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Umbilical cord-derived mesenchymal stem cells improves hepatic fibrosis in rat model of liver fibrosis

Taufik Sungkar, Rosita Juwita Sembiring, Dharma Lindarto

Universitas Sumatera Utara, 20155 Medan, Indonesia
taufik.sungkar@usu.ac.id, rositasembiring@ymail.com Agung Putra,
dharma_lindarto@yahoo.com

Abstract. Hepatic fibrosis frequently associated with culminating in tissue scarring, loss of normal parenchyma, and eventually organ failure. Stem cell-derived circulating cells particularly endogenous umbilical cord-derived mesenchymal stem cells (UC-MSCs) can play several roles in rapid regeneration process that regress hepatic fibrosis. The aim of this study was to investigate the effect of UC-MSCs on repair of liver damage in a carbon tetrachloride (CCl4) induced rat model of liver fibrosis.
Methods: UC-MSCs isolated from Sprague-Dawley (SD) rats. Liver fibrosis model was established by intraperitoneal injection of CCl4 (1 ml/kg body weight twice a week for 12 weeks) in SD rats. The survived 18 rats were randomly divided into 3 groups: sham group, untreated liver fibrosis group and UC-MSCs group. At 12 weeks after establishment of animal model, 1×10^6 UC-MSCs were intravenously injected. The histopathology of the liver specimens was evaluated using hematoxylin and eosin (H&E) staining.
Results: Following intravenous UC-MSCs transplantation, histopathology showed less fibrotic areas (F1) in rat liver fibrosis model as compared to positive control group (F3).
Conclusion: Our study indicates that UC-MSCs treatment improves hepatic fibrosis.

Keywords: CCl4, liver fibrosis, rats, umbilical-cord derived mesenchymal stem cells

Intruduction

Liver fibrosis is a result of sustained and chronic liver injury, which is the final common pathway of chronic hepatic injury stimulated by many factors such as viral hepatitis, alcohol, drugs, metabolic diseases, and autoimmune attacks. Liver fibrosis leads to the development of liver cirrhosis and hepatocellular carcinoma at the end stage[1]. According to global statistics, liver disorders have been the predominant cause of over 2 million deaths annually to 2015; approximately 1,200,000 deaths have occurred from chronic liver diseases and liver cirrhosis, and about 800,000 deaths from liver cancer[2]. In Adam malik General Hospital, The most common cause of liver cirrhosis was hepatitis B virus infection (53.7%), unknown causative agent (Non-B and C), 44.4% and hepatitis C infection (1.9%) [3]. Liver fibrosis is characterized by the excess accumulation of collagens and other extracellular matrix (ECM) proteins in pathology and by the clinical impairment of liver function [4].

The most effective therapy for acute liver failure and advanced hepatic fibrosis is liver transplantation, but its use is limited because of organ donor shortage, financial considerations, and requirement for lifelong immunosuppression[5]. Recently, stem cell transplantations, including embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), hematopoietic stem cells and mesenchymal stem cells (MSCs), have been suggested as effective and alternative therapy for hepatic diseases[1].

MSCs might differentiate to ectodermal (neurons), endodermal (hepatocytes) as well as other mesodermal lineages (cardiomyocytes)[6]. Umbilical cord (UC) contains multi-potent stromal cells also known as umbilical cord mesenchymal stem cells (UC-MSCs). UC-MSCs have several advantages, including easier accessibility, more primitive properties, higher proliferation capacity and lower immunogenicity. Due to these advantages, UC-MSCs are being explored as a promising candidate for many potential clinical applications[7]. A recent investigation found that UC-MSCs can accelerate the resolution of acute liver injury without any differentiation and manipulation. Additionally, it has been confirmed histologically that transplantation of UC-MSCs into rats with CCl4-induced liver fibrosis results in significant reduction of hepatic fibrosis[8]. Therefore, the aim of this study is to investigate the ability of UC-MSCs to improve hepatic fibrosis in rat chronic liver fibrosis model by histopathological analyses.

Methods

A. Animal model of liver fibrosis

To induce the liver fibrosis model, Sprague-Dawley (SD) rats were injected intraperitoneally with carbon tetrachloride (CCl4) (Sigma–Aldrich, USA) at dose 1 ml/kg body weight twice weekly for 12 weeks. The animals were randomly allocated into three groups (each group, n =6) as follows: Group I (G1, sham group); Group II (G2, untreated liver fibrosis group), which received the CCl4 injection; and Group III (G3, UC-MSCs treated group), which received the CCl4 injection and the UC-MSCs treatment (1×10^6 cells per rat) via the tail vein.

B. Isolation of umbilical cord derived mesenchymal stem cells

Fresh umbilical cords derived from pregnant sprague-dawley rats. Umbilical cords were rinsed twice in phosphate-buffered saline (PBS) containing 5% penicillin and 5% streptomycin until the cord blood was cleared, and cord vessels were removed. Cords were cut into pieces of 1–3 mm^3 and adhered to flasks for 30 min. Cord pieces were then floated in a low-glucose Dulbecco's modified Eagle's medium containing 10% fetal bovine serum (Gibco), 1% penicillin, and 1% streptomycin (Gibco). Cord pieces were subsequently incubated at 37°C in humid air with 5% CO2. The medium was changed every 3 days after initial plating. When well-developed colonies of fibroblast-like cells reached 80% confluence, cultures were trypsinized with 0.25% trypsin-EDTA (Invitrogen) and passaged into new flasks for further expansion.

C. Immunoprofiling of umbilical cord derived mesenchymal stem cells

Flowcytometry was used to assess the MSCs immunoprofile of UC cells, using the standard for MSCs described by the position paper of the International Society for Cellular Therapy (ISCT) [9]. UC cells at 4th passage were harvested, filtered through a cell strainer (70 µM), pelleted, resuspended in 2% bovine serum albumin (BSA in PBS), and counted. One million cells of each population were used for flowcytometry. Cells were stained with directly (phycoerythrin) conjugated antibodies against CD73, CD90, and CD 105. An appropriate isotype-matched control antibody was used in all analyses. Cells were analysed using flowcytometry.

D. Osteogenic differentiation of umbilical cord derived mesenchymal stem cells in vitro

UC cells at 4th passage were assessed for osteogenic differentiation potentials. Cells were grown in monolayer DMEM and FBS (10%) until reaching 80% confluency, and at this point the cells were given osteogenic differentiation medium for 21 days. Medium was changed every 2-3 days. Osteogenic differentiation medium contained DMEM, FBS (10%), β-glycerophosphate (10 mM), dexamethasone (10 nM), and L-ascorbic-acid (50 µM). Differentiated cells were analyzed by alizarin red staining.

E. Histopathological staining

The rats in each group were anesthetized and sacrificed on days 14 after UC-MSCs injection. Liver tissues were processed for paraffin embedding and were sectioned into 4-µm sections. The sections were stained with hematoxylin and eosin (HE) according to standard protocols.. The degree of hepatic fibrosis was based on a histological grading scale using the METAVIR score [10]. The fibrosis was graded on a 5-point scale from F0 to F4.

F. Statistical analysis

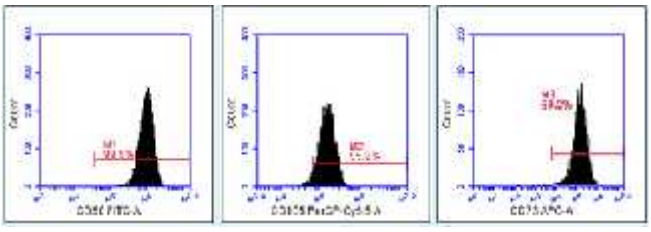
All data were presented as mean ± standard deviation. The differences between different groups were analyzed by a one-way ANOVA with an LSD comparison post hoc test. A $p < 0.05$ was considered as statistically significant.

Results

G. UC-MSCs were isolated and purified successfully

UC-MSCs derived from the umbilical cord express CD73 (99.2%), CD90 (99.9%), and CD105 (95.9%). UC-MSCs were long spindle-shaped cells. In addition, UC-MSCs had osteogenic capabilities. These findings suggested that cells were UC-MSCs (Fig. 1).

A **B** **C**



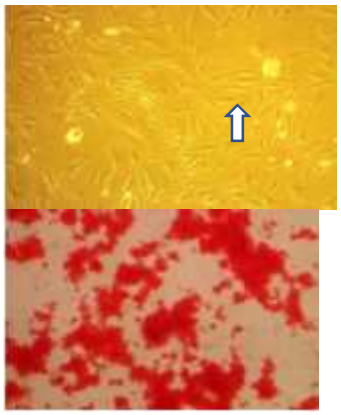


Fig. 1. Characteristics, Isolation and differentiation of UC-MSCs. (A) Flowcytometry immunoprofiling demonstrated immunopositive for markers characteristic of UC-MSCs (CD73, CD90, and CD105); (B) Umbilical cord mesenchymal stem cells were cultured to 80% confluence, and became elongated and similar to fibroblasts which in parallel or spiral-like growth (magnification10x, scale bar 200 µm); (C) Osteogenic differentiation was examined by alizarin red staining (magnification 40x, scale bar 50 µm).

H. UC-MSCs transplantation reduce liver fibrosis

CCl4 is a potent hepatotoxin that causes centrilobular necrosis following peritoneal injection in rats. After treatment, rats were sacrified and liver was extracted from each animal for histology. When the liver tissue was processed with hematoxylin and eosin (HE) staining, periportal fibrosis was observed. fibrous expansion of most portal areas with marked bridging (F3), which indicated the successful establishment of an animal model of hepatic fibrosis in untreated liver fibrosis group, and there is reduction of liver fibrosis (F1) in UC-MSCs treated group (Fig. 2).

Table i. histological stage of hepatic fibrosis

	G1 Mean± SD	**G2 Mean± SD**	**G3 Mean± SD**	**p**	
METAVIR Score	1.00 ±0.00 #*	3.83±0.41 ##*	2.17±0.41	0.000	

G1 (Sham group); G2 (untreated liver fibrosis group); G3 (UC-MSCs treated group).
METAVIR Score; F0 (1), F1 (2),F2(3),F3(4),F4(5).
Significantly lower than untreated liver fibrosis group; *p=0, 00
Significantly higher than UC-MSCs treated group; *p=0, 0

Table I. showed hepatic fibrosis scoring was significantly reduced by UC-MSCs treatment, compared to the untreated liver fibrosis group.

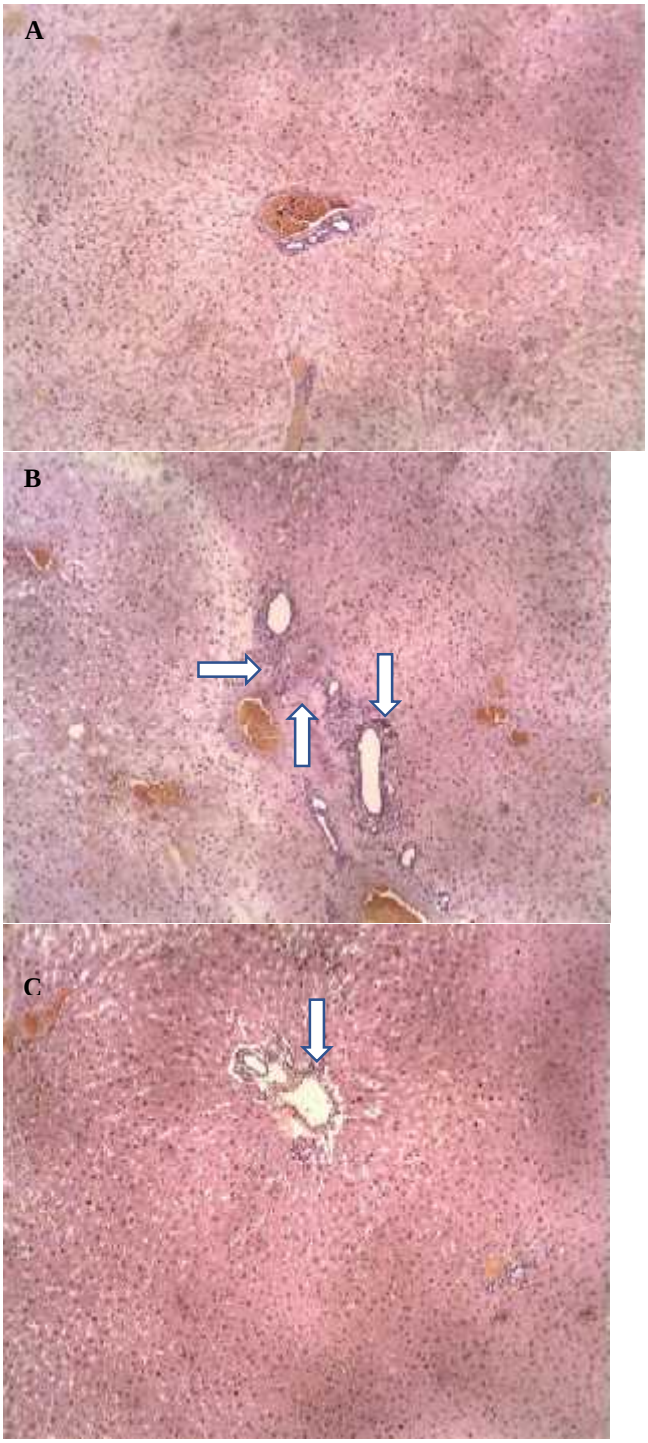


Fig. 2. Histology of liver sections recovered from rat in the various treatment groups. (A) sham group: no fibrosis; (B) untreated liver fibrosis group: histological alterations, fibrous expansion of most portal areas with marked bridging (F3); (C) UC-MSCs treated group: reduction of liver fibrosis (F1) (magnification 100x).

Discussions

Liver injury results in inflammation and the release of inflammatory mediators from inflammatory cells and liver parenchyma, which

further trigger HSCs and increase their multiplication and survival, thereby worsening ECM deposition with an exacerbation of fibrosis [11]. Umbilical cord blood is a very rich source of stem cells that is accessible, easy to obtain and of multipotential differentiation ability[12]. Previous study showed that UC-MSCs administration may accelerate liver regeneration of acute liver failure through platelet derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) regulation [13].

In this study, histopathological analysis of liver sections showed greater necrosis, inflammation, and fibrosis in the rat with CCl4-induced chronic liver fibrosis. Whereas, the liver fibrosis was significantly improved after UC-MSCs administration. The beneficial effect of MSC therapy described in these previous studies could have a different explanation. MSCs can secrete a number of bioactive molecules that affect to biological changing of other cells or known as paracrine effect[14]. MSCs express various trophic factors, such as growth factors, cytokines and chemokines which are known to not only reduce inflammation, apoptosis, and fibrosis of damaged tissues, but also stimulate angiogenesis and regeneration of injured cells[15]. Additionally, MSCs have ability to transdifferentiation into hepatocyte-like cells within liver fibrosis conditions, and regulation of immunomodulatory function[16].

Conclusion

Our study showed that UC-MSCs transplantation improves hepatic fibrosis. Acknowledgment None

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