Dulbecco’s modified Eagle’s medium) with L-glutamine (Lonza), Penicillin/streptomycin (Sigma), Fluorescein isothiocyanate (FITC) anti-human CD29 (Bio-science), Phycoerythrin (PE)-conjugated anti-human CD45 (Bioscience), Kits for estimation of serum albumin (Human kit, Germany), liver en-
zymes (BioMerieux. kits, France), total billirubin (Roche Diagnostic, Germany), superoxide dismutase (Bio-dignostic), malondialdehyde MDA (Bio-dignostic) and transforming growth factor beta (TGF-ß1) platinum ELISA (eBioscience). Hema-toxylin and Eosin stain for histopathological evaluation, Mouse monoclonal anti human hepatocyte (ready to use, 7ml, Labvision).

Measurement of portal blood flow velocity and resistance parameter:

Each rat was anaesthetized with urethane (25%) in a dose of (0.6ml/100gm) by intraperitoneal injection then mid line laparotomy was made to expose the portal vein. After setting the mode of pulsed blood flow meter (Bi-Directional blood flow meter with FFT-analysis (HADECO, Japan), we used ultrasonic gel on the probe top and turned the volume control to the maximum. The probe pressed softly to the measured area at an angle of 45-50º. After hearing the optimal sounds, we wait for 5 second without moving the probe then press the freeze key to freeze the waveform [18]. The portal blood flow velocity & resistance were measured to study changes that occurred in liver fibrosis before and after stem cells therapy.

Sample preparation and analysis:

Retro-orbital blood samples (each 2ml) were obtained through heparinized capillary tubes [19], samples were allowed to clot at room temperature in water bath for 10 minutes and then centrifuged at 3000 r.p.m. (rotation per minute) for 20 minutes. The supernatant serum was collected in a dry clean tube to estimate serum ALT, serum AST, serum albumin and Total billirubin. Rats were scarified by cervical dislocation, the livers were excised, before and after stem cells therapy.

MDA level in liver homogenate:

Colorimetric method for estimation of malondialdehyde (MDA) was done by using the protocol described in Satoh [20], by using thiobarbituric acid reactive substance for measuring the peroxidation of fatty acids as oxidative stress marker.

SOD level in liver homogenate:

Colorimetric method for estimation of superoxide dismutase (SOD) as described by Nishikimi et al., [21] depend on the ability of SOD to inhibit the initial rate of photo activatedphenazine-methosulfate mediated reduction of O$_2^{-}$ to O$_2$ which then reduce nitrobluetetrazolium dye.

TGF -ß1 level in liver homogenate:

The level of TGFß1 was estimated from liver homogenate using Elisa kit as per manufacturer protocol. Briefly, standard, blank and samples were pipetted into the respective micro wells. After stored in foil bag with desiccant provided at 2-8°C sealed tightly, the wells was washed twice with 400 µl wash buffer per well with thorough aspiration of micro wells contents between washes. The microwellwas then covered and incubated at room temperature for 2 hours on a microplate shaker set at 100 r.p.m., followed by washing 5 times with diluted wash buffer. Biotin-conjugate was then added into each well and incubated for 1 hour, and then washed 5 times. Diluted streptavidin-HRP was added into each well and incubated for 2 hour, after washing. TMP substrate solution was added into each well and incubated for 30 minutes. The reaction is terminated by addition of acid and absorbance is measured at 450nm. A standard curve is prepared from rat TGFß1 standard dilutions and rat TGFß1 sample concentration determined.

Histopathological evaluation of liver specimens in different groups:

Liver specimens were submitted to routine tissue processing ending with paraffin embedded blocks formation. Several sections were cut from the paraffin embedded blocks 4 µm thicknesses; one section was stained with hematoxylin & eosin staining for evaluation of degree of fibrosis according to Ishak scoring system [22]. The other sections were cut onpoly L lysine coated slides for immunohistochemical staining procedure.

Immunohistochemical staining for detection of human hepatocyte antigen:

The method used for immunostaining was streptavidin-biotin amplified system. Paraffin-embedded tissue sections were deparaffinized in xylene, rehydrated in a graded series of ethanol, and then incubated with 3% hydrogen peroxide.
Slides were rinsed in phosphate-buffered saline (PBS) and then exposed to heat-induced epitope retrieval in citrate buffer solution (pH 6) for 20 minutes. After cooling, the slides were incubated overnight at room temperature with ready to use mouse monoclonal anti-human hepatocyte (LabvisionNeomarker, Fremont, USA). Detection of immunoreactivity was carried out using the ultravision detection system, ready-to-use anti-polyvalent horseradish peroxidase/diaminobenzidine (Neomarker, Fremont, USA). Finally, the reaction was visualized by an appropriate substrate/chromogen (diaminobenzidine) reagent. Counter stain was carried out using Mayer’s hematoxylin. The staining procedure included negative controls obtained by substitution of primary antibodies with PBS. Brown cytoplasmic staining in any number of cells was considered positive expression. The percentage of positive expression for human hepatocyte cells in relation to total cells was considered and evaluated as mean ± standard error of mean (X ± S.E.M).

Statistical analysis:

The data were tabulated and analyzed by SPSS (statistical package for the social science software) using statistical package version 16.0 on IBM compatible computer. Quantitative data were expressed as mean ± standard error of mean (X ± S.E.M). Data from control and test groups were compared using one way ANOVA, followed by post Hoc test, Probability value of less than 0.05 was considered as statistically significant (p<0.05).

Results

Results of cell cultures:

In this study, we successfully isolated MSCs from 16 out of 20 umbilical cords samples (16/20, 80%). The onset of fibroblast like cell formation could be observed approximately during 5 to 7 days after first seeding of UC Wharton’s jelly. Attached cells were observed at 5-7 days after the initial plating. The adherent mesenchymal-like cells grew as spindle shaped cells, which developed into multi-polar fibroblastoid cells. These cells then gradually reached 50%-60% confluency at about 14 days (Fig. 1).

Characteristics of adherent cells of MSCs culture: Fibroblast like cells, adherent to the base of the flask and not removed except by trypsinization, the mean±S.E.M. of the percentages of cell showed immunophenotypic marker positivity for MSCs related antigen CD29 and lack of expression of CD45 a marker of hematopoietic stem cells was 86.09±3.09.

Portal blood flow velocity and resistance:

Results of Portal blood flow velocity (MV, cm/sec) showed that the mean values in fibrosis non treated group (3.73±0.14cm/sec), was significantly lower (p<0.001) when compared to the corresponding mean values in the normal control group (9.27±0.31). While MSCs-treated group, the mean value of Portal blood flow velocity (8.71±0.36 cm/sec), was significantly higher (p<0.001) compared to fibrosis non treated group (Fig. 2).

Results of portal vascular resistance (RP, PRU) showed that the mean value in fibrosis non-treated group (1.09±0.32), were significantly higher (p<0.001) when compared to the corresponding mean values in the normal control group (0.76±0.023). While MSCs-treated group, the mean value of resistance (0.8±0.017), were significantly lower (p<0.001) compared to fibrosis non treated group (Fig. 2).

Biochemical results:

Serum ALT, AST and total bilirubin level in fibrosis non-treated group (154.15±7.14U/L, 178.85±9.8U/L & 1.68±0.05mg/dl) respectively were significantly higher (p<0.001) compared to normal control group (37.36±1.3U/L, 47.37±1.42U/L & 0.47±0.04mg/dl) respectively, but these serum parameters in MSCs-treated group (74.74±2.14U/L, 101.2±3.09U/L & 0.65±0.05mg/dl) respectively, were significantly low (p<0.001) compared to fibrosis non-treated group while these parameters still significantly high (p<0.001) in MSCs-treated group compared to normal control group (Fig. 3-A,B).

Serum albumin level in fibrosis non-treated group (2.84±0.08gm/dl) was significantly low (p<0.001) compared to corresponding value in normal control group (4.11±0.17gm/dl). This parameter in MSCs-treated group (3.9±0.08gm/dl) was significantly low (p<0.001) compared to fibrosis non-treated group (Fig. 3-B).

MDA and TGF-β1 levels in liver homogenate (X±S.E.M) in fibrosis non-treated group (49.83±4.01Nmol/gm and 104.35±3.87Pg/10mg tissue) respectively, were significantly high (p<0.001) compared to corresponding values in normal control group (17.5±0.49Nmol/gm and 27.86±1.02Pg/10mg tissue) respectively. These parameter in MSCs-treated group (24.18±1.18 Nmol/gm and 28.89±2.15) was significantly low (p<0.001) compared to corresponding parameter in fibrosis non-treated group (Fig. 3-C,E).
SOD levels in liver homogenate (X±S.E.M) in fibrosis non-treated group (0.17 ±0.009a/gm) was significantly low (p<0.001) compared to corresponding value in normal control group (0.88 ±0.01a/gm). This parameter in MSCs-treated group (0.75±0.02a/gm) was significantly low (p<0.001) compared to corresponding parameter in fibrosis non-treated group, while still significantly low (p<0.001) in MSCs-treated group compared to normal control group (Fig. 3-D).

Histopathological changes and immunohistochemical results:

Normal control rat’s liver showed normal configuration, manifested by central vein with radiating cords of hepatocytes. Hepatocytes were seen to be polyhedral in shape with acidophilic granular cytoplasm. They showed large, rounded and vesicular nuclei. Blood sinusoids with kupffer cells were detected between the plates of hepatocytes (Fig. 4-A).

Rats treated with CCl4 for 4 weeks showed mild to moderate fibrosis with expansion of portal tract by fibrous tissue together with inflammatory changes confined to portal tract or extended into hepatic parenchyma (Fig. 4-B).

Rats treated with CCl4 for 8 weeks showed massive destruction of the liver tissue with loss of lobular pattern. Extensive fibrosis in the form of bridging fibrosis and pseudo lobular formation (Fig. 4-C).

MSCs-treated rat’s liver showed liver architecture ranged from normal to mild periportal fibrosis with minimal collagen fibers were seen deposited around central vein and in the portal tracts (Fig. 4-D).

There was significant elevation (p<0.01) in fibrotic score in fibrosis non-treated group (5.7 ±0.15) compared to normal control group (0 ±0) while MSCs-treated showed significant lower fibrosis score (0.7±0.27) compared to fibrosis non-treated group (p< 0.001) (Fig. 4-E).

The mean ± S.E.M of percentage of immunopositive cells for human hepatocyts detected in mesenchymal stem cells treated group was 13.1% ±3.5417 (Fig. 5).

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Fig. (1): Inverted microscope image of establishment of primary culture of MSCs. (A) Rounded cells floated in the media 3 days after 1st seeding (B) Adherent MSCs with floating non adherent cells (C) MSCs after removal of non-adherent cells with heterogeneous morphology (round and epithelioid cells) 7 days after the initial plating (D) The adherent fibroblast-like cells grew as spindle shaped cells at 50% - 60% confluency at about 14 day.
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Fig. (2): Portal blood flow velocity (MV) and portal vascular resistance (RP) recorded by Bi-Directional blood flow meter with FFT-analysis (HADECO, Japan) in (A) Normal control group (B) Fibrosis non treated group (C) Mesenchymal stem cells treated group (D) Portal blood flow velocity (cm/sec) among groups (E) Portal vascular resistance (RP) among groups.

*: Significant compared to normal control group.
#: Significant compared to fibrosis non-treated group.

Fig. (3): Serum and liver homogenate biochemical results in the normal control group, fibrosis non-treated group, MSCs-treated group (A) Serum levels of ALT and AST (U/L) (B) Total serum bilirubin (mg/dl) and serum albumin (gm/dl) (C) Liver MDA level (Nmol/gm) (D) Liver SOD level (u/gm) (E) Liver TGF-β1 level (Pg/10mg).

*: Significant compared to normal control group.
#: Significant compared to fibrosis non-treated group.
Discussion

In this study we successfully managed to extract mesenchymal stem cells from human umbilical cord Wharton’s jelly. The percentages of cell population showed immunophenotypic marker positivity for MSCs related antigen CD29 and lack of expression of CD45 a marker of hematopoietic stem cells was in accordance with Oh et al., [23].

Administration of CCL4 to rats caused liver fibrosis evidenced by estimated fibrotic score of Ishak fibrotic score.

H&E stained sections of liver tissue with significant deterioration in liver functions (serum ALT, serum AST, serum albumin and total serum bilirubin) and oxidative stress evaluated by imbalance in tissue level of MDA and SOD, also increased tissue inflammatory marker TGF-β1. The mean velocity of portal blood flow decreased and vascular resistance increased compared to normal control group. The MSCs which was extracted from human umbilical cord Wharton’s jelly when intravenously injected in rat’s tail significantly improved the
previously mentioned parameters with effective homing in rat’s liver evidenced by the percentage of immunopositive cells for human hepatocytes.

Several studies indicated that CCl4-intoxication for 8 weeks has a bad significant effect on the liver function, since the activities of serum AST and ALT were significantly higher than those of normal value. Leakage of large quantities of enzymes into the blood stream is often associated with massive fibrosis of the liver [24]. The CCl4 model resembles all important properties of human liver fibrosis, including inflammation, regeneration, fiber formation and potentially fibrosis regression [25], elevation of serum bilirubin which is also a measure of hepatotoxicity [26]. Reduced serum albumin and protein synthesis [24], our study concluded that these altered liver parameter caused by the extensive liver fibrosis detected by our results of histopathology and fibrotic score evaluation.

Our results detected oxidative imbalance in the CCl4 treated rats as the elevated MDA and reduced SOD which may be involved in cellular damage. The high significant elevation of MDA level in liver homogenate of rats treated with CCl4 indicated excessive formation of free radicals and activation of lipid peroxidation of the hydropic core and cell damage [26,27]. Toxic influence of CCl4 metabolites increasing reactive oxygen species causing oxidation of cellular proteins and extensive mitochondrial DNA damage thus impaired mitochondrial synthesis by the liver cells [28]. The interpretation for the present depletion of serum protein in CCl4-intoxicated animals may be due to the relationship between the damage occurring to DNA and consequently the protein synthesis [24].

Decrease in enzyme activity of superoxide dismutase (SOD) is a sensitive index in hepatocellular damage and is the most sensitive enzymatic index in live injury. SOD has been reported as one of the most important enzymes in the enzymatic antioxidant defense system. It scavenges the superoxide anion to form hydrogen peroxide and thus diminishing the toxic effect caused by this radical-Palanivel et al., [29].

The significant increase of tissue TGF-ß1 level in CCL4 induced liver fibrosis compared to the corresponding values in normal control group was in agreement with Liu et al., [30] who concluded that there was a significant increase in protein expression of TGF-ß1 in model group treated by CCL4. The free radicals may activate myofibroblasts to produce growth factors [31], Various cytokines and polypeptide growth factors are implicated in the pathogenesis of hepatic fibrosis/cirrhosis, among the peptide mediators; TGF-ß is the profibrogenic master cytokine [32]. These previous results are supported by our finding of elevated TGF-ß1 level associated with increased MDA and oxidative stress.

In the present investigation, there was significant decrease of velocity of portal blood flow and significant increase in resistance parameter in CCL4 induced liver fibrosis compared to the corresponding values in normal control group this result may be explained by the detected elevation of MDA and reduction of SOD. Due to oxidative stress, the endothelium loses its normal vasodilatory and anti-thrombotic properties and becomes vasoconstrictive and pro-thrombotic. These dynamic and reversible components further increase the resistance in the liver [26,27].

Moreover, our histopathological findings and the raised fibrotic score play a key role in increased portal resistance and decreased portal blood flow. Oxidative stress induces vascular occlusion and fibrosis in the liver and activates hepatic stellate cells, which display contractile properties and produce extracellular matrix and collagen [33]. These architectural changes determine an increase in intrahepatic resistance and consequently portal pressure.

The significant increase in fibrotic score in CCL4 induced liver fibrosis compared to the corresponding values in normal control group was due to marked accumulation of collagen fibers [34], this histopathologically detected liver damage may occurred due to oxidative stress and elevated TGF-ß1 levels detected in this study. Oxidative stress and associated damage could represent a common link between different forms of chronic liver injury and hepatic fibrosis. For example, oxidative stress contributing to lipid peroxidation is one of the critical factors involved in the genesis and the progression of nonalcoholic steatohepatitis and liver cancer. TGF-ß1 which was considered the profibrogenic master cytokine as mentioned before, [35], induce fibrosis through autocrine and paracrine effects on various hepatic and infiltrating cell types [36].

MSCs extracted from human umbilical cord Wharton’s jelly succeeded to implant in rat’s liver which is evidenced in our study by immunopositive staining for human hepatocyte treatment. Cell homing and engraftment into the injured liver is an integral steps in cell-based therapies [37]. Growth
factors have been shown to contribute to the recruitment of stem cells in the injured liver [38]. The elevated TGF-β1 which is increased in CCL4 induced liver fibrosis in this study may play a role in attraction of MSCs to the damaged liver tissue. TGF-β may act as an injury/stress-activated messenger to recruit MSCs for tissue repair, regeneration and pathological remodeling [39].

Implanted MSCs improved liver fibrosis and liver function effectively which was in agreement with other studies using other sources for MSCs as bone marrow [40,41]. MSCs have a significant antifibrotic effect as evidenced by the disappearance of septalcollagen deposition. This improved liver architecture and reduction of fibrotic score occurred after it underwent trans-differentiation into hepatic oval cells and then to hepatocyte-like cells. During this process, inflammation was reduced, damaged hepatocytes were repaired, and fibrosis was resolved, resulting in an overall improvement in liver function with a definite increase in intracellular glycogen storage [42].

Also MSCs implantation restored liver function through the improved portal blood flow velocity and reduced resistance with MSCs treatment observed in this investigation. Angiogenic support provided by MSC can be considered one more supportive effect, since the re-establishment of blood supply is fundamental for recovery of damaged tissues [43].

In the present investigation MSCs succeeded to restore the oxidative balance by reducing MDA and elevating SOD in liver. In other studies human mesenchymal stem cells injection decreased oxidative stress induced by irradiation significantly [44] by inducing Nrf2, a transcription factor that positively regulates the basal and inducible level of cytoprotective genes, Nrf2 activation is protective against oxidative stress and induced SOD production which decreased ROS in liver [48].

MSCs significantly decreased liver TGF-β1 level compared to the corresponding value in fibrosis non treated group. This result was in agreement with Sakaida [46], who indicated a reduced mRNA expression of type I procollagen and TGF-β in the liver one week after BM-MSCs infusion. TGF-β1-Smad signaling pathway, which is the main message pathway in liver fibrosis, is affected by MSCs treatment [47].

MSCs transplantation effectively increased portal blood flow velocity and reduced vascular resistance in fibrotic livers mainly due to reduction of liver fibrosis and decrease inflammatory changes confined to portal tract which was seen in CCL4 treated rats, caused removal of mechanical impediment of intrahepatic blood flow. The blockage of liver microcirculation due to collagen deposit and active remodeling may increase intrahepatic vascular resistance and exacerbate the disease progression [48]. The role of oxidative stress is obvious in inducing portal resistance and reduced blood flow as previously mentioned. MSCs restore oxidative balance and hence improve the previous parameters.

As seen in the present investigation, there was significant decrease of fibrosis score with MSCs implantation when compared to the corresponding values in fibrosis non treated group. The disappearance of collagen content in the cirrhotic rats treated with MSCs was apparently due to the lysis of fibrotic tissue which was accomplished by Metalloproteinase activity [49]. MSC-derived HGF (hepatic cell growth factor) was responsible for the marked induction of apoptosis of hepatic stellate cells which cause liver fibrosis [50]. Also bone marrow (BM)-derived MSCs showed high proliferation rates and hepatogenic differentiation, leading to tissue remodeling and the resolution of fibrosis [51].

The reduced TGF-β1 in this investigation detected in MSCs treated group compared to normal control represent a mechanism of improvement of fibrosis and consequently improvement of liver functions. Several animal studies and clinical trials have demonstrated that MSCs have the potential to reverse the fibrotic process by inhibiting collagen deposition and transforming growth factor-β1 production [6].

The previously mentioned mechanisms describing MSCs’ role in decreasing fibrosis, explain the better fibrotic score in MSCs group compared to CCl4-8 weeks treated and even better than CCl4-4 weeks tressed group, which is considered the basal level of fibrosis at which MSCs started working, this means that MSCs not only stop fibrosis but also reverse the already present fibrotic changes.

In Conclusion:

MSCs extracted from human umbilical cord Wharton’s jelly injected intravenously in rat tail succeeded to implant in rat liver with improvement of liver function and reduction of induced fibrosis and improved portal blood flow velocity and vascular resistance by different mechanisms including cell replacement, antioxidant and anti-inflammatory effect.
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