**Name of Journal: *World Journal of Gastroenterology***

**Manuscript NO: 39310**

**Manuscript Type: REVIEW**

**Taking advantage of the potential of mesenchymal stromal cells in liver regeneration: Cells and extracellular vesicles as therapeutic strategies**

FioreEJ *et al*. Mesenchymal stromal cells and extracellular vesicles in liver regeneration

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**Conflict-of-interest** The authors indicate no potential conflicts of interest.

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**Manuscript source:** Invited manuscript

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**Received:** April 13, 2018

**Peer-review started:** April 13, 2018

**First decision:** April 27, 2018

**Revised:** May 8, 2018

**Accepted:** June 2, 2018

**Article in press:**

**Published online:**

**Abstract**

Cell-based therapies for acute and chronic liver diseases are under continuous progress. Mesenchymal stem/stromal cells (MSCs) are multipotent cells able to migrate selectively to damaged tissue and contribute to its healing and regeneration. The MSC pro-regenerative effect occurs due to their immunomodulatory capacity and their ability to produce factors that promote cell protection and survival. Likewise, it has been observed that part of their paracrine effect is mediated by MSC-derived extracellular vesicles (EVs). EVs contain proteins, lipids and nucleic acids (DNA, mRNA, miRNA, lncRNA) from the cell of origin, allowing for intercellular communication. Recently, different studies have demonstrated that MSC-derived EVs could reproduce, at least in part, the biological effects obtained by MSC-based therapies. Moreover, due to EVs’ stability for long periods of time and easy isolation methods they have become a therapeutic option to MSCs treatments. This review summarizes the latest results achieved in clinical trials using MSCs as cell therapy for liver regeneration, the role of EVs in liver physiopathology and the potential of MSC‑derived EVs as intercellular mediators and therapeutic tools in liver diseases.

**Key words:** Mesenchymal stem cells; Extracellular vesicles; Cirrhosis; Liver; Acute damage; Regeneration

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**Core tip:** Cell-based therapies for acute and chronic liver diseases are very attractive strategies. In particular, mesenchymal stem/stromal cells (MSCs) are multipotent cells able to induce protective and pro-regenerative effects in different liver diseases. The mechanism through which MSCs support tissue regeneration is *via* secretion of paracrine factors, and solid evidence supports that part of these effects is mediated by extracellular vesicles (EVs). Therefore, EVs have become an attractive option in the research for new treatments in liver diseases.

Fiore EJ, Domínguez LM, Bayo J, García MG, Mazzolini GD. Taking advantage of the potential of mesenchymal stromal cells in liver regeneration: Cells and extracellular vesicles as therapeutic strategies. *World J Gastroenterol* 2018; In press

**INTRODUCTION**

A diverse set of toxic, metabolic, and inflammatory insults result in liver diseases and imply different degrees of inflammation, apoptosis, and necrosis of parenchymal cells[[1-4](#_ENREF_1)]. For example, acute liver failure (ALF) is characterized by a massive and sudden death of hepatocytes that lead to abrupt hepatocellular and systemic dysfunction[[3](#_ENREF_3)]. Similarly, in patients with chronic liver disease an important loss of viable parenchymal cells is observed[[1](#_ENREF_1),[2](#_ENREF_2),[4](#_ENREF_4)]. Cirrhosis is caused by diverse chronic liver diseases, such as viral hepatitis and chronic alcoholism[[1](#_ENREF_1),[2](#_ENREF_2)]. Moreover, increases in the prevalence of hypertriglyceridemia, obesity and diabetes in developed countries have resulted in an increase in the incidence of non-alcoholic fatty liver disease (NAFLD)[[4](#_ENREF_4),[5](#_ENREF_5)]. This condition is characterized by a lipid accumulation in the liver that could lead to hepatocytes apoptosis and inflammation. Regardless the liver chronic disease origin, the apoptosis of hepatocytes results in extracellular matrix accumulation that will affect the liver histoarchitecture of liver and ultimately impair its function[[4](#_ENREF_4)]. It is well known that mesenchymal stem/stromal cells (MSCs) migrate toward injured organs where they can provide tissue protection and promote liver regeneration[[6-8](#_ENREF_6)]. These properties make MSCs interesting tools to carry therapeutic genes in modern cellular-based therapeutic strategies[[6](#_ENREF_6)]. It is accepted that the main mechanism through which MSCs support tissue regeneration is *via* secretion of paracrine factors[[7](#_ENREF_7),[9](#_ENREF_9)]. However, solid evidence supports that part of these effects are mediated by extracellular vesicles (EVs)[[10](#_ENREF_10)]. In this review, we first provide an update on clinical trials using MSCs in different liver diseases; second, the mechanisms involved in the therapeutic effects of MSCs; third, general EVs characteristics and their role in liver diseases, and finally, the role of MSC-derived EVs as therapeutic tools for liver regeneration.

**CLINICAL TRIALS INVOLVING THE USE OF MSCS IN LIVER DISEASES**

Clinical investigations using MSCs to treat a broad spectrum of degenerative diseases, including liver diseases, are increasing steadily in recent years[[11](#_ENREF_11),[12](#_ENREF_12)]. The first clinical trial using MSCs was started in 2005 and 52 trials are registered up to now (CinicalTrial.gov and reviewed by Tsuchiya 2017[[13](#_ENREF_13)]). MSCs are obtained from bone marrow in most of the studies, but other sources such as umbilical cord, adipose tissue and menstrual blood has also been tested (Figure 1A). It should be noted that, allogeneic transplantation is more commonly used than autologous (Figure 1B). Between liver diseases, most of the trials are destined to the treatment of liver cirrhosis (Figure 1C) and only 2 of them are in phase II/III (CinicalTrial.gov). Unfortunately, only 22 of 52 registered clinical trials have published their results (Table 1). It is important to mention that MSCs were administered after culture *in vitro* between passages 3 to 6. Regarding the administration route, MSC transplant was performed by peripheral vein[[14-28](#_ENREF_14)], hepatic artery[[29-33](#_ENREF_29)], portal vein[[15](#_ENREF_15),[27](#_ENREF_27)] or directly into the spleen[[16](#_ENREF_16),[34](#_ENREF_34),[35](#_ENREF_35)]. One study performed on 12 patients showed similar therapeutic effects when MSCs were injected into the spleen or intravenously[[17](#_ENREF_17)].

In general, MSC administration in patients with different liver pathologies proved to be feasible and safe (Table 1). Regarding their efficacy, studies demonstrated that MSCs exert positive effects on liver function by increase in serum levels of albumin and improving coagulation or decreasing bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and γ-glutamyltransferase (γ-GT)[[17-19](#_ENREF_17),[21-24](#_ENREF_21),[26](#_ENREF_26),[29](#_ENREF_29),[31](#_ENREF_31),[33-35](#_ENREF_33)]. In addition, the MELD (Model for End-stage Liver Disease) and Child-Pugh scores were improved after MSC treatment[[14-19](#_ENREF_14),[23](#_ENREF_23),[28-32](#_ENREF_28),[34](#_ENREF_34)] (Table 1). Furthermore, in some studies it was demonstrated that the application of MSCs not only improved patient’s quality of life but also modulated the immune response of patients[[22](#_ENREF_22),[26](#_ENREF_26),[31](#_ENREF_31)]. In this sense, a randomized clinical trial using autologous BM-derived MSCs in patients with hepatitis B virus-related liver cirrhosis resulted in an improvement of liver function, an increase in Treg cells, and a decrease in Th17 cells, serum levels of interleukin (IL)-17, tumor necrosis factor alpha (TNF-α) and IL-6; in addition, tumor growth factor beta (TGF-β) levels were increased in comparison with control group[[31](#_ENREF_31)]. Similar results were reported in a phase I/II clinical trial in patients with primary biliary cirrhosis (PBC) transplanted with allogeneic MSCs that showed a reduction in the number of CD8+ T-cells and an increment of Treg cells and IL-10 serum levels[[22](#_ENREF_22)].

Recently, Suk *et al*[[32](#_ENREF_32)]reported a phase II clinical trial comparing the effects of one or two doses of autologous BM-derived MSC therapy with a control group in alcoholic patients. The authors observed that liver fibrosis quantification diminished after cell treatment, although no significant differences in fibrosis area were found between one and two doses. Importantly, no evidence of tumor formation was found during the follow-up in the MSC-treated groups[[32](#_ENREF_32)].

On the other hand, MSCs were used to diminish hepatocellular damage in acute liver diseases. Recently, a phase II clinical trial in acute-on-chronic liver failure compared the standard medical treatment with one dose of allogeneic BM-derived MSC therapy[[28](#_ENREF_28)]. In this study, MSC treatment demonstrated to be safe and able to improve bilirubin levels and MELD score. Interestingly, MSC transplant increased survival rate and decreased severe infection events[[28](#_ENREF_28)].

Long-term immunosuppression is frequently associated with impairment in patients´ quality of life and increased risk of infection or cancer. Therefore, considering their immunomodulatory capacity, MSCs can be used to induce tolerance after liver transplantation. In this regard, Detry *et al*[[25](#_ENREF_25)] reported that MSC infusion 3 d after liver transplant was feasible, safe and well tolerated. No opportunistic infections or *de novo* cancer were detected in 12 mo follow-up. However, no difference was observed in peripheral blood CD4+T cell proportion and immunosuppression could not be withdrawn in MSC-treated patients. In addition, the administration of umbilical cord-derived MSCs as therapy to prolong graft-survival in patients with severe ischemic-type biliary lesions after liver transplantation was also evaluated[[24](#_ENREF_24)]. Six doses of MSCs were infused intravenously every 2-4 wk, and patients were followed-up for 2 years and compared with a prospective cohort of patients treated with standard therapy. Remarkably, MSCs were safe, improved hepatic function, prolonged graft-survival and did not induce cancer or increase microbial infection events[[24](#_ENREF_24)]. In conclusion, these results demonstrated that MSC therapy is a safe procedure, especially in the field of solid organ transplant where intense and prolonged immunosuppression is required. Moreover, MSC administration could prevent the development of opportunistic infections or *de novo* tumor formation. All considered, the promising information generated in the clinic opens the possibility for further studies to determine the optimal protocol conditions of MSC application needed to induce tolerance after liver transplant.

Application of MSCs for treatment of either acute or chronic liver diseases has a promising future in the clinic. In acute liver diseases, MSCs could have a role decreasing liver damage progression or as a bridge for liver transplant. In chronic liver diseases, MSCs could contribute to decrease liver damage, to ameliorate the degree of fibrosis, and even to avoid the need for transplant in some particular cases. On the other hand, in the post-transplant setting, MSC therapy could extend the graft survival and/or decrease the amount of immunosuppression. However, it is extremely important to understand that therapeutic potential of MSCs is an open question, and the information regarding source of MSCs, culture conditions, pre-condition protocols before cell transplantation, administration route, number of doses, and time of treatment are very heterogeneous and standardization is needed.

**MECHANISM OF ACTION OF MSCS IN LIVER REGENERATION**

As detailed above, is necessary to understand the mechanisms that mediate MSCs therapeutic effects prior to continuing with its clinical application. The understanding of MSC biology has grown considerably. In the last decade, many mechanisms involved in their regenerative potential have been identified. These mechanisms involve, at least in part, migration toward injured tissues, immunomodulatory properties, differentiation and/or secretion of regenerative factors, which induce cell survival and proliferation[[7](#_ENREF_7),[9](#_ENREF_9),[36](#_ENREF_36),[37](#_ENREF_37)]. It has been described that MSCs can be recruited to inflamed tissue by classic mechanism of blood stream migration: rolling, adherence to endothelium and transmigration[[8](#_ENREF_8),[36](#_ENREF_36)]. The injured liver produces signals that induce migration and homing of different cell types[[38](#_ENREF_38),[39](#_ENREF_39)]. Both, chronic and acute liver injury induce apoptosis and necrosis of hepatocytes and/or cholangiocytes, infiltration of leucocytes, monocytes and activation of Kupffer cells (KCs), liver sinusoidal endothelial cells (LSECs) and hepatic stellate cells (HeSCs)[[2](#_ENREF_2),[3](#_ENREF_3),[40](#_ENREF_40)]. In this context, MSCs could be attracted by several chemokines, cytokines and factors secreted by the damaged liver microenvironment, such as IL-1β, IL-6, IL-8, monocyte chemoattractant protein-1 (MCP-1), growth-regulated protein (GRO), TNF-α, TGF-β1, platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and stromal cell-derived factor 1 (SDF-1) among others. Receptors for these chemoattractants were found to be expressed in MSCs allowing them to migrate to the injured liver[[11](#_ENREF_11),[36](#_ENREF_36)].

The differentiation potential of MSCs has been considered one of the advantages for their regenerative application[[7](#_ENREF_7),[41-43](#_ENREF_41)]. By definition, MSCs give rise to cells of mesodermal lineages including osteoblasts, adipoblasts and chondroblasts[[44](#_ENREF_44)]. Furthermore, there are many reports demonstrating that MSCs might differentiate to ectodermal (neurons), endodermal (hepatocytes) as well as other mesodermal lineages (cardiomyocytes), but this field remains very controversial[[7](#_ENREF_7),[41](#_ENREF_41)]. In this context, a putative mechanism postulated for liver regeneration was the MSC differentiation into hepatocyte-like cells[[45](#_ENREF_45)]. A number of differentiation *in vitro* protocols were explored in MSCs obtained from bone marrow, adipose tissue or umbilical cord demonstrating that MSC-derived cells acquired characteristic markers and functions of immature and mature hepatocytes (reviewed in Fiore *et al*[[7](#_ENREF_7)]). In fact, a clinical trial compared the effect of autologous BM-MSCs undifferentiated or differentiated into hepatocyte-like cells in patients with cirrhosis. Despite the improvement of liver function, there was no significant difference between undifferentiated or differentiated MSC-based treatment[[17](#_ENREF_17)]. However, difficulties to study and reproduce the *in vitro* differentiation and *in vivo* tracking after their administration, makes the MSC differentiation to hepatocytes a controversial field. This difficulty is due, at least in part, to unspecificity and/or unreliability of methods/trackers/reagents frequently used for the identification of cells or the phenotype they might acquire after their transplantation[[46](#_ENREF_46),[47](#_ENREF_47)]. It should be noted that track able MSCs by GFP expression is detectable in the liver up to 7 d after transplant and then the signal rapidly decreases in animal models[[48](#_ENREF_48)]. Moreover, some evidence indicated that in spite of the MSC homing to the liver, the rate of differentiation to hepatocyte-like cells is very low (less to 1%)[[49](#_ENREF_49),[50](#_ENREF_50)]. Together, this information demonstrates that the main regenerative effect of MSCs cannot be explained by the differentiation to hepatocyte-like cells.

Broad scientific consensus states that the regenerative effect of MSCs is due to paracrine mechanisms. The versatility of MSCs makes them able to differentially express factors depending on the surrounding microenvironment[[51](#_ENREF_51)]. In the context of liver fibrosis, it was observed that MSCs produce high levels of anti-apoptotic growth factors such as SDF-1, VEGF, hepatocyte growth factor (HGF) and insulin-like growth factor (IGF)-I[[9](#_ENREF_9)]. Also, the release of HGF, fibroblast growth factor (FGF), IL-6, fibrinogen and TGF-α can induce hepatocyte proliferation[[51](#_ENREF_51)]. In addition, HGF and epidermal growth factor (EGF) can induce hepatic progenitor cell proliferation and differentiation[[52](#_ENREF_52)] and VEGF increases angiogenesis, an important event for liver regeneration[[53](#_ENREF_53)]. Moreover, IL-10, HGF and IGF-I produced by MSCs can reduce fibrogenesis by inhibition of HeSCs activation and proliferation[[7](#_ENREF_7),[9](#_ENREF_9)]. MSCs also secrete factors involved in extracellular matrix remodeling and chemokines that attract immune cells which could modulate their function[[51](#_ENREF_51)]. In this way, *in vivo* administration of conditioned medium (CM) obtained from MSC cultures can be effective to reduce liver injury. It was reported that the administration of MSC-derived CM significantly improved short-term survival in a D-galactosamine-induced rat model of fulminant hepatic failure[[54](#_ENREF_54),[55](#_ENREF_55)]. Furthermore, MSC-CM therapy had great inhibitory effects on hepatocellular death reducing hepatocyte apoptosis in a 90%. In addition, an increase in liver regeneration programs and number of proliferating hepatocytes was observed[[55](#_ENREF_55)]. Subsequently, it was demonstrated that EVs, present in CM, are partly responsible of the therapeutic effects of MSCs[[10](#_ENREF_10),[56](#_ENREF_56)].

**EXTRACELLULAR VESICLES**

Although, EVs were described as intracellular mediators many years ago, they have recently generated great interest as therapeutic and diagnostic tools. Initially, “extracellular vesicle” was used to refer to all kind of vesicles released by cells. Nevertheless, the increased knowledge of their biology allowed to distinguish between different types of vesicles[[57](#_ENREF_57)]. Exosomes (50-100 nm in diameter) are homogenous and the largest family of EVs and are different from microvesicles (100-1000 nm) and apoptotic bodies (500-2000 nm) in size and biogenesis[[58](#_ENREF_58)]. While exosomes are originated from multivesicular bodies (MVBs), microvesicles are originated directly from plasma membrane and released to extracellular space[[58](#_ENREF_58)]. At present, vesicles can only be fractioned according to their sizes and no specific markers have been described. Due to a large heterogeneity in methods of isolation and terminology in the past published results, in this review we will refer to exosomes and microvesicles as “EVs”. In order to define a minimal criteria for EV characterization, the International Society for Extracellular Vesicles (ISEV) suggests a semi-quantitative analysis for typical protein marker, such as CD9, CD63, CD81, Alix or TSG101, size analysis and morphology examination[[59](#_ENREF_59)]. In addition to these specific proteins, EVs contain a large number of proteins (growth factors, cytokines, vesicles proteins), DNAs, mRNAs, microRNAs (miRNAs), long non-coding RNAs (lncRNAs)[[57](#_ENREF_57),[59](#_ENREF_59)]. An interesting characteristic of EVs is that they can be charged with specific components of the cell of origin, and this “cargo” could be modified by different stimuli and microenvironment conditions[[58](#_ENREF_58)]. As mentioned above, it has been demonstrated that EVs are involved in the paracrine effects of MSCs, but as EVs are implicated in many intercellular communications, we will first describe their implication in liver diseases.

**EVS IN LIVER DISEASES**

EVs have been implicated in a number of physiological and pathophysiological processes, such as immune response, angiogenesis, tissue regeneration, tumorigenesis/metastasis and neurodegenerative diseases[[60-62](#_ENREF_60)]. In patients with primary biliary cirrhosis it was demonstrated that serum exosomes are taken up by peripheral monocytes and dendritic cells, resulting in the up regulation of co-stimulatory molecules[[63](#_ENREF_63)]. Interestingly, serum circulating EVs present different miRNA composition in cirrhotic patients when compared with healthy controls[[63](#_ENREF_63),[64](#_ENREF_64)]. Recent findings demonstrated that EVs are implicated in viral hepatitis, drug-induced injury, alcohol injury, non-alcoholic steatohepatitis and biliary injury[[65](#_ENREF_65),[66](#_ENREF_66)].

***EVs derived from parenchymal cells***

Exosomes transport a variety of macromolecules that could act as signals between donor and recipient cells. *In vitro* studies demonstrated that liver parenchymal cells produce EVs that are involved in many physiological and pathophysiological processes[[65](#_ENREF_65),[66](#_ENREF_66)]. For instance, it was demonstrated that there is an increase in the number of circulating exosomes with proliferative effect on hepatocytes after ischemia/reperfusion injury[[67](#_ENREF_67)]. Nojima *et al*[[67](#_ENREF_67)] reported that EVs derived from hepatocytes, but no other liver cells can induce hepatocyte proliferation *in vitro*. It should be noted that hepatocyte-derived EVs exert their effect in a dose-dependent manner. Furthermore, their administration in mice under ischemia/reperfusion liver injury or after 70% hepatectomy promotes hepatocyte proliferation and liver regeneration. Similarly, Herrera *et al*[[68](#_ENREF_68)]showed that hepatic progenitor cell-derived EVs promote hepatocyte proliferation, suppress cell death, and accelerate liver regeneration in rats after hepatectomy. The author suggested that this effect is mediated by the delivery of RNA by EVs to target cells. It is worth mention, that current data demonstrated that hepatocyte-derived exosome properties are mediated by fusion with target hepatocytes transferring their cargos[[67](#_ENREF_67),[69](#_ENREF_69),[70](#_ENREF_70)]. For example, hepatocyte-derived EVs transfer neutral ceramidase and sphingosine kinase 2 (SK2) within hepatocytes resulting in the induction of sphingosine-1-phosphate (S1P), a demonstrated promoter of cell proliferation[[67](#_ENREF_67)] (Figure 2). Further studies of the same group showed that CXCR1 is required for packaging of SK2 into exosomes and CXCR2 regulates neutral sphingomyelinase activity and there by neutral ceramidase production[[71](#_ENREF_71)].

On the other hand, hepatocyte-derived EVs in a nonalcoholic steatohepatitis (NASH) model interact with macrophages inducing an inflammatory phenotype[[72](#_ENREF_72)] (Figure 2). Activation of the death receptor 5 (DR-5) expressed on hepatocytes by free fatty acid induces there lease of EVs that could stimulate IL‑1β and IL-6 expression on macrophages[[72](#_ENREF_72)]. On the same line, EVs derived from palmitic acid (PA) stimulated hepatoma cells (Huh7 and HepG2) induce a profibrogenic phenotype on HeSCs[[73](#_ENREF_73)]. Moreover, the activation of HeSCs seems to be related with the presence of miRNA-122 and miRNA-192 in the EVs[[73](#_ENREF_73)] (Figure 2). Similarly, an increase in the number of circulating exosomes in mice is observed after chronic alcohol consumption[[74](#_ENREF_74)]. Furthermore, *in vitro* incubation of hepatocytes with ethanol not only increases the release of exosomes but also allows monocyte activation through miRNA-122 horizontal transfer[[74](#_ENREF_74),[75](#_ENREF_75)]. Remarkably, circulating EVs obtained from patients with alcoholic hepatitis show higher levels of miRNA-30a, miRNA-192 and, in particular, miRNA-122 than those obtained from heathy donors[[74](#_ENREF_74)] (Figure 2).

EVs have also been implicated in horizontal transfer of information in chronic hepatitis C infection between hepatocytes and HeSCs. Devhare *et al*[[76](#_ENREF_76)] demonstrated that EVs derived from HCV-infected hepatocytes transfer viral RNAs that increases the expression of profibrogenic markers on HeSCs. Interestingly, these EVs carry miR-19a that activates the STAT3 signaling, enhancing the expression of TGF-β and IL-6. Importantly, miR-19a up-regulation was observed in HCV-infected hepatocytes and in sera of chronic HCV patients with fibrosis[[76](#_ENREF_76),[77](#_ENREF_77)] (Figure 2). Moreover, Seo *et al*[[78](#_ENREF_78)] demonstrated that EVs are implicated in HeSCs activation *via* toll-like receptor 3 with the subsequent activation of γδ T cell population exacerbating liver fibrosis. Regarding other liver parenchymal cells, the role of cholangiocyte-derived EVs has been less studied. However, the presence of circulating EVs with altered miRNA composition and immunostimulatory functions on patients with PBC could suggest a role of cholangiocyte-derived EVs on this disease[[63](#_ENREF_63),[64](#_ENREF_64)].

***EVs derived from non-parenchymal cells***

Non-parenchymal cells, including LSECs, Kupffer cells, lymphocytes, and HeSCs play a critical role in many liver diseases and use EVs for communication with neighbor cells during liver damage[[65](#_ENREF_65),[66](#_ENREF_66)]. For instance, connective tissue growth factor (CCN2), a pro-fibrogenic mediator, is packaged into EVs produced by activated HeSCs. Then, exosome CCN2 can be delivered to other quiescent or activated HeSCs to induce trans-activation[[79](#_ENREF_79)]. On the other hand, EVs derived from quiescent but not from activated HeSCs transport Twist 1 that suppress CCN2 on target cells through miR-214 induction[[80](#_ENREF_80)]. Furthermore, Chen *et al*[[81](#_ENREF_81)]showed that miR-199a-5p is loaded in quiescent HeSCs-derived EVs and also regulates CCN2 expression and activity on target cells (Figure 2). It seems to be a balance between EVs derived from active or quiescent HeSCs that promotes or inhibits liver damage. In addition, LSECs are known to maintain the HeSC quiescence through direct cell-to-cell contact and paracrine factors secretion[[82](#_ENREF_82)]. Wang *et al*[[83](#_ENREF_83)] demonstrated that LSEC-derived EVs have the ability to transfer Sphingosine kinase 1 (SK1) to regulate HeSC activation. In contrast, Ichinohe *et al*[[84](#_ENREF_84)] demonstrated that progenitor hepatic cell-derived EVs stimulate LSECs and Kupffer cells to produce IL17B and IL25 resulting in proliferation of small progenitor hepatic cells (SPHCs) and liver regeneration (Figure 2). These findings evidence the importance of EVs derived from LSECs in affecting the activation state of neighboring cells. However, the mechanisms by which exosomes reach and attach target cells are not well understood. It has been reported that endocytosis of LSEC-derived EVs is mediated by the interaction between exosomal fibronectin and its ligand on the surface of HeSCs[[83](#_ENREF_83)]. Additionally, integrin αvβ3 or α5β1 and heparan sulfate proteoglycan are ligand for EVs-HeSCs interaction and allow information transfer from endothelial cells[[85](#_ENREF_85)].

Other important non-parenchymal cells involved in fibrogenesis are Kupffer cells and infiltrating macrophages. These cells are key players not only in fibrogenesis but also in fibrosis resolution and regeneration[[86](#_ENREF_86),[87](#_ENREF_87)]. In alcoholic liver injury, hepatocyte-derived EVs enriched in miR‑122 sensitize macrophages to LPS and induce their production of pro-inflammatory cytokines[[75](#_ENREF_75)]. Moreover, CD40L presence on hepatocyte-derived EVs during alcoholic hepatitis promotes macrophage activation and the switch to a pro-inflammatory profile[[88](#_ENREF_88),[89](#_ENREF_89)]. In contrast, *in vitro* exposure of monocytes to alcohol increased their release of EVs, which in turn induce an anti-inflammatory M2 profile of naïve monocytes[[90](#_ENREF_90)]. Interestingly, EVs derived from alcohol exposed monocytes contain high levels of miR-27a that is known to induce M2 polarization[[90](#_ENREF_90),[91](#_ENREF_91)] (Figure 2).

In summary, it has been determined that EVs play a key role on the pathophysiological response to liver damage. EVs allow the interaction between parenchymal cells and non-parenchymal cells, mainly HeSCs, Kupffer cells and LSECs, mediating their anti/pro-fibrogenic state. The role of EVs in cell-to-cell communication during liver damage and the transfer of molecules, proteins and miRNAs is gaining importance in the field. Furthermore, this mechanism can be exploited for new therapeutic approaches or used as biomarkers in non-invasive methods. In line with this, it has recently been reported that MSCs release high levels of EVs that can mediate part of their therapeutic effects. Thus, considering the key role of EVs in liver cell communication, MSC-derived EVs (MSC-EVs) could be studied as a new therapeutic approach for hepatic regeneration strategies.

**MSC-EVS AND THEIR POTENTIAL FOR LIVER REGENERATION**

As described above, the main mechanism by which MSCs support there pair and regeneration of injured tissues is by releasing paracrine factors[[7](#_ENREF_7)]. Recently, this paracrine mechanism was described to be partially mediated by EVs released by MSCs[[10](#_ENREF_10),[56](#_ENREF_56)]. *In vitro* assays demonstrated that MSC-EVs induce hepatocyte proliferation and dedifferentiation into progenitor oval cells[[92](#_ENREF_92),[93](#_ENREF_93)]. Therapeutic effects of MSC-EVs were demonstrated in pre-clinical models of acute kidney injury[[94](#_ENREF_94)], and then in pathologies of heart, brain, kidney, muscle and liver[[95](#_ENREF_95)].

Li *et al*[[96](#_ENREF_96)] demonstrated that MSC-EVs reduced the degree of hepatic injury, collagen deposition and inflammation in mice with fibrosis induced by carbon tetrachloride (CCl4). The antifibrotic effect observed by MSC-EVs is mediated by the inactivation of TGF-β1/Smad signaling pathway. Moreover, a reversion of the epithelial-to-mesenchymal transition (EMT) both *in vivo* and *in vitro* was observed after the EV treatment[[96](#_ENREF_96)] (Figure 2). Therefore, EVs derived from human MSCs were effective in mice demonstrating that they preserve at least part of the immunomodulatory properties of the cells of origin.

As for liver fibrosis, the therapeutic capacity of MSC-EVs was also assessed in acute models of liver injury. Cheng *et al*[[97](#_ENREF_97)]studied the effect of EVs derived from menstrual blood MSCs in a galactosamine/LPS mice model. MSC-EVs were able to enhance animal survival and reversion of liver failure through hepatocyte apoptosis inhibition and systemic inflammation reduction. In addition, *in vitro* assays showed that EVs are taken up by AML12 cells (hepatocyte cell line) resulting in the inhibition of apoptosis induced by galactosamine/LPS[[97](#_ENREF_97)]. Similar results were observed by Haga *et al*[[98](#_ENREF_98)]using EVs derived from bone marrow MSCs in an animal model of acute liver failure. A relevant finding was the preservation of the biological activity of cryopreserved-EVs m up to 3 mo, indicating the stability of EVs[[98](#_ENREF_98)].

It is known that induction of oxidative stress in the liver results in severe hepatic diseases by inducing cell apoptosis. A protective and proliferative effect of MSC-EVs in a lethal mice model induced by a single dose of CCl4 has been reported[[99](#_ENREF_99)]. In addition, MSC-EVs inhibited hepatocyte apoptosis induced by acetaminophen and H2O2 and increased cell viability *in vitro*[[99](#_ENREF_99)]. In this line, Yan *et al*[[100](#_ENREF_100)] demonstrated that EVs derived from umbilical cord MSCs induced an antioxidant effect on hepatocytes. Glutathione peroxidase 1 (GPX1) delivered on MSC-EVs reduces the reactive oxygen species (ROS) intracellular levels and inhibits the oxidative stress-induced apoptosis *in vitro* and *in vivo.* Remarkably, authors demonstrated that a low dose of EVs (16 mg/kg of body weight) resulted in similar effects either by tail vein administration or oral gavage. In addition, Nong *et al*[[101](#_ENREF_101)]tested the effect of EVs generated from MSC-derived Induced pluripotent stem cells (iPSCs) (MSC-iPSC-EVs) in hepatic ischemia-reperfusion (I-R) injury models. *In vivo* administration of MSC-iPSC-EVs in I-R injury mice model resulted in a decrease of oxidative stress response and apoptosis, and an increase of hepatocyte proliferation (Figure 2).Consistently, an amelioration of hepatic damage and inflammatory response was observed after EV treatment. It should be noted that MSC-iPSC-EVs keep the characteristics of EVs usually obtained from tissue-derived MSCs (bone marrow, adipose tissue and umbilical cord)[[101](#_ENREF_101)]. In addition, it has been reported that MSC-iPSC-EVs could directly fuse with hepatocytes increasing the activity of sphingosine kinase (SK1). Moreover, the increase in SK activity will in turn increase the sphingosine-1-phosphate (S1P) levels affecting hepatocyte proliferation[[93](#_ENREF_93)] (Table 2).

Although there is a great therapeutic potential of MSC-EVs in liver protection and regeneration, it is mandatory to understand the mechanisms involved in their biological effects. One key point of research is to know the bio distribution of EVs after systemic administration *in vivo*. *In vitro* assays showed that EVs are taken up *via* integrin mediated endocytosis by target liver cells[[67](#_ENREF_67),[85](#_ENREF_85),[90](#_ENREF_90),[97](#_ENREF_97)]. In addition, MSC-EV biodistribution after intravenous (IV) administration shows EVs uptake as fast as 3 to 6 h after injection in liver, spleen and lung cells[[97](#_ENREF_97)]. Furthermore, Haga *et al*[98]reported that liver, spleen and lung from mice with fulminant hepatitis exert a higher uptake of MSC-EVs in comparison with organs from healthy mice. However, since these experiments have been carried out using lipophilic tracers, confirmation of these results with specific markers is necessary. The MSC-EV survival in circulation after being administered and the recognition pathways used by the target cells need to be studied in depth. Moreover, this knowledge could help to define the better scheme of doses, the time of treatment and the route of administration depending of the type of liver damage. In addition, conformation details of proteins and nucleic acids loaded in the EVs is required.

**ENGINEERING MSC-EVS FOR LIVER REGENERATION**

Recently, an increased interest has been shown by the research community to improve the efficiency and potency of EVs loading specific cargos[[102](#_ENREF_102)]. As described above, the EVs produced by MSCs have useful properties that would allow them to be used as therapies in different liver pathologies[[62](#_ENREF_62)], and advances in nanomedicine would allow to improve the technology to generate more effective EVs[[102](#_ENREF_102),[103](#_ENREF_103)]. Thereby, engineered EVs can be generated with strategies based on covalent surface chemistry, hydrophobic insertions or membrane permeabilization. Moreover, modification of the parental cells through metabolic labeling, genetic modification or by insertion of exogenous material could also produce modified EVs. These engineered EVs could carry specific DNAs, mRNAs or non-coding RNAs to the specific cell[[102](#_ENREF_102)].

Lou *et al*[[104](#_ENREF_104)]demonstrated that miRNA-122 expression in MSCs by lentiviral infection increased their therapeutic effect in a fibrosis model in mice. MiR-122 positively regulates proliferation and trans differentiation of HeSCs having an important role in liver fibrogenesis[[105](#_ENREF_105)]. Authors demonstrated that *in vitro* miRNA-122 is transferred to HeSCs through MSC-EVs resulting in the regulation of genes involved in collagen maturation and cell proliferation[[104](#_ENREF_104)]. Another strategy uses EVs derived from MSCs with transient expression of miRNA-181-5p as a therapeutic option in a liver fibrosis model[[106](#_ENREF_106)]. As for miRNA-122, miRNA-181-5p was delivered by MSC-EVs reducing the fibrosis and the inflammatory state of fibrotic mice[[106](#_ENREF_106)]. Moreover, *in vitro* experiments show that after reaching the target cell, miRNA-181-5p binds to3´-UTR of STAT3 and Bcl-2, which in turn down regulates TGF-β1 expression and induces autophagy in HeSCs[[106](#_ENREF_106)]. Finally, in a model of autoimmune hepatitis, a cytoprotective effect of MSC-EVs engineered to charge miRNA-223 was observed. The *in vitro* experiments demonstrated that miRNA-223 levels were increased in hepatocytes after their incubation with the engineered EVs[[107](#_ENREF_107)]. Similar results were observed *in vivo* with an increase of this miRNA in the liver and a reduction of its target gene NLRP3, and therefore a decrease in hepatocyte apoptosis[[107](#_ENREF_107)] (Figure 2).

In summary, more information is needed not only to develop more efficient therapies for liver diseases based on MSC-EVs but also to engineer them to increase their efficacy and potency.

**CONCLUSION**

MSCs-based therapy has emerged as a potent and innovative treatment for acute and chronic liver diseases. The safety and feasibility observed in the early clinical trials using MSCs have increased the interest to translate the use of these cells to the clinic. Moreover, pro-regenerative results and an improvement in the life quality of patients were observed. In ALF, MSCs could have a role decreasing liver damage progression due their immunomodulatory properties. In chronic liver diseases, MSCs could contribute to decrease liver damage and to ameliorate the degree of fibrosis. Even more, in both case MSC treatment could not only delay the transplant but also to avoid it in some particular cases. In addition, in the post-transplant setting, MSC therapy could extend the graft survival and/or decrease the amount of immunosuppression required. Although the main mechanism by which MSCs support the repair and regeneration of injured livers is by releasing paracrine factors, strong evidences demonstrated that this paracrine mechanism is mediated by EVs released by MSCs. Therefore, due to EVs’ stability for long periods of time and easy isolation methods they have become a therapeutic option to MSCs treatments in liver diseases. At present, EVs are strongly explored for therapeutic or diagnostic application, and more information is needed to develop more efficient tools for liver diseases based on MSC-EVs. However, it is important to understand that therapeutic potential of MSCs or its EVs is still a matter of debate. In addition, standardization of source of MSCs, culture conditions, pre-condition protocols for cell transplantation, administration route, doses and time of treatment is required. Nevertheless, considering that development of new therapeutic approaches for liver diseases is urgent, MSCs emerge as potent innovation. Thus, take advantage of the therapeutic potential of MSCs as promising tool for liver regeneration could attend to an important worldwide human health problem.

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**P-Reviewer:** Musumeci G, Miloso M, Scarfì S **S-Editor:** Wang XJ

**L-Editor:** **E-Editor:**

**Specialty type:** Gastroenterology and hepatology

**Country of origin:** Argentina

**Peer-review report classification**

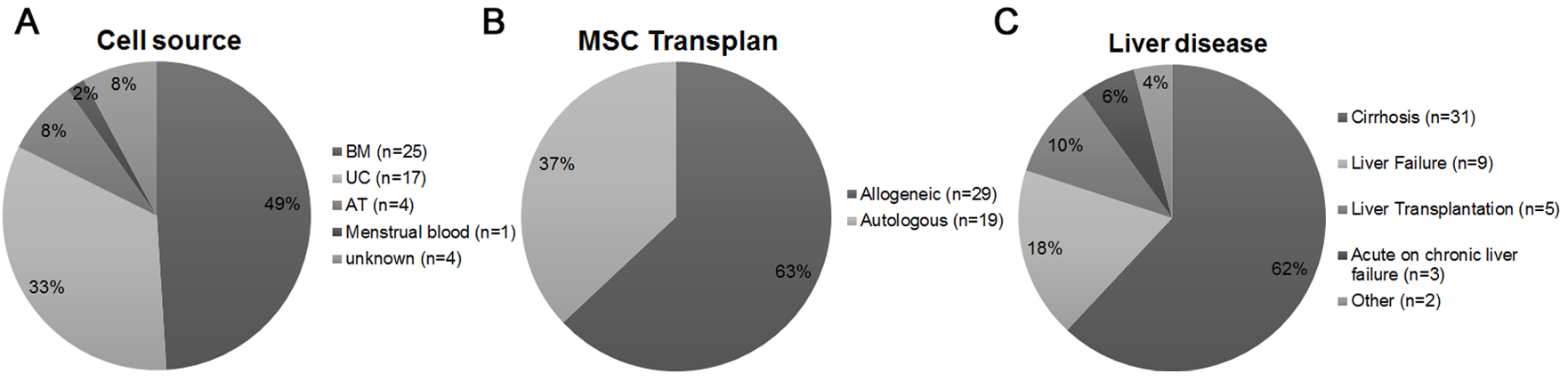
Grade A (Excellent): A

Grade B (Very good): B

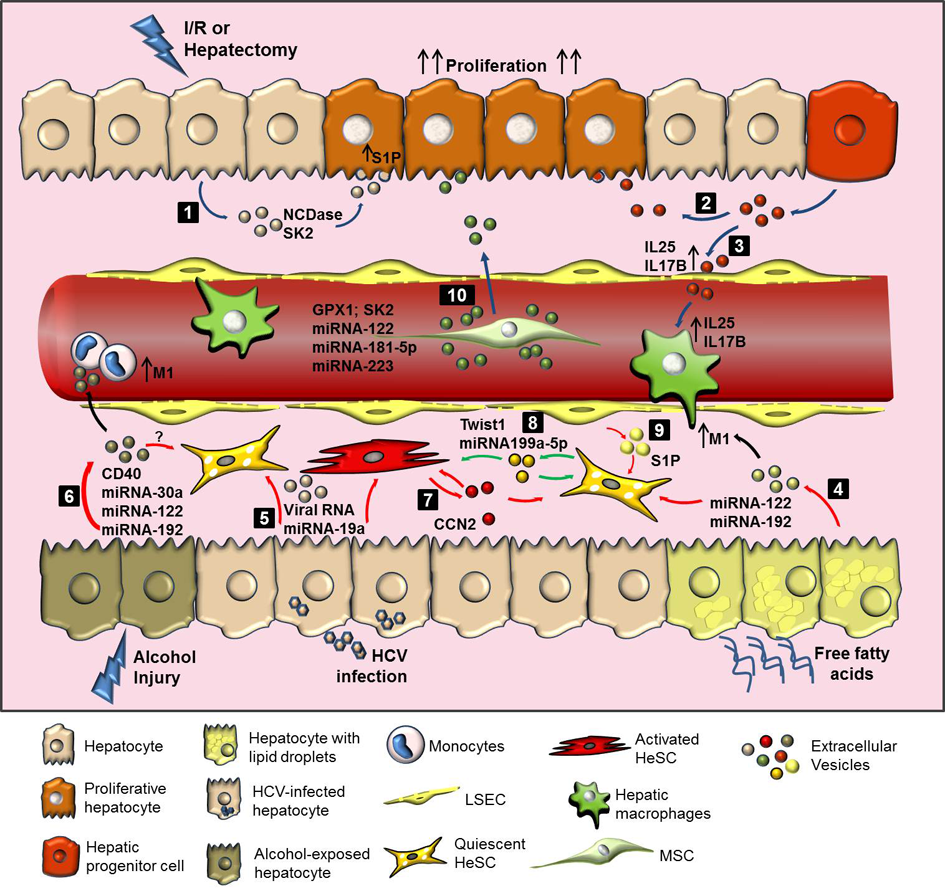
Grade C (Good): 0

Grade D (Fair): D

Grade E (Poor): 0



**Figure 1 Mesenchymal stem/stromal cells clinical trials in liver disease.** A: MSCs clinical trials classified by cell source; B: MSCs clinical trials classified by transplant type; C: MSCs clinical trials classified by liver disease treated. Data from http//[www.clinicaltrial.gov](http://www.clinicaltrial.gov). *n*: Number of clinical trials; MSCs: Mesenchymal stem/stromal cells; BM: Bone marrow; UC: Umbilical cord; AT: Adipose tissue.

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**Figure 2 Extracellular vesicles as paracrine mediator in liver disease and therapeutics potential of mesenchymal stem/stromal cells**. After ischemia reperfusion injury (I/R) or hepatectomy, hepatocytes (1) HPCs (2) release EVs with the ability to induce hepatocyte proliferation. (3) HPC-derived EVs stimulate LSECs and macrophages production of proliferative cytokines such as IL25 and IL17B. (4) On the other hand, free fatty acids induce the production of hepatocyte-derived EVs that result in the activation of quiescent HeSCs and pro-inflammatory macrophages (M1). (5) During chronic hepatitis C virus infection, EVs secreted by HCV-infected hepatocytes induce activation of HeSCs. (6) EVs secreted by hepatocytes after alcohol injury (containing CD40L and miRNAs) induce activation of monocytes and HeSCs. It seems to be a balance between EVs derived from active or quiescent HeSCs that promotes or inhibits fibrogenesis. Activated HeSC-derived EVs induce activation of quiescent HeSCs trough CCN2 (7) and quiescent HeSCs inhibit activated HeSCs transferring Twist1 or miRNA199a-5p (8). LSEC-derived EVs could also regulate HeSC activation (9). MSC-EVs induce hepatocyte proliferation, reduce oxidative stress and apoptosis, and modulate inflammatory response by carrying GPX1 or SK2 (10). Engineered MSC-EVs transferring miRNA-122, miRNA‑181‑5p and miRNA-223 have potential effects. The effects of MSC-EVs on HeSCs, hepatic macrophages, LSEC and infiltrated cells populations remain poorly explored. Green arrows: Inactivation of HeSCs; Red arrows: Activation of HeSCs; Blue arrow: Proliferative effect; Colors spots represent EVs from different cell origin; NCDase: Neutral ceramidase; SK2: Sphingosine kinase 2; S1P: Sphingosine-1-phosphate; IL: Interleukin; SK1: Sphingosine kinase 1; CCN2: Connective tissue growth factor; Twist1: Basic helix-loop-helix transcription factor; GPX1: Glutathione peroxidase 1; HCV: Hepatitis C virus; EVs: Extracellular vesicles.

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Table 1 Mesenchymal stem/stromal cells clinical trials for liver diseases** | | | | | | | | | | |
| **Reference** | **Etiology**  **or disease** | **Cell source/origen** | **Study design (*n*; groups)** | **Cell administration condition** | | | **Phase** | **Results** | **Follow up** | **Side effects** |
| **Dose** | ***P* value** | **Route** |
| Mohamadnejad *et al*[14] 2007 | Cirrhosis | BM/Auto | *n* = 4 | 1, 0.6 × 107 | 2-4 | IV | I | MELD ↓ | 12 mo | None |
| Kharaziha *et al*[15] 2009 | Cirrhosis | BM/Auto | *n* = 8;  4 HBV  1 HCV  1 Alcohol  2 Control | 3.5 × 107 | 3-4 | PV/IV | I/II | MELD ↓ | 24 wk | None |
| El-Ansary*et al*[16]2010 | Cirrhosis | BM/Auto | *n* = 12 | 10 × 106 | 1 | IS/IV | I | MELD ↓; no differences between IS *vs* IV | 6 mo | NA |
| Peng *et al*[29]2011 | Cirrhosis (HBV) | BM/Auto | *n* = 158;  53 MSC  105 Control | 3.4-3.8 × 108 | 3 | HA | I/II | ALB ↑, MELD ↓ | 48 mo | None |
| Amer*et al*[34]2011 | Cirrhosis (HCV) | BM/Auto | *n* = 40;  0 MSC  20 Control | 2 × 107 | NA | IS/IH | I/II | ALB ↑, C.P ↓, MELD ↓ | 6 mo | Fever (50%), transient shivering (15%) |
| El-Ansary *et al*[17]2012 | Cirrhosis (HCV) | BM/Auto | n=25;  9 MSC  6 Hep. Diff.  10 Control | 1 × 106/kg MSC or 40% HLCs and 60% MSCs | 5 | IV | II | ALB↑, MELD ↓, no differences between HLCs *vs* MSCs. | 6 mo | NA |
| Zhang *et al*[18]2012 | Cirrhosis (HBV) | UC/Allo | *n* = 45;  30 MSC  15 Control | 0.5 × 106/kg every 4 wk, 3 times | 3-4 | IV | I/II | ALB ↑, MELD↓, ascites ↓ | 48 wk | None |
| Shi *et at*[19]2012 | Acute-on-chronic  Liver failure (HBV cirrhosis) | UC/Allo | *n* = 43;  34 MSC  9 Control | 0.5 × 106/kg every 4 wk, 3 times | 3-4 | IV | I/II | ALB ↑, PT ↑, MELD ↓, SR↑ | 72 wk | None |
| Mohamadnejad *et al*[20]2013 | Cirrhosis | BM/Auto | *n* = 25;  14 MSC  11 Control | 2 × 108 | 3-4 | IV | II | No beneficial effect | 12 mo | None |
| Wang *et al*[21]2013 | UDCA-resistant PBC | UC/Allo | *n* = 7 | 0.5 × 106/kg every 4 wk, 3 times | 4 | IV | I/II | ALP↓, γ-GT ↓, quality of life↑ (fatigue↓, pruritus↓) | 48 wk | None |
| Amin *et al*[35]2013 | Cirrhosis | BM/Auto | *n* = 20 | 10 × 106 | 2 | IS | I/II | ALT ↓,AST ↓,BIL ↓, PT ↓, ALB↑, PT↑ | 24 wk | None |
| Jang *et al*[30]2014 | Cirrhosis | BM/Auto | *n* = 11 | 5 × 107 every 4 wk, 2 times | 4-5 | HA | II | C.P ↓, TGF- ↓, ‑SMA ↓, collagen‑1 ↓, fibrosis ↓, | 20 wk | None |
| Wang *et al*[18]2014 | UDCA-resistant PBC | BM/Allo | *n* = 10 | 3-5 × 105/kg | 3-5 | IV | I/II | ALT ↓, AST ↓, γ‑GT, BIL ↓, IgM ↓, Tregs ↑, IL-10↑, CD8+T cells↓ | 12 mo | None |
| Salama *et al*[23]2014 | Cirrhosis | BM/Auto | *n* = 40;  20 MSC  20 Control | 1 × 106/kg | 0 | IV | II | ALT↓, AST↓,BIL↓, ALB↑, PT↑, C.P↓, ascites ↓ | 26 wk | NA |
| Xu *et al*[31]2014 | Cirrhosis | BM/Auto | *n* = 56;  27 MSC  27 Control | 0.75 × 106/kg | NA | HA | II/III | ALB↑, MELD↓, ↑ Tregs/Th17 cell ratio, IL-17↓, TNF‑↓, IL-6↓, TGF- ↑ | 24 wk | NA |
| Suk *et al*[32]2016 | Cirrhosis | BM/Auto | *n* = 55;  18 MSC (× 1)  19 MSC (× 2)  18 Control | 5 × 107 (1 m post BM asp.) /5 × 107 (1 and 2 m post BM asp.) | 4-5 | HA | II | C.P ↓, fibrosis ↓ | 12 mo | None |
| Zhang *et al*[24]2017 | Ischemic-type biliary lesions | UC/Allo | *n* = 82;  12 MSC  70 Control | 1 × 106/kg; week 1, 2, 4, 8, 12 and 16 | 4 | IV | II/III | BIL↓,ALP↓, γ‑GT ↓, graft survival ↑ | 24 mo | None |
| Detry *et al*[25]2017 | Liver transplantation | BM/Allo | *n* = 20;  10 MSC  10 Control | 1.5-3 × 106/kg; day 3 post-transplant | 2-3 | IV | I/II | No beneficial effect | 6 mo | None |
| Sakai *et al*[33]2017 | Cirrhosis | AT/Auto | *n* = 4 | 6.6 × 105/kg | 0 | HA | I | ALB ↑, PT↓ | 1 mo | None |
| Shi *et al*[26]2017 | Liver transplantation | UC/Allo | *n* = 20;  14 MSC  13 Control | 1 × 106/kg; every 4 wk, 3 times | 3-4 | IV | I | ALT↓, AST↓, BIL ↓, improve liver allograft histology, acute rejection↓ (↑ peripheral Tregs, ↑ Tregs/Th17 cell ratio). | 12 wk | None |
| Hartleif *et al*[27]2017 | Pediatric liver transplantation | BM/Allo | *n* = 7 | 1 × 106/kg; day 0 and day 2 post-transplantation | 2-3 | PV/IV | I | NA | 24 mo | None |
| Lin *et al*[28]2017 | Acute-on-chronic | BM/Allo | *n* = 110;  56 MSC  54 Control | 1-10 × 105 cells/kg; 1/wk,  4 wk | 5-6 | IV | I/II | MELD↓, SR↑, infections↓ | 24 wk | None |
| Cirrhosis (HBV) |  |  |

P: Passage; BM: Bone Marrow; Allo: Allogeneic; Auto: Autologous; IV: Intravenous Infusion; MELD: Model for end-stage Liver Disease; HBV: Hepatitis B Virus; HCV: Hepatitis C Virus; IS: Intrasplenic; IH: Intrahepatic, NA: Not Available; HA: Hepatic Artery; CP: Child-Pugh score; HLC: Hepatocyte-Like Cells; UC: Umbilical cord; SR: Survival Rate; Cr: Creatinine; BIL: Bilirubin; PBC: Primary biliary cirrhosis; UDCA: Ursodeoxycholic acid*;* Diff: Hepatocyte differentiated; PT: Prothrombin time; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; γ-GT: γ-glutamyltranspeptidase.

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| --- | --- | --- | --- | --- |
| **Table 2 Mesenchymal stem/stromal cells-derived extracellular vesicles in experimental models of liver disease** | | | | |
| **Reference** | **EVs Isolation/characteristics** | **Experimental model** | **Protocol** | **Biological effects** |
| Haga *et al*[98]2016 | Ultracentrifugation  Size: 46-116 nm  Alix+ CD9+, CD81+ | C57Bl mice. ALF, i.p. 20mg/body D‑GalNAc + 0,3mg/body TNF-α | 2 × 108 to 2 × 1010 i.p./i.v. | ↑ Survival, ↑ F4/80, ↑ inhibitor MMP-1 and IL-6, ↓ inflammation and apoptosis, ↓ ALT/AST, ↓ ALP, ↓ EGF, SCF, IFN-γ, IP-10, IL-1α, MIP‑3, MCP-1/3 |
| Yan *et al*[100]2016 | Ultracentrifugation  Size: 30-100 nm  CD9+, CD61+, CD63+ | BALB/c-nu/nu mice, *i.p.*  CCL4‑induced ALF, 0.15-0.35 mL/kg | 8, 16, and 32 mg/kg i.v./oral | ↓ Oxidative stress and apoptosis Induces ERK1/2 phosphorylation and Bcl2 expression  Inhibits IKKB/NFkB/casp9/3 pathway |
| Tan *et al*[99]2014 | TFF, 100.kDa MWCO filter Size: 55-100 nm | C57BL/6 mice. CCL4-induced ALF, i.p*.* 0.05 mL CCL4/kg | 0.4 μg (100 μl) i.s. | ↑ Cell viability: TAMH, THLE-2, and Huh-7  ↑ Hepatocytes proliferation  ↓ ALT/AST ↓ Casp 3/7  ↑ antiapoptoticBcl-xL |
| Chen *et al*[97]2017 | Centrifugation and Exoquick-TC  Size: 30-100 nm  CD63+ and tsg101+ | C57BL/6 mice. ALF, i.p. D-GalNAc 800 mg/kg and LPS 50 μg/kg | 1 μg/μL i.v. | ↑ Liver function and survival ↓ Apoptosis  ↓ TNF-, IL-6 and IL-1  ↓ Casp-3  ↓ Necrosis and inflammation |
| Nong *et al*[101]2016 | Ultracentrifugation and ultrafiltration  Size: 50-60 nm  CD9+, CD63+ and CD81+ | Rats I/R injury | 600 μg suspended in 400 μL of PBS i.v. | ↑ GSH, GSH-PX and SOD  ↓ AST/ALT  ↓ TNF-, IL-6 and HMGB1  ↓ Casp-3 and Bax |
| Du *et al*[93]2017 | Centrifugation and filtered by 0, 45-μm PVDF filter ExoQuick  Size: 100-200 nm  Alix+, CD63+ and CD81+ | C57 mice I/R injury | 2.5 × 1012 particles in 500 μL of PBS i.v. | ↑ Hepatocytes proliferation  ↑ SK activity and S1P formation. Hepatoprotective and proliferative effect abolished by the inhibition of SK or S1P receptor 1 |

i.p.: Intraperitoneal injection; i.v.: Intravenous injection; i.s.: Intrasplenic; MMP: Metalloproteinases; CCL4: Corbon tetrachloride; D-GalNAc: N‑Acetylgalactosamine; IL: Interleukins; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; EGF: Epidermal growth factor; SCF: Stem cell factor; TNF: Tumor necrosis factor; IFN: Interferon; IP: Inducible protein; MIP: Macrophage inflammatory protein; MCP: Monocyte chemotactic protein; ERK: Extracellular signal-regulated kinase; Bcl: B-cell lymphoma; TFF: Tangential flow filtration; MWCO: Molecular weight cut-off; ALF: Acute liver failure;TAMH: Transgenic mouse hepatocyte; THLE: T-antigen immortalized human liver epithelial; Casp: Caspase; GSH: Glutathione; GSH-PX: Glutathione peroxidase; SOD: Superoxide dismutase; HMGB: High mobility group box; PBS: Phosphate-buffered saline; SK: Sphingosine kinase; S1P: Sphingosine 1-phosphate.