

**COMPARTIVE STUDY BETWEEN THE EFFECTS OF
HUMAN CD34 AND RAT BONE MARROW MESENCHYMAL
STEM CELLS ON AMELIORATION OF CCL4 INDUCED
LIVER FIBROSIS.**

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ABSTRACT

Human umbilical cord blood (UCB) cells and rat bone marrow mesenchymal stem cells (BM-MSCs) have many advantages as grafts for cell transplantation. The aim of this study was to investigate the impact of UCB cells and BM-MSCs on reversal of hepatic injury and revival of hepatic function in a rat model of carbon tetrachloride (CCl₄)-induced liver fibrosis.

Keywords: CCl₄ liver fibrosis, Umbilical cord blood CD34⁺, Bone marrow stem cell, Stem cell therapy.

Stem cells administration recovered liver function that confirmed by histopathological examination of liver tissue. GFP labeled cells were detected in groups injected with stem cells. Concerning gene expression, stem cells administration reduced gene expressions of collagen 1 α , TGF- β 1, and TNF- α with lower expression in CCl₄/CD34⁺ group. Albumin, MMP-2 and MMP-9 genes were highly

expressed in liver tissues in the groups that received stem cells with higher expression in the group that received undifferentiated CD34⁺. Human UCB CD34⁺ stem cells were more effective in alleviation of CCl₄-induced liver injury in rat than BM-MSCs. This study highlights an important role of human UCB CD34⁺ stem cells in liver fibrosis therapy.

INTRODUCTION

Liver fibrosis is a progressive disease that included disorder of standard hepatic tissue construction in addition to tremendous accretion of extracellular matrix in reply to chronic damage (**Nasir et al., 2013**). Inflammation is compulsory to originate and conserve of liver fibrosis. As soon as grievance turn up, scratched epithelial and/or endothelial cells ooze inflammatory mediators that draw inflammatory cells from the blood to the offended area and release mediators that persuade fibrosis such as TGF-β1 and TNF-α, turn on hepatic stellate cells and gather extracellular matrix (**Zheng et al.,2013**). Besides, collagens, the main protein of connective tissues, correspond to a group of obviously occurring proteins, and comprising 25-35% of the total body protein. It is conventional in liver fibrosis that there is tremendous collagen creation (**El-Mahdi., 2014**). As fibrotic liver diseases advancement, disease advanced from collagen bands to bridging fibrosis subsequently to frank cirrhosis. Extracellular matrix (ECM) declaration resulted from increased synthesis as well as decreased degradation that due to decreased ECM removing matrix metalloproteinases (MMPs) activity (**Puglisi., 2011& Radwa.,2012**). Liver transplantation has been used in the management of liver fibrosis. Nonetheless, the escalating number of patients and insufficient availability of donors, morbidity and mortality from liver fibrosis continue to be increased. Consequently, alternative therapies are immediately required. Mesenchymal stem cells (MSCs) have involved attention as a possible cell source for repair of damaged liver (**Kanazawa et al., 2011 & Xu et al., 2012**). Recently, stem cells, undifferentiated cells, hold promising therapeutic results since they undergo self- renewal and differentiation into one or more cell types, and are appropriate to human disorders(**Abdel Aziz et al., 2014**). Umbilical cord blood (UCB) contained mostly hematopoietic stem cells (HSCs)(**De Coppi et al.,2007**) .The stem cells and early progenitors expressed CD34, a surface membrane glycoprotein, and is

the characteristic of HSPCs. The bulk of CD34⁺ cells expressed HLA-DR (a major histocompatibility complex class II) and CD38 (a surface antigen on leukocytes) antigens. The number of CD34⁺HLADR⁻ and CD34⁺CD38⁻ cells in UCB is greater than in adult bone marrow (BM). Thus, UCB contains a higher amount of immature HPCs than adult BM (Radwa et al., 2012). In bone marrow (BM) there are several populations of stem cell including hematopoietic stem cells (HSCs), marrow mesenchymal stem cells (MSCs), and multi-potent adult progenitor cells. Many authors accounted that CD34⁺ cells and BM-MSC could assuage chemically-induced liver fibrosis (Abdel Aziz et al., 2014), however, which cell type is more effective in alleviating liver fibrosis and the mechanism by which MSC repair the fibrosis is unclear and their results seem notorious. In the present study, we employed an experimental model of liver fibrosis induced with CCl₄ and compared between the ameliorative effects of human CD34⁺ and rat bone marrow derived mesenchymal stem cells on functional revival of the liver and management of injury in a rat model of hepatic fibrosis. Also, we studied the mechanism that induces the protective activity of stem cells by examining the expression of key genes in the pathogenesis of hepatic fibrosis such as collagen-1-alpha, transforming growth factor beta (TGFβ1), matrix metalloproteinase (MMP-2), MMP-9, and tumor necrosis factor-alpha (TNF-α) by real-time PCR. Here, we transplanted UCB cells and BM-MSCs into injured liver fibrosis and investigated the reversal of hepatic fibrosis after in vivo transplantation.

MATERIALS AND METHODS

Experimental Animals: Forty female white Albino rats were 6 weeks old, weighing between 150 and 200g. Rats were bred and maintained in an air-conditioned animal house with specific pathogen-free conditions. All experimental animals have ethical approval from the institutional animal care committee. They were subjected to a normal light/dark cycle and allowed unlimited access to chow and water. All experimental protocols followed the guidelines of the Animal Committee of the Faculty of Medicine of Assiut University.

Induction of Liver Fibrosis: Liver fibrosis was induced by subcutaneous injection of carbon tetrachloride (CCl₄) at a dose of 0.2 ml/100 g body weight of 40 ml/L CCl₄ (Sigma, St Louis, USA) dissolved in equal volume of castor oil (Sigma, St. Louis, USA). The

injection was given twice weekly for 6 weeks (**Zhao., et al 2005**).

Experimental Design Rats were divided into the following groups:

Control group 1: 10 rats received 0.2 ml/100 g body weight of castor oil twice weekly for 6 weeks. **CCl₄ group 2:** 10 rats received 0.2 ml/100 g body weight of CCl₄. Liver fibrosis was determined by histopathological examination. **CCl₄/CD34⁺ group 3:** 10 rats received CCl₄ as previous. The rats were infused with 10⁷ isolated CD34⁺ cells/rat intravenously (through tail vein) and scarified after 3 months. **CCl₄/BM-MSCs group 4:**, 10 rats received CCl₄ as previous and followed by injection of 10⁷ BM-MSCs and scarified after 3 months.

At 4, 8 and 12 weeks from stopping CCl₄ and administration of stem cells, venous blood was collected from the retro-orbital vein to assess serum albumin and alanine transaminase (ALT). All rats were sacrificed with CO₂ narcosis, and liver tissue was harvested for histopathological examination and real time PCR analysis.

Isolation and Culture of BM-MSCs: Bone marrow cells were flushed from tibia and fibula of rat bones with phosphate-buffered saline (PBS) containing 2 mM EDTA. Over 15 ml Ficoll-Paque (Gibco-Invitrogen, Grand Island, NY), 35 ml of the diluted sample was carefully layered, centrifuged for 35 minutes at 400xg rpm and the upper layer was aspirated leaving undisturbed mononuclear cell (MNC) layer at the interphase. This MNC layer was aspirated, washed twice in PBS containing 2 mM EDTA and centrifuged for 10 minutes at 200xg rpm at 10 °C. The cell pellet was re-suspended in a final volume of 300 µl of buffer. Isolated MSCs were cultured on 25 ml culture flasks in minimal essential medium(MEM) supplemented with 15% fetal bovine serum (FBS) and incubated for 2 hours at 37°C and 5% CO₂. Adherent MSCs were cultured in MEM supplemented with 30% FBS, 0.5% penicillin, streptomycin and at 37°C in 5% CO₂ in air (**Abdel Aziz., et al 2007**). Cultured MSCs was confirmed by morphology (Fig. 1) and Florescent Analysis Cell Sorting (FACS) by detection of CD29⁺ and CD44⁺ specific to MSCs (Fig. 2).

Collection of Human Umbilical Cord Blood: Human umbilical cord blood withdraws immediately after normal vaginal delivery within 24 hours after rupture of membranes and before separation of the placental. Written informed consent was obtained from each woman after a full explanation of the study. Ethical standard approval was

maintained throughout the present work. Participants considered suitable for the study according to the following exclusion and inclusion criteria. Women with the following criteria were excluded: Family history of gene based disorders or maternal fever during delivery. The umbilical cord clamped one inch or less apart at the infant's abdomen. Umbilical cord was sterilized with 70% alcohol followed by betadine at the needle insertion site. The needle inserted just above the clamp. Blood allowed pouring as much as possible. The collection normally took about 3-5 minutes.

Cell Sorting of Human UCB CD34⁺: Anti-coagulated human umbilical cord blood (UCB) was diluted 1:4 with PBS containing 2 mM EDTA (Gibco-Invitrogen, Grand Island, NY). As prescribed in our previous work [12]. Briefly, the MNCs were separated by centrifugation over a Ficoll-Paque (Gibco-Invitrogen, Grand Island, NY) density gradient at 400 xg rpm for 35 minutes at 10°C. The MNC fraction was washed first in PBS, then with MACS (magnetic cell sorting) buffer (PBS supplemented with 0.5% bovine serum albumin and 2 mM EDTA, pH 7.2). CD34⁺ cells were isolated from MNCs, using the CD34⁺ positive cell selection kit (MiniMacs; Miltenyi Biotec, Bergisch Gladbach, Germany). Percentage of isolated CD34⁺ cells was characterized by flow cytometry.

Labeling Stem Cells with GFP: At 4th passage, MSCs were harvested and labeled with GFP (amaxa GmbH, amaxa Inc. Europe/World USA Scientific Support). Human MSC were nucleofected using the Human MSC Nucleofector Kit and a plasmid encoding the fluorescent protein GFP. Cells were centrifuged, washed twice in serum free medium, pelleted and suspended in nucleofector solutions. A final concentration of 4-5x 10⁵ cells/100µl nucleofector solutions was applied. The sample was placed in cuvette of electroporation transfection instrument at program U-23 (for high transfection efficiency) or C-17 (for high cell survival). 24 hours post-nucleofection cells were analyzed by light and fluorescence microscopy. Transfection efficiencies of around 80% can be reached with GFP. Labeled cells were injected intravenously in rat with CCL₄-induced liver fibrosis. After 12 weeks, liver tissue was examined with a fluorescence microscope (Leica, Germany) to detect and trace the cells stained with GFP.

Serum Biochemical Assessment: Blood sample was driven from rats

at 4, 8 and 12 weeks. ALT and albumin were assessed using (Randox laboratories limited, Country Antrim, UK) colorimeter kits according to manufacture instructions.

Histopathological Examination: Liver tissues were collected and divided into two sections. The first section was assessed for tracing of injected labeled cells with GFP. The second section was washed with PBS and fixed overnight in 40 g/L paraformaldehyde at 4°C. Serials μm sections of the dissected liver tissues were stained with hematoxylin and eosin (H&E) and Masson Trichrome for evaluation of fibrosis.

Immunohistochemistry: Immunohistochemical staining was performed on 5- μm , formalin-fixed, paraffin-embedded sections using the CD34 & Albumin antibodies at 1:50 dilution (DAKO, Carpinteria, CA). Antigen retrieval was performed in all cases by steam heating the slides in a 1-mmol/L solution of EDTA (pH 8.0) for 30 minutes. After blocking of endogenous biotin, staining was performed using an automated immunostainer (DAKO) followed by detection by using a streptavidin-biotin detection system (DAKO). Positive and negative control sections were used for each assay.

RNA Extraction: Liver tissue of all studied groups was homogenized and total RNA was isolated with RNAeasy Mini Kit (Qiagen) and further analyzed for quantity and quality with Beckman dual spectrophotometer (USA). Real Time PCR (qRT-PCR) for Quantitative Expression of Collagen I α , TGF- β , Albumin, MMP-2 and 9 and TNF- α .

The mRNA expression level was quantified by qRT-PCR (Real time PCR). 1000 ng of the total RNA from each sample were used for cDNA synthesis by reverse transcription using High capacity cDNA Reverse Transcriptase kit (Applied Biosystem, USA). The cDNA was subsequently amplified with the Syber Green I PCR Master Kit (Fermentas) in a 48-well plate using the Step One instrument (Applied Biosystem, USA) as follows: 10 minutes at 95 °C for enzyme activation followed by 40 cycles of 15 seconds at 95 °C, 20 seconds at 55 °C and 30 second at 72 °C for the amplification step. Changes in the expression of each target gene were normalized relative to the mean critical threshold (CT) values of GAPDH housekeeping gene by the $\Delta\Delta\text{Ct}$ method. We used 1 μM of both primers specific for each target gene. Primers sequence and annealing temperature specific for each gene demonstrated in Table (1).

Statistical analysis

The collected data was organized, tabulated and statistically analyzed using prism software statistical computer package version 5. Mean and standard deviation were calculated; one-way ANOVA (Analysis of variance) was used to test the difference about mean values of measured parameters among groups. For interpretation of results of tests of significance, significance was adopted at $P < 0.05$.

RESULTS

BM -MSCs showed positive expression of the $\beta 1$ -integrin CD29 and the endoglin receptor CD44 in **Figure 1 (a, b)**. Liver function was monitored by the serum level of albumin and alanine aminotransferase (ALT) as displayed in **Figure 2**. At the end of the 1st month, both CCl₄/CD34⁺ and CCl₄/BM-MSCs groups showed non-significant increase in the serum level of albumin as compared to the CCl₄ group. At the 2nd and 3th months, serum level of albumin in rats injected with CD34⁺ and BM-MSCs increased significantly as compared to CCl₄ group ($P < 0.001$). Highest level was demonstrated in the CCl₄/CD34⁺ group but there was non-significant difference with CCl₄/BM-MSCs group. Regarding to the liver enzymes, ALT, at the 1st month there was non-significant reduction of its level in CCl₄/CD34⁺ or CCl₄/BM-MSCs groups as compared to the CCl₄ group. At 2nd and 3th months, serum level of ALT showed significant decrease in comparison to the CCl₄ group ($P < 0.001$). However, its level was still significantly higher than control level and there was no significant difference between the groups that received stem cells. Concerning gene expression, the rat collagen 1 α , TGF- β and TNF- α gene were highly expressed in CCl₄ group and expression of collagen 1 α was significantly decreased after stem cells administration. Least expression was in the CCl₄/CD34⁺ group. However, there was no significant difference between the different groups that received stem cells. Whereas, TGF- β was still significantly expressed in CCl₄/CD34⁺ and CCl₄/BM-MSCs groups ($P < 0.05$ and $P < 0.001$, respectively). As regard to gene expressions of albumin, MMP-2 and MMP-9. They were expressed in liver tissues in the groups that received stem cells but the expressions not reach a significant level in MMP genes. Highest expression was in CCl₄/CD34⁺ group (**Table3**). Histopathological examination of liver tissue showed that stem cells

have a significant anti-fibrotic effect as evidenced by the decrease in liver collagen compared to the CCl₄ group together with improvement of liver histopathological picture as detected by Hematoxylin & Eosin and Masson Trichome (**Figure 3**). Albumin is normally expressed by hepatocytes indicating functioning hepatocytes. It exhibits cytoplasmic expression. Bile ducts & portal mononuclear cells don't express albumin. In this study, albumin expressing cells was detected in the portal areas indicating differentiation of injected BM-MSCs and CD34⁺ cells into hepatocytes (**Figure 4**).

Table (1): Primers sequence and annealing temperature specific for each gene

Target gene	Primer sequence: 5' - 3'	Gene bank accession number
Collagen I α	Forward: AGAGCATGACCGATGGATTC Reverse: CCTTCTTGAGGTTGCCAGTC	KJ696743.1
Albumin	Forward: TTTACGAGAAGCTTGGAGAG Reverse: TGTGCAGATATCAGAGTGGA	FQ210445.1
TGF- β	Forward: TGCGCCTGCAGAGATTCAAG Reverse: AGGTAACGCCAGGAATTGTTGCTA	NM_021578.2
MMP-2	Forward: CTATTCTGTCAGCACTTTGG Reverse: CAGACTTTGGTTCTCCA ACTT	NM_031054.2
MMP-9	Forward: AAATGTGGGTGTACACAGGC Reverse: TTCACCCGGTTGTGGAAACT	NM_031055.1
TNF- α	Forward: AACTCGAGTGACAAGCCCGTAG Reverse: GTACCACCAGTTGGTTGTCTTTGA	XM_008772775.1
GAPDH	Forward : CACCTGTTGCTGTAGCCATATTC Reverse: GACATCAAGAAGGTGGTGAAGCAG	XR_598347.1

Table (2): Effects of human CD34⁺ and rat bone marrow mesenchymal stem cells on the gene expression of collagen 1 α , TGF- β , albumin, MMP-2, MMP-9 and TNF- α in CCl₄-induced liver fibrosis in rats

Groups Parameters	Group 1 (Control group) n = 10	Group 2 (CCL4 group) n =10	Group 3 (CCl₄/CD34⁺ group) n =10	Group 4 (CCl₄/BM- MSCs group) n =10
Albumin gene	3.85 \pm 1.06	0.89 \pm 0.35***	2.93 \pm 0.85†††	1.88 \pm 0.71***†
Collagen1 α gene	0.33 \pm 0.14	1.81 \pm 0.63***	0.62 \pm 0.21†††	0.76 \pm 0.33†††
TGF- β gene	0.21 \pm 0.09	1.08 \pm 0.39***	0.59 \pm 0.26*	0.81 \pm 0.26***
MMP-2 gene	1.46 \pm 0.68	0.70 \pm 0.27*	1.09 \pm 0.50	0.93 \pm 0.51
MMP-9 gene	1.63 \pm 0.49	0.77 \pm 0.27***	1.12 \pm 0.50	1.00 \pm 0.37*
TNF- α gene	1.03 \pm 0.55	1.66 \pm 0.36**	0.93 \pm 0.22†††	0.93 \pm 0.32†††

Data were expressed as mean \pm SD. TGF- β : Transforming growth factor-beta, MMP-2: Matrix metalloproteinase-2, MMP-9: Matrix metalloproteinase-9, TNF- α : Tumor necrosis factor-alpha. Analysis between groups was done using one way ANOVA test followed by Bonferroni test. *: P<0.05 and ***: P<0.001 compared to the control group; †: P<0.05, †††: P<0.001 compared to the CCl₄ group.

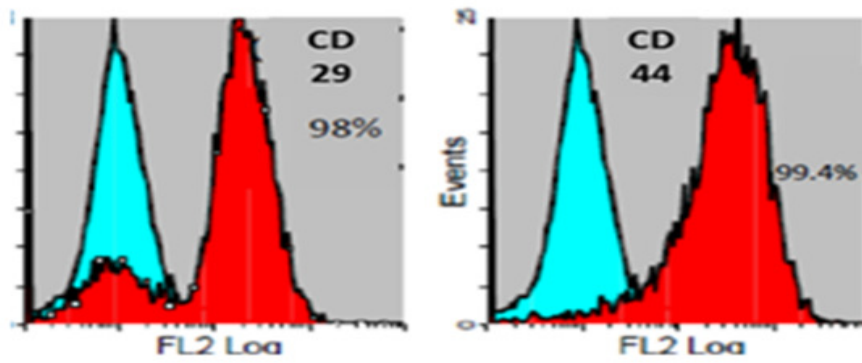
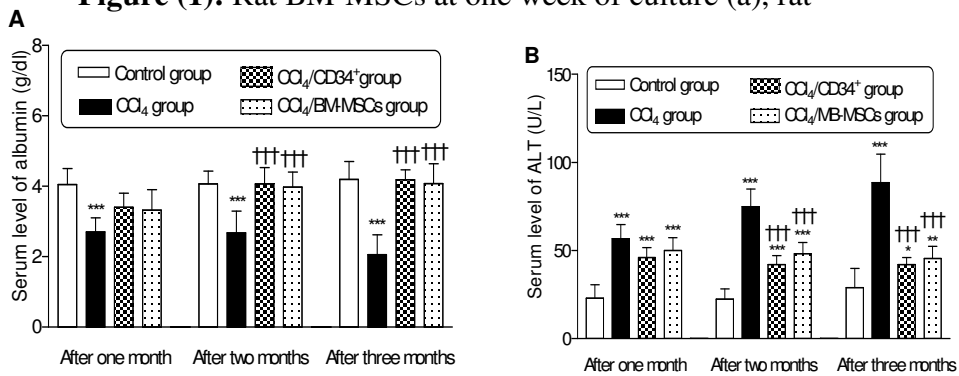


Figure (1): Rat BM-MSCs at one week of culture (a), rat



BM-MSCs at two weeks of culture (80-90% confluent) (b)

Figure (2): Effects of human CD34⁺ and rat bone marrow mesenchymal stem cells on the serum level of albumin and ALT in CCl₄-induced liver fibrosis in rats. Data were expressed as mean ± SD. LH: ALT: alanine transaminase. Analysis between groups was done using one way ANOVA test followed by Bonferroni test. *: P<0.05, **: P<0.01, and ***: P<0.001 compared to the control group; †††: P<0.001 compared to the CCl₄ group.

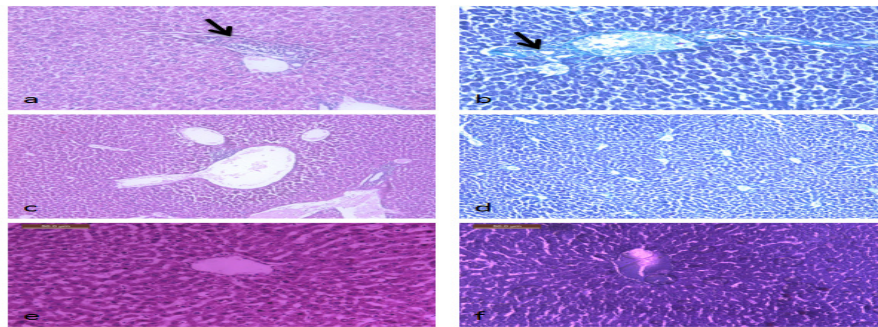


Figure (3): Histopathological examination of liver tissue showed that stem cells have a significant antifibrotic effect as evidenced by the decrease in liver collagen compared to the CCl₄ group. Mild focal portal fibrosis was detected in CCl₄ model (black arrow) a-H&E x200 and b- Masson Trichrome x200. Normal liver was evidenced with no fibrosis after BM-MSCs injection in CCl₄ model c- H&E x40 and d- Masson Trichrome x40. Normal liver was assessed showed no fibrosis CD34⁺ injection in CCl₄ model e- H&E x200 and f- Masson Trichrome x200.

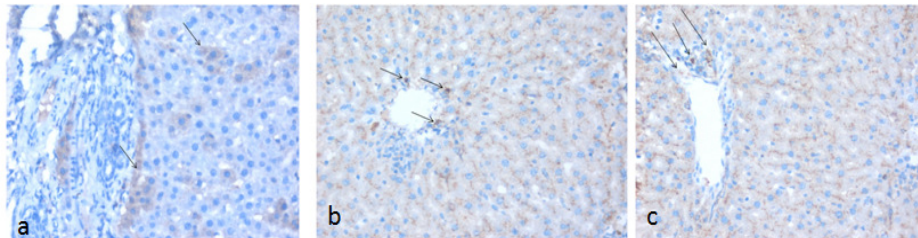


Figure (4): a-Showed immunohistochemistry (400x) positive expression of CD34⁺ differentiated hepatocytes in portal area (arrow). Some hepatocytes showed expression of CD34 positivity around portal tract. b- Showed immunohistochemistry (400x) positive expression of albumin for injected BM-MSCs in portal area (arrows). c- Showed immunohistochemistry (400x) positive expression of albumin for injected CD34⁺ in portal area (arrows).

DISCUSSION

Owing to a grave scarcity of liver donors and the high-risk of transplant rejection, a surrogate therapeutic approach is advantageous for patients with liver failure. Stem cell therapy demonstrated a great talented outcome for repair of tissues and organs injury, together with the liver. Stem cells are undifferentiated cells that have the capability

to self-renewal and multi-lineage differentiation (**Abdel Aziz et al., 2014**). Accordingly, the present study compared the ability of human CD34⁺ and rat BM-MSCs injection to ensure the functional revival of the liver and improvement of fibrosis in an experimental model of liver fibrosis in rats induced by CCl₄.

The chief findings of this study were: 1) both human umbilical cord blood derived CD34⁺ and rat bone marrow-derived MSCs improved liver function in rats with CCl₄-induced liver fibrosis as indicated by increased serum albumin and decreased ALT levels and this confirmed by histopathological assessment of liver tissue. 2) Administration of human CD34⁺ stem cells more effectively decreased collagen 1 α , TGF β 1 and TNF- α and increased albumin, MMP-2, and MMP-9 gene expression than bone marrow-derived MSCs. In contrast to BM-MSCs, UCSCs have higher division, homing, differentiation and extension opportunity and susceptibility to genetic treatment, and they create a lesser frequency of graft-vs.-host disease (**Sáez-Lara et al., 2006**). In the present study, injection of human CD34⁺ and rat BM-MSCs increased albumin and decreased ALT levels in the serum and this indicated normalization of liver function with stem cells injection. In agreement with the present results, **Abdel Aziz et al. 2012**, showed that human CD34⁺ cells derived from human UCB capable to recover liver purpose as indicated by increasing serum albumin and decreasing ALT level in rats with liver fibrosis. Also, intravenously infused BM-MSCs resulted in upgrading of liver functions (**Abdel Aziz et al., 2012 and Zhao et al., 2012**). This occurred concurrently with increased albumin gene expression with higher expression with human CD34⁺ cells injection. This is consistent with the finding of **Abdel Aziz et al., 2012**, who's found increased albumin gene expression in liver tissues in the group that received human CD34⁺ stem cells. Also, **Ali and Masoud., 2012**, demonstrated a marked increase in the albumin gene expression after transplantation of BM-MSCs. The ability of stem cells to recover liver function in rats with CCl₄-induced liver fibrosis was long-established by histopathological examination of liver tissue. Treatment with human CD34⁺ and rat BM-MSCs showed a return of liver design to normal and anti-fibrotic effect as evidenced by the decrease in liver collagen compared to the CCl₄ group together with upgrading of liver histopathological picture. The recognized reduction

in the liver fibrosis might contribute to inhibition of collagen formation or breakdown of collagen. Many researchers observed the anti-fibrotic effect of BM-MSCs through embarrassment of collagen formation and they speculated that BM-MSCs have an effective therapeutic effect against fibrosis (El-Mahdi et al.,2014; Abdel Aziz, et al ., 2014 and Abdel Aziz., et al ., 2007). Moreover, Hong et al.,2014, observed that human UCBMSCs capable to recover liver cirrhosis by breaking down collagen fibers. TGF β 1 is believed to be the key fibrogenic cytokine in liver fibrosis that plays a noteworthy role in the metabolism of ECM (Hong et al.,2014, Crosby et al., 2009;Hernandez-Gea et al.,2011 and Mannaerts et al ., 2013). Augmented expression of TGF β 1 related to HSCs commencement and ECM deposition during initiation and progression of fibrosis (Hong et al., 2014). Besides, collagens are the major constituents of the ECM. Collagen I α , another pro-fibrotic factor produced by activated hepatic stellate cells, is a significant indicator of collagen metabolism in the liver(Ali and Masoud., 2012).The tendency in gene expression levels of TGF- β 1 paralleled those of collagen 1 α (Presser et al ., 2013). Our results are consistent with those in humans and mammalian models in which increased gene expression of collagen and collagen deposition in liver fibrosis be an adjunct to increased TGF- β 1 gene expression (Presser et al ., 2013 and Li et al., 2013). In the present study, we found that treatment of CCl₄-induced rat liver fibrosis with stem cells resulted in a marked reduction in the gene expression levels of pro-fibrogenic factors, including collagen 1 α and TGF- β 1 with higher reduction in CCl₄/CD34⁺ group. This is in accordance to a study conducted by Ali and Masoud., 2012 who's found reduction of collagen I α gene expression after 4 weeks of bone marrow derived MSCs transplantation in comparison with CCl₄ treated group. Also, Li et al., 2013, who's demonstrated successful reduction of TGF- β 1 level in serum after transplantation of human umbilical cord MSC-exosomes into mouse liver fibrosis induced with CCl₄. MMP-2 is secreted by HSCs (Li et al ., 2013), it degrades collagen IV, a chief constituent of the vascular basement membrane (Gressner et al., 2002 and Li et al ., 2013). MMP-9 help migration of bone marrow derived MSCs to the site of inflammation (Ali and Masoud ., 2012). The results of the current study showed that administration of stem cells reduced gene expression of MMP-2 and MMP-9 with higher expression in CCl₄/CD34⁺ group. This is consistent with the finding of

Abdel Aziz et al., 2010, who's found enhanced MMP-2 gene expression in liver tissues in the group that received human CD34⁺ stem cells. Also, **Li et al., 2013**, observed that combined treatment liver fibrosis with taurine, epigallocatechin gallate and genistein resulted in marked increase in the expression of MMP-2 in the HSC cells. During progression of liver fibrosis diverse cytokines, chemokines and growth factors are secreted as part of the inflammatory reaction (**Van Wettere et al., 2013**). Furthermore TNF- α is a pro-inflammatory cytokines produced basically by macrophages (**Zhou et al., 2014 and Li et al., 2013**). In the present study, administration of human CD34⁺ and BM-MSCs stem cells similarly reduced TNF- α gene expression. This effect claimed to anti-inflammatory and immunosuppressive properties of MSCs. This is in accordance with the finding of **Huang et al.2014.**, who's revealed that co-culture of MSC with neuron deprived from oxygen and glucose alleviated neuronal recovery with decreasing TNF- α . Moreover, MSCs administration were effective in decreasing the level of TNF- α and treating antigen-induced arthritis in mice (**Kehoe et al., 2014**) and sheep model of osteoarthritis (**Song et al.,2014**).

Wang et al.,2014 demonstrated that human albumin RNA was detected specifically in the livers of the mice that had received CCl₄ injury, but not in other tissues such as spleen and BM. The human albumin-expressing cells, stained blue, were found to be dispersed throughout some areas of the liver. Normally albumin is expressed by hepatocytes indicating a functioning hepatocytes .it exhibits cytoplasmic expression. Bile ducts & portal mononuclear cells don't express albumin. In this study, albumin expressing cells were detected in the portal areas indicating that injected stem cells were transdifferentiated in both CD34⁺ve and BM-MSCs groups into functioning mature hepatocytes indicated by immunohistochemistry.

Crosby et al., 2009, have shown that c-kit and CD34⁺ positive cells isolated from human liver are able to differentiate into biliary epithelial cells and endothelial cells. Thus, biliary cells and endothelial cells may also share some common precursors. Additionally, some antigens traditionally associated with hematopoietic cells (c-kit and CD34⁺) can also be expressed by oval cells. Our study showed positive staining CD34⁺ differentiated injected stem cells in portal area and this explain that oval cells gradually transform themselves into small basophilic hepatocytes,

which then become fully mature hepatocytes and replace the lost liver mass. Thus, the precursor-product relationship between oval cells and basophilic hepatocytes has been proved in our study. Furthermore the adhesion of human haematopoietic (CD34+) stem cells to human liver compartments is integrin and CD44 dependent and modulated by CXCR3 and CXCR4. Barely is the form of liver injury probable to be significant in settling on which stem cell repair mechanism is triggered, although it is also likely to control how hastily liver repair or reconstitution arises.

Our work advocates that in chronic liver injury models, liver repair is a slow process and may take more than 6 weeks. Cells conscripted to the site of regeneration may need to reveal a survival growth advantage over the endogenous liver cells that are affected by the disease process. In conclusion, the present study revealed that human CD34⁺ is more effective in amelioration of CCl₄-induced liver injury in rat than BM-MSCs by reducing fibrosis, expressing liver-specific genes, decreasing gene expression of pro-fibrotic genes (collagen 1 α and TGF β 1), and increasing anti-fibrotic genes (MMP-2, MMP-9 and TNF- α). This work also confirm long term safety efficacy of human cord blood CD34⁺ and potential therapeutic abilities of these cells provided by higher significant synthetic function of CD34⁺ for albumin compared to BM-MSCs.

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Conflict of interest: All authors declare that they don't have conflict of interest in any kind.

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الملخص العربي

دراسة مقارنة بين آثار سى دي ٣٤ + والفئران نخاع العظام الوسيطة الخلايا الجذعية البشرية على تحسين من رابع كلوريد الكربون الناجم عن تليف الكبد

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يحتوى دم الحبل السري (يو سي بي) فى الإنسان والفئران على خلايا نخاع العظم الجذعية الوسيطة التى لديها العديد من المزايا لزرع الخلايا. وكان الهدف من هذه الدراسة التعرف على تأثير الخلايا على عكس الإصابة الكبدية وإحياء وظيفة الكبد في نموذج الفئران من رابع كلوريد الكربون المتسبب فى تليف الكبد. تم عزل (الاحبال السريه) سى دي ٣٤ + الخلايا الجذعية مع الخلايا المغناطيسية. تم عزل الفئران وزراعته الانسجه . وصفت الخلايا في المختبر بواسطة بروتين الفلورية الخضراء. ثم قسمت الفئران إلى ٤ مجموعات كل مجموعة تحتوى على ١٠ فئران. المجموعة ١ (المجموعة الأصحاء)، المجموعة ٢ (مجموعة رابع كلوريد الكربون): الفئران المحقونة برابع كلوريد الكربون ، مجموعة ٣

رابع كلوريد الكربون مجموعة حقن الجرذان رابع كلوريد الكربون وخلايا / (سى دي ٣٤ / مجموعة خلايا نخاع العظم غير متمایزة الإنسان) ، ومجموعة ٤ (رابع كلوريد الكربون الجذعية الفئران حقنها بخلايا غير متمایزة رابع كلوريد الكربون والعظام في الفئران نخاع). ويفصل عينة الدم من الفئران بعد ٤ و ٨ و ١٢ أسابيع لقياس مستوى المصل من ، الزلال I، TGF- β ، الزلال والانين ترانساميناز. تم قياس التعبير الكمي الكولاجين باستخدام الزمن الحقيقي- TNF- α و MMP-9، MMP-2، ومجموعه الميتلاوبروتيناز (في امصال جميع المجموعات التجريبيه بالاضافه RT-PCRتفاعل -البلمرة المتسلسل) الى إجراء فحص التشريح من أنسجة الكبد. ووجد أن الخلايا الجذعية قد استعادت وظيفة المسمى في GFPالكبد التي أكدها فحص الأنسجة من أنسجة الكبد. وتم الكشف عن خلايا مجموعات حقنها بخلايا جذعية. وفيما يتعلق بالتعبير الجيني، والخلايا الجذعية وخلايا إدارة مع انخفاض التعبير TNF- α ، و TGF- β 1 الكولاجين، α تخفيض تعبيرات الجينات من ١ و MMP-2+/ رابع كلوريد الكربون مجموعة. وأعرب عن الزلال، في سى دي ٣٤ جينات غاية في أنسجة الكبد في المجموعات التي تلقت الخلايا الجذعية مع ارتفاع MMP-9 التعبير في المجموعة التي تلقت مشوه سى دي ٣٤+. والخلاصة: أن الخلايا الجذعية من + الإنسان أكثر فعالية في التخفيف من اصابة الكبد الناجم UCB CD34 الاحبال السريه في الفئران من خلايا نخاع العظم الجذعية. وتسلط هذه الدراسة الضوء على CCI4 عن الدور الهام للخلايا الجذعية من دم الاحبال السريه في علاج تليف الكبد.