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**Multipotent mesenchymal stromal cell: A promising strategy to manage alcoholic liver disease**

Ezquer F *et al*. MSC for treatment of alcoholic liver disease

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**Abstract**

Chronic alcohol consumption is a major cause of liver disease. The term alcoholic liver disease (ALD) refers to a spectrum of mild to severe disorders including steatosis, steatohepatitis, cirrhosis, and hepatocellular carcinoma. With limited therapeutic options, stem cell therapy offers significant potential for these patients.

In this article, we review the pathophysiological features of ALD and the therapeutic mechanisms of multipotent mesenchymal stromal cells, also referred to as mesenchymal stem cells (MSCs), based on their potential to differentiate into hepatocytes, their immunomodulatory properties, their potential to promote residual hepatocyte regeneration and their capacity to inhibit hepatic stellate cells.

The perfect match between ALD pathogenesis and MSC therapeutic mechanisms, together with encouraging pre-clinical data available, allow us to support the notion that MSC transplantation is a promising therapeutic strategy to manage ALD onset and progression.

**Key words**: Alcoholic steatohepatitis; Alcoholic liver disease; Mesenchymal stem cells; Hepatic function recovery; Cellular therapy

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**Core tip:** Chronic alcohol consumption is a major cause of liver disease. Stem cells, in particular multipotent mesenchymal stromal cells (MSCs), have been envisioned as a promising tool for the development of therapeutic strategies to treat alcoholic liver diseases (ALD). The advantages of MSC include the regulation of exacerbated inflammatory process, their differentiation into hepatocytes, the production of trophic factors that prevent the apoptosis of parenchymal cells, and the induction of the proliferation of endogenous progenitors. Here, we revise the pathophysiology of ALD to identify therapeutic targets for MSCs. Also, we discuss the rationale to propose a MSC-based therapy to treat ALD.

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**ALCOHOLIC LIVER DISEASE**

Chronic alcohol consumption is a major cause of liver disease[1-3]. Moreover, alcohol consumption negatively impacts the natural history of other types of chronic liver diseases such as nonalcoholic steatohepatitis, and hepatitis B and C, favoring fibrosis progression[3-5]. Alcoholic liver disease (ALD) has a broad spectrum of disorders, encompassing simple steatosis, steatohepatitis, and cirrhosis. The patho-mechanism associated to ALD involves complex interactions between the deleterious effects of alcohol and its toxic metabolites on various cell types in the liver, the induction of reactive oxygen species (ROS), and the up-regulation of the pro-inflammatory cascade[1,3].

Alcoholic steatosis, the earliest manifestation of ALD is present in more than 90% of heavy drinkers, and is pathologically characterized by microvesicular and macrovesicular fat accumulation within hepatocytes, minimal inflammatory reaction, and no hepatic fibrosis[1]. This stage is asymptomatic and reversible with alcohol abstinence[6]. Alcohol consumption increases the ratio of NADH/NAD+ in hepatocytes, which disrupts mitochondrial β-oxidation of fatty acids, leading to steatosis development[7]. Alcohol consumption also increases fatty acid triglycerides synthesis through the up-regulation of the sterol regulatory element binding protein ic (SREBP-1c)[8] and the down-regulation of peroxisome proliferator-activated receptor (PPAR)-α[9].

ALD progression is characterized by steatosis, a superimposed inflammatory infiltrate of predominantly polymorphonuclear leukocytes and hepatocellular damage. When the inflammation and hepatocellular injury are severe, the condition is termed steatohepatitis and is associated with high mortality rate[10,11].

The pathogenesis of alcoholic steatohepatitis is complex and multifactorial. In the liver, alcohol is metabolized primarily into acetaldehyde by the enzymes alcohol dehydrogenase in the cytosol, cytochrome P450 in microsomes, and catalase in peroxisomes[12]. Acetaldehyde is highly toxic to hepatocytes because it binds to proteins and DNA, forming adducts that promote glutathione depletion, lipid peroxidation, and mitochondrial damage[13,14]. Additionally, these adducts act as antigens that activate the adaptive immune response, leading to lymphocyte recruitment to the liver[15].

Acetate resulting from acetaldehyde breakdown is rapidly released from the liver into circulation and is then metabolized into CO2 *via* the tricarboxylic acid cycle in skeletal muscle, brain and heart. Although acetate has no direct hepatotoxicity, it is believed that it can regulate the inflammatory response in patients with alcoholic steatohepatitis through the up-regulation of pro-inflammatory cytokines released by macrophages[16].

Alcohol abuse also results in changes in colonic microbiota and increased gut permeability, leading to translocation of bacterial products such as lipopolysaccharide (LPS) into the portal circulation[17]. In Kupffer cells, LPS activates the MyD88-independent signaling pathway through TLR4, resulting in the production of oxidative stress and pro-inflammatory cytokines such as TNF-α contributing to hepatocellular damage[18,19].

Histological features of alcoholic steatohepatitis include inflammation and necrosis, which are more prominent in the centrilobular region of the hepatic acinus, while hepatocytes are classically ballooned, leading to compression of the sinusoid and portal hypertension[20,21].

Alcoholic cirrhosis is the end stage of ALD which is characterized by the distortion of the hepatic architecture, septum formations, rings of scars that surround the nodules of hepatocytes, the formation of regenerative nodules and the loss of liver function[22].

Extracellular matrix (ECM), particularly collagen type I, is mainly produced by activated hepatic stellate cells (HSCs), located in the space of Disse between the hepatocytes and sinusoids. HSCs can be activated by neutrophils, damaged hepatocytes, and activated Kupffer cells through various pro-fibrogenic mediators including TGF-β, TNF-α, and ROS[3,23]. Additionally, ROS down-regulate the action of metalloproteinases and up-regulate tissue inhibitor of metalloproteinase 1, resulting in greater collagen accumulation[24].

Along with other liver diseases, patients with cirrhosis are at risk for hepatic decompensation (ascites, variceal bleeding, and encephalopathy) and the development of hepatocellular carcinoma[25,26].

Although the most important risk factor for ALD is the absolute amount of alcohol intake, only about 35% of heavy drinkers develop advanced ALD, indicating that other factors are involved in host susceptibility to the disease. These factors include sex, obesity, drinking pattern, dietary factors, non-sex-linked genetic factors, and cigarette smoking[27-30].

**ALD CURRENT TREATMENT**

Despite the profound economic and health impact of ALD, little progress has been made in the management of patients with this condition, and medical treatment has not changed significantly in the last 45 years[10,31,32].

Although nutritional and supportive management are important, alcohol abstinence is the mainstay therapy for patients with all stages of ALD[33,34]. However, the benefits of alcohol abstinence may not be sufficient for patients with decompensated ALD like cirrhosis or severe alcoholic hepatitis[35,36].

Corticosteroids were one of the first pharmacological therapies investigated for the treatment of alcoholic hepatitis, despite the widespread awareness and use of this therapy, controversy still exists regarding its true efficacy[37].

Taking into account the participation of TNF-α in ALD pathogenesis, TNF-α antagonists have been studied for this condition, and even though the first studies were promising, larger clinical trials demonstrated an increased risk of infection and mortality with these agents[38]. In addition, pharmacologic therapy with medications such as disulfiram, bacoflen, colchicine, vitamin E and naltrexone have been considered, although their efficacy is limited[3,39,40].

The most effective therapy for advanced cirrhosis is liver transplant, however, the scarcity of donors, surgical complications, immunological suppression and rejection, and high medical cost, limit its availability and clinical utility[41].

No treatment has demonstrated superiority over steroids until now, and liver transplantation is not an option for most of these patients. Therefore, alternative therapies are needed. In this sense, in recent years alternative approaches that circumvent the use of the whole organ, such as transplantation of cells of diverse origins, have been proposed [42].

**CELLULAR THERAPY FOR LIVER REGENERATION**

It is well known that the liver has a high regenerative capacity. Under normal conditions, recovery of liver mass occurs mainly via proliferation of remaining adult hepatocytes. On the other hand, under pathological conditions in which the proliferation of hepatocytes is inhibited, liver progenitor cells (oval cells) proliferate and differentiate into hepatocytes or biliary epithelial cells[43]. It proposed that chronic ethanol exposure and sustained inflammation have been shown to inhibit DNA synthesis in the damaged liver[44,45]. This impaired hepatocyte proliferation is the consequence of oxidative damage by the ROS produced in alcohol metabolism[46]. Moreover, ethanol could inhibit early hepatic differentiation of hepatic progenitor cells into functional mature hepatocytes[47].

Cell therapy for the treatment of hepatic fibrosis has been evaluated in different animal models and some findings have been very encouraging. It was shown that the transplantation of mature hepatocytes into human patients has provided insights of the way in which human liver disease could be treated by cellular therapies[48]. However, the high number of cells needed for the transplantation, the availability of fresh cells or the quality of cryopreserved ones and the necessity of immunosuppression to avoid the rejection of transplanted cells, are the main limitations on adult hepatocyte transplantation[49,50]. Immunosuppression is a particularly important point, since the hepatic failure itself increases the risk of developing septic complications, which are worsened by the use of immunosuppressive drugs.

Therefore, numerous studies have focused on investigating the ability of a variety of stem cells that can be readily isolated using non-invasive procedures to give rise to hepatocytes both *in vitro* and *in vivo*[51]. Considering that some of these stem cell populations are present in adults, it would be possible to produce personalized immunologically matched hepatocytes[52]. Moreover, several adult stem cells have the ability to reduce the hepatic pro-inflammatory microenvironment, inhibit the activation of HSCs or induce apoptosis of these cells and promote the regeneration of residual hepatocytes[53,54].

**MSCS AS A TOOL FOR THE INDUCTION OF TISSUE REGENERATION**

Regenerative medicine pursues the development of therapeutic strategies aimed to manage severe injuries or chronic diseases presented by patients whose endogenous regenerative mechanisms fail to restore the impaired functions. Over the past years, stem cells have been envisioned as the best tool for this. According to this, stem cell-based intervention is known to act through multiple mechanisms, which makes a clear advantage when facing diseases with complex pathophysiology as is the case of ALD.

In general terms, adult stem cells are found in all-non-embryonic tissues; where they contribute to both, maintenance of cellular homeostasis and regeneration of damaged organs. These cells are multipotent and can be isolated from fetus, newborn, child and adult individuals, and due to their limited self-renewal potential, they are not teratogenic. Some of them also have plasticity, *i.e.,* they can differentiate into cells from lineages different from their origin[55].

The fact that adult stem cells pose less bioethical and technical concerns than embryonic stem cells, the first candidate for a stem cell-based strategy to treat liver regeneration was bone marrow-derived stem cells[53,56-58]. Bone marrow harbors at least two distinct adult stem cell populations; the hematopoietic stem cells that give rise to blood and endothelial cells[59] and the multipotent mesenchymal stromal cells, also referred to as mesenchymal stem cells (MSCs), that provides support to the hematopoietic stem cell and drives the process of hematopoiesis[60]. In addition to bone marrow, MSCs have now been isolated from numerous tissues, including liver, lung, umbilical cord, skeletal muscle, dental pulp, spleen and adipose tissue[61-63]. Thus, it has been postulated that MSCs play a critical role in organ homeostasis by providing supportive factors to the surrounding tissue.

One of the main technical difficulties associated to the therapeutic use of MSCs is the lack of a specific antigen for their identification. Therefore, in 2006 the International Society for Cellular Therapy proposed the minimal criteria to define human MSCs (hMSCs): (1) must be plastic-adherent when maintained under standard culture conditions; (2) must express CD105, CD73 and CD90, and lack the expression of CD45, CD34, CD14, CD11b, CD19 and HLA class II surface molecules; and (3) must differentiate into osteoblast, adipocytes and chondroblast under *in vitro* differentiating conditions[64,65].

Despite MSCs being scarce (< 0.01% of the mononuclear cells present in the bone marrow), they can be considered as ideal candidates for cell therapy because: (1) they can be obtained from donors without major complications; (2) they can be easily expanded *ex vivo*; (3) when MSCs are systemically administered they can selectively migrate and engraft into damaged tissue. The process involves the release of several molecules by the damaged tissues that can interact with different receptors expressed by the MSCs, facilitating the migration of the cells to the damaged tissue[66,67]; (4) it has been suggested that MSCs might cross the germ line barrier and generate cells from the endodermal and ectodermal lineages[55]; (5) MSCs secrete a broad range of bioactive growth factors (*i.e.,* VEGF, bFGF, IGF, HGF and EGF)[68]. Therefore, MSCs could provide trophic support for injured tissue modifying the microenvironment to induce local precursor proliferation and differentiation, improving damaged tissue irrigation and preventing parenchymal cell apoptosis[55,68]; and (6) MSCs are hypo-immunogenic[69], which represents the main advantage of MSCs over hematopoietic stem cells for clinical use, since histocompatibility between donor and receptor is not required and the recipient do not need to be conditioned before MSC transplantation[70]. Furthermore, MSCs have been administered to more than 1000 human patients with no evidence of adverse effects or tumor formation[70] (Table 1).

**MSC TRANSPLANTATION: A PROMISING STRATEGY TO TREAT PATIENTS WITH ALD**

Multiple mechanisms have been suggested to play a role in liver diseases amelioration after MSC administration, such as: trans-differentiation of MSCs into hepatocytes, immunomodulation, inhibition of fibrosis development, protective effects on hepatic cells and restoration of hepatic cell proliferation capacity (Figure 1).

***Differentiation of MSC into parenchymal cells***

The high degree of plasticity of MSCs has been widely described during the last years[55,71]. Therefore, MSCs might cross the germ line barrier and differentiate into non-mesodermal cells (such as hepatocytes and neurons)[72].

Is important to note, that MSC-derived hepatocytes will need to not only express the genes found in mature liver cells, but also the level of the gene expression needed to be closer to those found in the normal liver. Therefore, it is crucial to define which characteristics are needed for a differentiated cell to be comparable to a primary hepatocyte. The minimal set of functions of a true hepatocyte includes: (1) metabolic function (detoxification of xenobiotics and endogenous substances); (2) synthetic function (production of albumin, clotting factors, complement); and (3) storage function (storage of glycogen and fat-soluble vitamins)[73].

Although the protocols for hepatocyte induction have been standardized for cultured MSCs[74,75], an organ specific microenvironment is the most suitable place for them to differentiate into the required cell types. In this sense, Stato *el al*[76] were the first to demonstrate the *in vivo* hepatic differentiation potential of hMSCs. In this study, hMSC were directly xenografted to the liver of allylalcohol-treated rat, and they observed that some of the administered hMSC differentiated into hepatocyte-like cells one month later. Additionally, the *in vivo* hepatic differentiation potential of MSCs has been also demonstrated in rats[77], mice[78], sheep[79] and humans[51].

On the other hand*, in vitro* differentiated cells were found to express hepatocyte markers (AFP, albumin, CK18, CK19, CYP1A1, CYP3A4, G6P and HGRF)[80], to store glycogen[81], to clear ammonia and to produce urea[82], to secrete albumin and to uptake low density lipoprotein[83,84]. However, it is much more challenging to determine whether a cell is a true hepatocyte *in vivo*. Immunostaining for albumin, CK18 or hepatocyte nuclear factor are recognized indicators of hepatocyte trans-differentiation but not cellular functionality. It is important to note that differentiated MSCs still express mesenchymal markers such as CD90, α-SMA, vimentin, and fibronectin suggesting that complete trans-differentiation was not achieved[85].

The hepatic trans-differentiation potential is essential for MSCs-based therapies in the context of ALD, in which the injured hepatocyte cannot regenerate. However, this initial optimism has been tempered by the recognition of many groups that fusion of MSCs with endogenous hepatocytes is the main mechanism by which new hepatocytes are produced *in vivo*[86,87]. Hence, irrespectively if the mechanism is MSC trans-differentiation or fusion, these events do not occur at a sufficient high frequency to account for the observed functional improvement after MSC administration. Therefore, additional mechanisms may be involved in the regenerative process[88-90].

***Modulation of inflammation by MSCs***

Liver injury caused by persistent inflammation is accompanied with T cell, B cell and monocyte infiltration of the liver[91,92]. In this respect, MSC immunomodulatory and immunosuppressive properties could be potentially involved in the positive effects that MSC transplantation has in chronic and acute liver diseases.

MSCs regulate the activity of cells from both adaptive and innate immunity[93]. *In vitro*, they inhibit the differentiation of monocytic precursors into activated dendritic cells[94,95]. Thus, MSCs indirectly limit the cytotoxic expansion and activity of NK T lymphocytes[96]. Both *in vitro* and *in vivo*, MSCs down-regulate the expression of pro-inflammatory molecules (IL-1β, IL-12, TNF-α and INF-γ) and secrete anti-inflammatory factors (IL-4 and IL-10), shifting the immune response pattern toward a protective Th2 type, establishing a tolerogenic microenvironment where activated T cells are unable to proliferate and die by apoptosis[97].

Another candidate for the MSC suppressive effects is indoleamine 2,3-dioxygenase, which is expressed by MSCs upon INF-γ stimulation, leading to tryptophan depletion and thus inhibition of T cell proliferation[98]. This effect on T lymphocytes indirectly suppresses the function of B lymphocytes because their activation is mainly T cell dependent. Moreover, MSCs can also modulate B cell functions by inhibiting their proliferation, differentiation into antibody-secreting cells and chemotaxis[99].

MSCs also promote the appearance of regulatory T cells, inducing antigen-specific tolerance[100]. Interestingly, it has been shown that the immunological properties of undifferentiated MSCs are retained when they differentiate into parenchymal cells[101]. Therefore, both undifferentiated and differentiated MSCs will contribute to the maintenance of a microenvironment that allows tissue regeneration.

***Induction of endogenous regeneration by MSCs***

It is known that MSCs have the ability to secrete, *in vitro* and *in vivo,* a wide range of trophic factors, including VEGF, bFGF, HGF, PDGF, TGF-β, IGF-1 and EGF [68]. The biological effects of these factors can be both direct, by unleashing intracellular signalization pathways, as well as indirect, by inducing other cells from the microenvironment to secret other bioactive factors. Therefore, it has been proposed that MSCs have a catalytic role in tissue regeneration, since once in the damaged tissue they are able to modify the microenvironment by secreting factors that would: (1) prevent parenchymal cells from dying; (2) induce the proliferation and differentiation of endogenous progenitors; (3) promote neovascularization; and (4) avoid/revert fibrosis development[88,90].

Diverse studies have shown that less than 1% of systemically administered MSCs are still present in any organ including the lung, heart, kidneys, liver, spleen and gut one week after the administration[102-104]. However, clinically, the beneficial effects associated to MSC administration can be observed for much longer than just a week.

MSC-conditioned medium (MSC-CM) administration can recapitulate the beneficial effects of MSCs regarding tissue repair; for instance, data from Van Poll *et al*[105] has provided the first clear evidence that MSC-CM procures trophic support for injured liver by inhibiting hepatocellular death and by stimulating liver regeneration. Although no specific mechanisms of action have been identified, soluble factors including VEGF, HGF, IGF-1, EGF, IGF-BP and IL-6 have been implicated in those regenerative effects.

Microvesicles (MVs) have been recently considered important mediators of cell-to-cell communications, since they carry a complex load of proteins, lipids, mRNA and microRNA which might affect several cellular processes and pathways[106]. MVs account for around 10% of conditioned medium components in terms of protein amount; therefore MSC-CM therapeutic activity could thus be partially attributed to MVs[107,108].

In addition to the induction of liver regeneration, the MSC secretome has also been described to have anti-fibrotic properties. In this sense, Li *et al*[109] demonstrated that transplantation of MVs derived from human umbilical cord MSCs can alleviate liver fibrosis induced by carbon-tetrachloride (CCL4) administration. These results have also been recapitulated by the administration of *ex vivo* expanded MSCs[109-112]. However, other studies have reported that MSCs can be potentially fibrogenic and contribute to increased fibrosis[113-115] or have no effect whatsoever[116,117].

Even these experimental results propose two apparently contradictory scenarios; a great number of variables contribute to the inconsistences between the different observations. One of them, is the difference in the properties of MSCs prepared in different laboratories, due to differences in the protocols used for MSC isolation and *ex* *vivo* expansion. There are also important differences between human MSCs and rodent MSCs, and even between different mice strains[55]. Finally, another important factor is the dependence for the MSC differentiation process on most of the culture conditions or *in vivo* microenvironments, especially those developed in damage tissue. In most of the cases the signals that drive this differentiation process have not been characterized, so they cannot be replied *in vitro*.

**MSC TRANSPLANTATION IN ANIMAL MODEL OF LIVER INJURY**

Numerous studies have tried to demonstrate the therapeutic potential of MSCs in the treatment of acute and chronic liver diseases, however to date, there is a gap in the study of MSC administration for the treatment of ALD (Table 2). This gap is due, in part, to the lack of experimental animal models that recapitulate the full progression of ALD in human patients. Non-human primates are possibly the most similar model of human disease[118,119]. For example, exposure of baboons *to ad libitum* alcohol intake leads to the progression of all stages of liver damage associated with ALD in human. However, the relevance of non-human primates as a model of ALD is outweighed by the prodigious cost of maintaining them, which limits their utility to the field as a whole. Therefore, is not surprising that the majority of alcohol research performed in animal models involves rodents[118,119]. The major disadvantage of the rodent models with regard to experimental ALD is that the liver pathology obtained is limited predominantly to steatosis, with some necroinflammatory changes. More severe steatohepatitis and advanced liver damage observed in human patients (fibrosis and cirrhosis) is generally not observed in rodents[118,119].

Several *in vivo* studies have been performed to evaluate the therapeutic potential of MSCs in the context of liver fibrosis[54,56]. In most of the studies, liver fibrosis was induced by intraperitoneal or subcutaneous injection of CCL4, however, this model cannot provide a perfect simulation of a human etiology[120,121].

Application of MSCs in the *in vivo* models of liver fibrosis/cirrhosis ameliorates the development of the disease[54,56,111,112]. Similar results were obtained when MSC-CM or MVs have been applied instead[105,108,109,122] suggesting that MSCs long-term survival might not be necessary for their beneficial effects. In these studies, the reduction of fibrosis has been correlated with the decrease in the synthesis of collagen I and matrix metalloproteases inhibitors, with the concomitant decrease of activated HSCs. Multiple mechanisms have been suggested to participate, such as immunomodulation[123], selective apoptosis of HSCs[124,125] or the reversion of the activate state of these cells to a quiescent state and production of protective factors[126,127].

Studies of *in vitro* co-cultures of MSCs with activated stellate cells have shown that even in a small number, MSCs can paracrinally inhibit the fibrogenic activity of activated stellate cells. This inhibition can be the consequence of the secretion of IL-10 and TNF-α by MSCs. Moreover, MSCs are able to induce apoptosis in reactive stellate cells, process mediated in part by the secretion of HGF[125]. These results support the hypothesis that the therapeutic effects of MSCs on fibrosis inhibition is the result of the secretion of paracrine factors that modulate the proliferation, viability and function of resident stellate cells. The production of matrix metalloproteases (MMP) can also be effective at reverting hepatic fibrosis; MSCs are capable of secreting and inducing the expression of MMP-9 and MMP-13 in other cells, this last one being the main rodent and human interstitial collagenase[128,129].

In ALD, as well as in more prominent cirrhotic liver, hepatocytes are reported to have reduced proliferative capacity, which may reflect either the inhibitory effect of adjacent collagen I, or that they have reached replicative senescence after many rounds of injury and repair[44,45]. MSCs infusion may increase the intrinsic ability of hepatocytes to proliferate by the release of proliferative trophic factors and cytokines, or by facilitating the breakdown of the scar tissue, thereby removing a block to proliferation[130].

In our laboratory, we found that intravenous administration of bone marrow-derived MSCs into animals suffering from diet-induced metabolic syndrome and obesity, recovers liver function and avoids the progression of steatosis to non-alcoholic-steatohepatitis. Such MSCs-mediated hepatoprotection was unrelated to metabolic syndrome reversion, nevertheless, this has been associated with MSCs potential for enhancing liver regeneration and/or managing the second hit required for the transition of steatosis to non-alcoholic-steatohepatitis, since an increased hepatic proliferation rate was found as well as an increased expression of fatty-acid oxidation enzymes[110]. Thus, MSCs administration could prevent the evolution of ALD by reducing the impairment of fatty-acid oxidation.

Finally, the question of the ideal route of MSCs injection remains one of the main unsolved issues regarding efficient administration of MSCs. Even if the tail vein seems to be the most often used administration route in animals, the portal vein and intrahepatic injections also seems to be efficient[129,131]. The optimal dose of cells or conditioned medium, also needs to be evaluated because there are significant variations among studies in terms of the number of cells injected per animal.

**CLINICAL TRIALS USING MSCS**

MSCs have been successfully used in humans to treat different pathologies such as osteogenesis imperfecta[132], idiopathic aplastic anemia[133], graft-versus-host disease[134], and acute myelogenous leukemia[135]. Other applications to specifically, avoid lung fibrosis injury after bleomycin challenge[136], and in the protection of the cardiac function after a myocardial infarction[137]. In every case clear therapeutic effects with no complications have been reported.

In the same direction, the translation of preclinical research on MSCs to the clinical use for cirrhotic patients has generated great interest, due to the growing population of patients with advanced liver diseases and the critical shortage of available liver donors.

To date some clinical trials using hMSC to treat patients with liver fibrosis have been published[112,138-145]. Unfortunately, in general, the studies were heterogeneous in their design and have not distinguished between the various etiologies of cirrhosis. ALD patients and viral hepatitis patients have been mixed together in small case series.

Recently, Jan *et al*[140], evaluated the effect of autologous bone marrow-derived MSC transplantation on hepatic fibrosis in patients with alcoholic cirrhosis. After MSC administration, liver histological improvements were observed in 6 of 11 patients, and recovery of liver function in 10 patients associated with a decreased expression of TGF-β1, collagen type I and α-SMA, without significant complications or side effects during the study period [140]. These results support the use of these cells as a therapy for patients with alcoholic cirrhosis. However, further prospective controlled studies are needed before MSC administration could be accepted as new strategy for antifibrosis therapy.

**POTENTIAL LIMITATIONS TO CLINICAL TRANSLATION**

Knowledge regarding MSC biology and their application in liver fibrosis has significantly increased during the last years. Nevertheless, the clinical use of MSCs for liver regeneration, in particular ALD, is still in its beginnings, and fundamental questions remain to be addressed.

Although clinical trials have provided the hope that MSCs could be a valuable resource for cell-based therapies for liver fibrosis, these results must be interpreted with some caution given the limited number of patients enrolled in each trial and the lack of appropriate controls. For example, patients with acute alcoholic hepatitis normally receive a high dose of prednisone therapy. However, the effect of high-dose steroids on the transplantation of MSCs is not well studied. There is some evidence that MSCs are glucocorticoid sensitive and are induced to differentiate into adipocytes with steroid exposure[146].

Clinical trials have shown that MSCs based therapy is relatively safe and no serious detrimental effects have been reported in human to date. However, some concerns have arisen over the use of replicating cells which may scape the control as time elapses[147]. Some potential complications could also arise from the intravascular administration of MSCs leading to vascular occlusion. Preclinical studies have not excluded the differentiation of injected MSCs into ectopic structures[148], myocardial calcification[149], and enhanced accumulation of fibroblast and myofibroblast in the lungs[150] since all these events have been reported following MSC treatment.

**CONCLUSION**

Stem cell-based therapy represents a newly emerging therapeutic approach to treat ALD. MSCs become an attractive tool because they have proved to trigger the regeneration of damaged liver tissue, with no evidence of significant adverse effects both in preclinical and clinical studies.

Due to the relation between of pathological events that occur in ALD development and the cellular and molecular mechanisms associated to MSC therapeutic effects, we believe that MSC transplantation could be a promising therapeutic strategy to manage ALD progression.

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**REFERENCES**

1 **Gao B**, Bataller R. Alcoholic liver disease: pathogenesis and new therapeutic targets. *Gastroenterology* 2011; **141**: 1572-1585 [PMID: 21920463 DOI: S0016-5085(11)01228-5]

2 **Moon KM**, Kim G, Baik SK, Choi E, Kim MY, Kim HA, Cho MY, Shin SY, Kim JM, Park HJ, Kwon SO, Eom YW. Ultrasonographic scoring system score versus liver stiffness measurement in prediction of cirrhosis. *Clin Mol Hepatol* 2013; **19**: 389-398 [PMID: 24459644 DOI: 10.3350/cmh.2013.19.4.389]

3 **Orman ES**, Odena G, Bataller R. Alcoholic liver disease: pathogenesis, management, and novel targets for therapy. *J Gastroenterol Hepatol* 2013; **28** Suppl 1: 77-84 [PMID: 23855300 DOI: 10.1111/jgh.12030]

4 **Clouston AD**, Jonsson JR, Powell EE. Steatosis as a cofactor in other liver diseases: hepatitis C virus, alcohol, hemochromatosis, and others. *Clin Liver Dis* 2007; **11**: 173-89, x [PMID: 17544978 DOI: 10.1016/j.cld.2007.02.007]

5 **Gitto S**, Micco L, Conti F, Andreone P, Bernardi M. Alcohol and viral hepatitis: a mini-review. *Dig Liver Dis* 2009; **41**: 67-70 [PMID: 18602355 DOI: S1590-8658(08)00196-5]

6 **Pateria P**, de Boer B, MacQuillan G. Liver abnormalities in drug and substance abusers. *Best Pract Res Clin Gastroenterol* 2013; **27**: 577-596 [PMID: 24090944 DOI: 10.1016/j.dld.2008.05.009]

7 **Baraona E**, Lieber CS. Alcohol and lipids. *Recent Dev Alcohol* 1998; **14**: 97-134 [PMID: 9751944]

8 **You M**, Fischer M, Deeg MA, Crabb DW. Ethanol induces fatty acid synthesis pathways by activation of sterol regulatory element-binding protein (SREBP). *J Biol Chem* 2002; **277**: 29342-29347 [PMID: 12036955 DOI: 10.1074/jbc.M202411200]

9 **Wagner M**, Zollner G, Trauner M. Nuclear receptors in liver disease. *Hepatology* 2011; **53**: 1023-1034 [PMID: 21319202 DOI: 10.1002/hep.24148]

10 **Kim W**, Kim DJ. Severe alcoholic hepatitis-current concepts, diagnosis and treatment options. *World J Hepatol* 2014; **6**: 688-695 [PMID: 25349640 DOI: 10.4254/wjh.v6.i10.688]

11 **Spengler EK**, Dunkelberg J, Schey R. Alcoholic hepatitis: current management. *Dig Dis Sci* 2014; **59**: 2357-2366 [PMID: 24798996 DOI: 10.1007/s10620-014-3173-8]

12 **Rusyn I**, Bataller R. Alcohol and toxicity. *J Hepatol* 2013; **59**: 387-388 [PMID: 23391479 DOI: 10.1016/j.jhep.2013.01.035]

13 **Farfán Labonne BE**, Gutiérrez M, Gómez-Quiroz LE, Konigsberg Fainstein M, Bucio L, Souza V, Flores O, Ortíz V, Hernández E, Kershenobich D, Gutiérrez-Ruíz MC. Acetaldehyde-induced mitochondrial dysfunction sensitizes hepatocytes to oxidative damage. *Cell Biol Toxicol* 2009; **25**: 599-609 [PMID: 19137438 DOI: 10.1007/s10565-008-9115-5]

14 **Setshedi M**, Wands JR, Monte SM. Acetaldehyde adducts in alcoholic liver disease. *Oxid Med Cell Longev* 2010; **3**: 178-185 [PMID: 20716942 DOI: 10.4161/oxim.3.3.12288]

15 **Szabo G**. Gut-liver axis in alcoholic liver disease. *Gastroenterology* 2015; **148**: 30-36 [PMID: 25447847 DOI: 10.1053/j.gastro.2014.10.042]

16 **Kendrick SF**, O'Boyle G, Mann J, Zeybel M, Palmer J, Jones DE, Day CP. Acetate, the key modulator of inflammatory responses in acute alcoholic hepatitis. *Hepatology* 2010; **51**: 1988-1997 [PMID: 20232292 DOI: 10.1002/hep.23572]

17 **Rao R**. Endotoxemia and gut barrier dysfunction in alcoholic liver disease. *Hepatology* 2009; **50**: 638-644 [PMID: 19575462 DOI: 10.1002/hep.23009]

18 **Zhao XJ**, Dong Q, Bindas J, Piganelli JD, Magill A, Reiser J, Kolls JK. TRIF and IRF-3 binding to the TNF promoter results in macrophage TNF dysregulation and steatosis induced by chronic ethanol. *J Immunol* 2008; **181**: 3049-3056 [PMID: 18713975 DOI: 181/5/3049]

19 **Hritz I**, Mandrekar P, Velayudham A, Catalano D, Dolganiuc A, Kodys K, Kurt-Jones E, Szabo G. The critical role of toll-like receptor (TLR) 4 in alcoholic liver disease is independent of the common TLR adapter MyD88. *Hepatology* 2008; **48**: 1224-1231 [PMID: 18792393 DOI: 10.1002/hep.22470]

20 **Bataller R**, Rombouts K, Altamirano J, Marra F. Fibrosis in alcoholic and nonalcoholic steatohepatitis. *Best Pract Res Clin Gastroenterol* 2011; **25**: 231-244 [PMID: 21497741 DOI: S1521-6918(11)00036-9]

21 **Neuman MG**, French SW, French BA, Seitz HK, Cohen LB, Mueller S, Osna NA, Kharbanda KK, Seth D, Bautista A, Thompson KJ, McKillop IH, Kirpich IA, McClain CJ, Bataller R, Nanau RM, Voiculescu M, Opris M, Shen H, Tillman B, Li J, Liu H, Thomes PG, Ganesan M, Malnick S. Alcoholic and non-alcoholic steatohepatitis. *Exp Mol Pathol* 2014; **97**: 492-510 [PMID: 25217800 DOI: S0014-4800(14)00146-4]

22 **Bataller R**, Brenner DA. Liver fibrosis. *J Clin Invest* 2005; **115**: 209-218 [PMID: 15690074 DOI: 10.1172/JCI24282]

23 **Wang JH**, Batey RG, George J. Role of ethanol in the regulation of hepatic stellate cell function. *World J Gastroenterol* 2006; **12**: 6926-6932 [PMID: 17109512]

24 **Arthur MJ**, Iredale JP, Mann DA. Tissue inhibitors of metalloproteinases: role in liver fibrosis and alcoholic liver disease. *Alcohol Clin Exp Res* 1999; **23**: 940-943 [PMID: 10371419 DOI: 00000374-199905000-00027]

25 **Bolondi L**, Gramantieri L. From liver cirrhosis to HCC. *Intern Emerg Med* 2011; **6 Suppl 1**: 93-98 [PMID: 22009618 DOI: 10.1007/s11739-011-0682-8]

26 **Lee SS**, Shin HS, Kim HJ, Lee SJ, Lee HS, Hyun KH, Kim YH, Kwon BW, Han JH, Choi H, Kim BH, Lee JH, Kang HY, Shin HD, Song IH. Analysis of prognostic factors and 5-year survival rate in patients with hepatocellular carcinoma: a single-center experience. *Korean J Hepatol* 2012; **18**: 48-55 [PMID: 22511903 DOI: 10.3350/kjhep.2012.18.1.48]

27 **Altamirano J**, Bataller R. Cigarette smoking and chronic liver diseases. *Gut* 2010; **59**: 1159-1162 [PMID: 20650922 DOI: 10.1136/gut.2008.162453]

28 **Anstee QM**, Daly AK, Day CP. Genetics of alcoholic and nonalcoholic fatty liver disease. *Semin Liver Dis* 2011; **31**: 128-146 [PMID: 21538280 DOI: 10.1055/s-0031-1276643]

29 **Bellentani S**, Saccoccio G, Costa G, Tiribelli C, Manenti F, Sodde M, Saveria Crocè L, Sasso F, Pozzato G, Cristianini G, Brandi G. Drinking habits as cofactors of risk for alcohol induced liver damage. The Dionysos Study Group. *Gut* 1997; **41**: 845-850 [PMID: 9462221]

30 **Stroffolini T**, Cotticelli G, Medda E, Niosi M, Del Vecchio-Blanco C, Addolorato G, Petrelli E, Salerno MT, Picardi A, Bernardi M, Almasio P, Bellentani S, Surace LA, Loguercio C. Interaction of alcohol intake and cofactors on the risk of cirrhosis. *Liver Int* 2010; **30**: 867-870 [PMID: 20492499 DOI: 10.1111/j.1478-3231.2010.02261.x]

31 **Helman RA**, Temko MH, Nye SW, Fallon HJ. Alcoholic hepatitis. Natural history and evaluation of prednisolone therapy. *Ann Intern Med* 1971; **74**: 311-321 [PMID: 4928161]

32 **Jaurigue MM**, Cappell MS. Therapy for alcoholic liver disease. *World J Gastroenterol* 2014; **20**: 2143-2158 [PMID: 24605013 DOI: 10.3748/wjg.v20.i9.2143]

33 **Borowsky SA**, Strome S, Lott E. Continued heavy drinking and survival in alcoholic cirrhotics. *Gastroenterology* 1981; **80**: 1405-1409 [PMID: 6971772 DOI: S0016508581001303]

34 **Pessione F**, Ramond MJ, Peters L, Pham BN, Batel P, Rueff B, Valla DC. Five-year survival predictive factors in patients with excessive alcohol intake and cirrhosis. Effect of alcoholic hepatitis, smoking and abstinence. *Liver Int* 2003; **23**: 45-53 [PMID: 12640727]

35 **Menachery J**, Duseja A. Treatment of decompensated alcoholic liver disease. *Int J Hepatol* 2011; **2011**: 219238 [PMID: 21994849 DOI: 10.4061/2011/219238]

36 **Morgan MY**. The prognosis and outcome of alcoholic liver disease. *Alcohol Alcohol Suppl* 1994; **2**: 335-343 [PMID: 8974353]

37 **Singal AK**, Walia I, Singal A, Soloway RD. Corticosteroids and pentoxifylline for the treatment of alcoholic hepatitis: Current status. *World J Hepatol* 2011; **3**: 205-210 [PMID: 21954408 DOI: 10.4254/wjh.v3.i8.205]

38 **Boetticher NC**, Peine CJ, Kwo P, Abrams GA, Patel T, Aqel B, Boardman L, Gores GJ, Harmsen WS, McClain CJ, Kamath PS, Shah VH. A randomized, double-blinded, placebo-controlled multicenter trial of etanercept in the treatment of alcoholic hepatitis. *Gastroenterology* 2008; **135**: 1953-1960 [PMID: 18848937 DOI: 10.1053/j.gastro.2008.08.057]

39 **Mezey E**, Potter JJ, Rennie-Tankersley L, Caballeria J, Pares A. A randomized placebo controlled trial of vitamin E for alcoholic hepatitis. *J Hepatol* 2004; **40**: 40-46 [PMID: 14672612]

40 **O'Shea RS**, McCullough AJ. Treatment of alcoholic hepatitis. *Clin Liver Dis* 2005; **9**: 103-134 [PMID: 15763232 DOI: 10.1016/j.cld.2004.11.004]

41 **Singal AK**, Duchini A. Liver transplantation in acute alcoholic hepatitis: Current status and future development. *World J Hepatol* 2011; **3**: 215-218 [PMID: 21954410 DOI: 10.4254/wjh.v3.i8.215]

42 **Strom SC**, Bruzzone P, Cai H, Ellis E, Lehmann T, Mitamura K, Miki T. Hepatocyte transplantation: clinical experience and potential for future use. *Cell Transplant* 2006; **15** Suppl 1: S105-S110 [PMID: 16826802]

43 **Michalopoulos GK**. Liver regeneration. *J Cell Physiol* 2007; **213**: 286-300 [PMID: 17559071 DOI: 10.1002/jcp.21172]

44 **Duguay L**, Coutu D, Hetu C, Joly JG. Inhibition of liver regeneration by chronic alcohol administration. *Gut* 1982; **23**: 8-13 [PMID: 7056500]

45 **Wands JR**, Carter EA, Bucher NL, Isselbacher KJ. Effect of acute and chronic ethanol intoxication on hepatic regeneration. *Adv Exp Med Biol* 1980; **132**: 663-670 [PMID: 7191626]

46 **Dey A**, Cederbaum AI. Alcohol and oxidative liver injury. *Hepatology* 2006; **43**: S63-S74 [PMID: 16447273 DOI: 10.1002/hep.20957]

47 **Gao W**, Zhou P, Ma X, Tschudy-Seney B, Chen J, Magner NL, Revzin A, Nolta JA, Zern MA, Duan Y. Ethanol negatively regulates hepatic differentiation of hESC by inhibition of the MAPK/ERK signaling pathway in vitro. *PLoS One* 2014; **9**: e112698 [PMID: 25393427 DOI: 10.1371/journal.pone.0112698]

48 **Schneider A**, Attaran M, Meier PN, Strassburg C, Manns MP, Ott M, Barthold M, Arseniev L, Becker T, Panning B. Hepatocyte transplantation in an acute liver failure due to mushroom poisoning. *Transplantation* 2006; **82**: 1115-1116 [PMID: 17060866 DOI: 10.1097/01.tp.0000232451.93703.ab]

49 **Serralta A**, Donato MT, Orbis F, Castell JV, Mir J, Gómez-Lechón MJ. Functionality of cultured human hepatocytes from elective samples, cadaveric grafts and hepatectomies. *Toxicol In Vitro* 2003; **17**: 769-774 [PMID: 14599475]

50 **Serralta A**, Donato MT, Martinez A, Pareja E, Orbis F, Castell JV, Mir J, Gómez-Lechón MJ. Influence of preservation solution on the isolation and culture of human hepatocytes from liver grafts. *Cell Transplant* 2005; **14**: 837-843 [PMID: 16454358]

51 **Alison MR**, Poulsom R, Jeffery R, Dhillon AP, Quaglia A, Jacob J, Novelli M, Prentice G, Williamson J, Wright NA. Hepatocytes from non-hepatic adult stem cells. *Nature* 2000; **406**: 257 [PMID: 10917519 DOI: 10.1038/35018642]

52 **Hannan NR**, Segeritz CP, Touboul T, Vallier L. Production of hepatocyte-like cells from human pluripotent stem cells. *Nat Protoc* 2013; **8**: 430-437 [PMID: 23424751]

53 **Almeida-Porada G**, Zanjani ED, Porada CD. Bone marrow stem cells and liver regeneration. *Exp Hematol* 2010; **38**: 574-580 [PMID: 20417684 DOI: 10.1016/j.exphem.2010.04.007]

54 **Berardis S**, Dwisthi Sattwika P, Najimi M, Sokal EM. Use of mesenchymal stem cells to treat liver fibrosis: current situation and future prospects. *World J Gastroenterol* 2015; **21**: 742-758 [PMID: 25624709 DOI: 10.3748/wjg.v21.i3.742]

55 **Phinney DG**, Prockop DJ. Concise review: mesenchymal stem/multipotent stromal cells: the state of transdifferentiation and modes of tissue repair--current views. *Stem Cells* 2007; **25**: 2896-2902 [PMID: 17901396 DOI: 10.1634/stemcells.2007-0637]

56 **Fiore EJ**, Mazzolini G, Aquino JB. Mesenchymal Stem/Stromal Cells in Liver Fibrosis: Recent Findings, Old/New Caveats and Future Perspectives. *Stem Cell Rev* 2015; **11**: 586-597 [PMID: 25820543 DOI: 10.1007/s12015-015-9585-9]

57 **Dai LJ**, Li HY, Guan LX, Ritchie G, Zhou JX. The therapeutic potential of bone marrow-derived mesenchymal stem cells on hepatic cirrhosis. *Stem Cell Res* 2009; **2**: 16-25 [PMID: 19383405 DOI: 10.1016/j.scr.2008.07.005]

58 **Levine P**, McDaniel K, Francis H, Kennedy L, Alpini G, Meng F. Molecular mechanisms of stem cell therapy in alcoholic liver disease. *Dig Liver Dis* 2014; **46**: 391-397 [PMID: 24440312 DOI: 10.1016/j.dld.2013.11.015]

59 **He N**, Zhang L, Cui J, Li Z. Bone marrow vascular niche: home for hematopoietic stem cells. *Bone Marrow Res* 2014; **2014**: 128436 [PMID: 24822129 DOI: 10.1155/2014/128436]

60 **Friedenstein AJ**, Chailakhyan RK, Latsinik NV, Panasyuk AF, Keiliss-Borok IV. Stromal cells responsible for transferring the microenvironment of the hemopoietic tissues. Cloning in vitro and retransplantation in vivo. *Transplantation* 1974; **17**: 331-340 [PMID: 4150881]

61 **De Ugarte DA**, Morizono K, Elbarbary A, Alfonso Z, Zuk PA, Zhu M, Dragoo JL, Ashjian P, Thomas B, Benhaim P, Chen I, Fraser J, Hedrick MH. Comparison of multi-lineage cells from human adipose tissue and bone marrow. *Cells Tissues Organs* 2003; **174**: 101-109 [PMID: 12835573]

62 **in 't Anker PS**, Noort WA, Scherjon SA, Kleijburg-van der Keur C, Kruisselbrink AB, van Bezooijen RL, Beekhuizen W, Willemze R, Kanhai HH, Fibbe WE. Mesenchymal stem cells in human second-trimester bone marrow, liver, lung, and spleen exhibit a similar immunophenotype but a heterogeneous multilineage differentiation potential. *Haematologica* 2003; **88**: 845-852 [PMID: 12935972]

63 **Lee OK**, Kuo TK, Chen WM, Lee KD, Hsieh SL, Chen TH. Isolation of multipotent mesenchymal stem cells from umbilical cord blood. *Blood* 2004; **103**: 1669-1675 [PMID: 14576065 DOI: 10.1182/blood-2003-05-1670]

64 **Dominici M**, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop Dj, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006; **8**: 315-317 [PMID: 16923606 DOI: 10.1080/14653240600855905]

65 **Minguell JJ**, Erices A, Conget P. Mesenchymal stem cells. *Exp Biol Med (Maywood)* 2001; **226**: 507-520 [PMID: 11395921]

66 **Chen J**, Li Y, Katakowski M, Chen X, Wang L, Lu D, Lu M, Gautam SC, Chopp M. Intravenous bone marrow stromal cell therapy reduces apoptosis and promotes endogenous cell proliferation after stroke in female rat. *J Neurosci Res* 2003; **73**: 778-786 [PMID: 12949903 DOI: 10.1002/jnr.10691]

67 **Rüster B**, Göttig S, Ludwig RJ, Bistrian R, Müller S, Seifried E, Gille J, Henschler R. Mesenchymal stem cells display coordinated rolling and adhesion behavior on endothelial cells. *Blood* 2006; **108**: 3938-3944 [PMID: 16896152 DOI: 10.1182/blood-2006-05-025098]

68 **Caplan AI**, Dennis JE. Mesenchymal stem cells as trophic mediators. *J Cell Biochem* 2006; **98**: 1076-1084 [PMID: 16619257 DOI: 10.1002/jcb.20886]

69 **Chen PM**, Yen ML, Liu KJ, Sytwu HK, Yen BL. Immunomodulatory properties of human adult and fetal multipotent mesenchymal stem cells. *J Biomed Sci* 2011; **18**: 49 [PMID: 21762539 DOI: 10.1186/1423-0127-18-49]

70 **Uccelli A**, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. *Nat Rev Immunol* 2008; **8**: 726-736 [PMID: 19172693 DOI: 10.1038/nri2395]

71 **Pittenger MF**, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999; **284**: 143-147 [PMID: 10102814]

72 **Jiang Y**, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, Reyes M, Lenvik T, Lund T, Blackstad M, Du J, Aldrich S, Lisberg A, Low WC, Largaespada DA, Verfaillie CM. Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature* 2002; **418**: 41-49 [PMID: 12077603 DOI: 10.1038/nature00870]

73 **Hengstler JG**, Brulport M, Schormann W, Bauer A, Hermes M, Nussler AK, Fandrich F, Ruhnke M, Ungefroren H, Griffin L, Bockamp E, Oesch F, von Mach MA. Generation of human hepatocytes by stem cell technology: definition of the hepatocyte. *Expert Opin Drug Metab Toxicol* 2005; **1**: 61-74 [PMID: 16922653 DOI: 10.1517/17425255.1.1.61]

74 **Taléns-Visconti R**, Bonora A, Jover R, Mirabet V, Carbonell F, Castell JV, Gómez-Lechón MJ. Human mesenchymal stem cells from adipose tissue: Differentiation into hepatic lineage. *Toxicol In Vitro* 2007; **21**: 324-329 [PMID: 17045453 DOI: 10.1016/j.tiv.2006.08.009]

75 **Schwartz RE**, Reyes M, Koodie L, Jiang Y, Blackstad M, Lund T, Lenvik T, Johnson S, Hu WS, Verfaillie CM. Multipotent adult progenitor cells from bone marrow differentiate into functional hepatocyte-like cells. *J Clin Invest* 2002; **109**: 1291-1302 [PMID: 12021244 DOI: 10.1172/JCI15182]

76 **Sato Y**, Araki H, Kato J, Nakamura K, Kawano Y, Kobune M, Sato T, Miyanishi K, Takayama T, Takahashi M, Takimoto R, Iyama S, Matsunaga T, Ohtani S, Matsuura A, Hamada H, Niitsu Y. Human mesenchymal stem cells xenografted directly to rat liver are differentiated into human hepatocytes without fusion. *Blood* 2005; **106**: 756-763 [PMID: 15817682 DOI: 10.1182/blood-2005-02-0572]

77 **Shu SN**, Wei L, Wang JH, Zhan YT, Chen HS, Wang Y. Hepatic differentiation capability of rat bone marrow-derived mesenchymal stem cells and hematopoietic stem cells. *World J Gastroenterol* 2004; **10**: 2818-2822 [PMID: 15334677]

78 **Theise ND**, Badve S, Saxena R, Henegariu O, Sell S, Crawford JM, Krause DS. Derivation of hepatocytes from bone marrow cells in mice after radiation-induced myeloablation. *Hepatology* 2000; **31**: 235-240 [PMID: 10613752 DOI: 10.1002/hep.510310135]

79 **Chamberlain J**, Yamagami T, Colletti E, Theise ND, Desai J, Frias A, Pixley J, Zanjani ED, Porada CD, Almeida-Porada G. Efficient generation of human hepatocytes by the intrahepatic delivery of clonal human mesenchymal stem cells in fetal sheep. *Hepatology* 2007; **46**: 1935-1945 [PMID: 17705296 DOI: 10.1002/hep.21899]

80 **Liang XJ**, Chen XJ, Yang DH, Huang SM, Sun GD, Chen YP. Differentiation of human umbilical cord mesenchymal stem cells into hepatocyte-like cells by hTERT gene transfection in vitro. *Cell Biol Int* 2012; **36**: 215-221 [PMID: 21988655 DOI: 10.1042/CBI20110350]

81 **Bornstein R**, Macias MI, de la Torre P, Grande J, Flores AI. Human decidua-derived mesenchymal stromal cells differentiate into hepatic-like cells and form functional three-dimensional structures. *Cytotherapy* 2012; **14**: 1182-1192 [PMID: 22900961 DOI: 10.3109/14653249.2012.706706]

82 **Ayatollahi M**, Soleimani M, Tabei SZ, Kabir Salmani M. Hepatogenic differentiation of mesenchymal stem cells induced by insulin like growth factor-I. *World J Stem Cells* 2011; **3**: 113-121 [PMID: 22224170 DOI: 10.4252/wjsc.v3.i12.113]

83 **Piryaei A**, Valojerdi MR, Shahsavani M, Baharvand H. Differentiation of bone marrow-derived mesenchymal stem cells into hepatocyte-like cells on nanofibers and their transplantation into a carbon tetrachloride-induced liver fibrosis model. *Stem Cell Rev* 2011; **7**: 103-118 [PMID: 20182823 DOI: 10.1007/s12015-010-9126-5]

84 **Prasajak P**, Leeanansaksiri W. Developing a New Two-Step Protocol to Generate Functional Hepatocytes from Wharton's Jelly-Derived Mesenchymal Stem Cells under Hypoxic Condition. *Stem Cells Int* 2013; **2013**: 762196 [PMID: 23818908 DOI: 10.1155/2013/762196]

85 **Campard D**, Lysy PA, Najimi M, Sokal EM. Native umbilical cord matrix stem cells express hepatic markers and differentiate into hepatocyte-like cells. *Gastroenterology* 2008; **134**: 833-848 [PMID: 18243183 DOI: 10.1053/j.gastro.2007.12.024]

86 **Berisio R**, Schluenzen F, Harms J, Bashan A, Auerbach T, Baram D, Yonath A. Structural insight into the role of the ribosomal tunnel in cellular regulation. *Nat Struct Biol* 2003; **10**: 366-370 [PMID: 12665853 DOI: 10.1038/nature01539]

87 **Wang X**, Willenbring H, Akkari Y, Torimaru Y, Foster M, Al-Dhalimy M, Lagasse E, Finegold M, Olson S, Grompe M. Cell fusion is the principal source of bone-marrow-derived hepatocytes. *Nature* 2003; **422**: 897-901 [PMID: 12665832 DOI: 10.1038/nature01531]

88 **Prockop DJ**, Kota DJ, Bazhanov N, Reger RL. Evolving paradigms for repair of tissues by adult stem/progenitor cells (MSCs). *J Cell Mol Med* 2010; **14**: 2190-2199 [PMID: 20716123 DOI: 10.1111/j.1582-4934.2010.01151.x]

89 **Caplan AI**, Correa D. The MSC: an injury drugstore. *Cell Stem Cell* 2011; **9**: 11-15 [PMID: 21726829 DOI: 10.1016/j.stem.2011.06.008]

90 **Lee T**. Stem cell therapy independent of stemness. *World J Stem Cells* 2012; **4**: 120-124 [PMID: 23516128 DOI: 10.4252/wjsc.v4.i12.120]

91 **Szabo G**, Petrasek J, Bala S. Innate immunity and alcoholic liver disease. *Dig Dis* 2012; **30** Suppl 1: 55-60 [PMID: 23075869 DOI: 10.1159/000341126]

92 **Bala S**, Petrasek J, Mundkur S, Catalano D, Levin I, Ward J, Alao H, Kodys K, Szabo G. Circulating microRNAs in exosomes indicate hepatocyte injury and inflammation in alcoholic, drug-induced, and inflammatory liver diseases. *Hepatology* 2012; **56**: 1946-1957 [PMID: 22684891 DOI: 10.1002/hep.25873]

93 **Prockop DJ**, Oh JY. Mesenchymal stem/stromal cells (MSCs): role as guardians of inflammation. *Mol Ther* 2012; **20**: 14-20 [PMID: 22008910 DOI: 10.1038/mt.2011.211]

94 **Liu YL**, Jiang XX, Su YF, Huo SW, Zhu H, Wu Y, Mao N, Zhang Y. Endothelial cells from human umbilical vein inhibit generation of monocyte-derived dendritic cells. *Zhongguo Shi Yan Xue Ye Xue Za Zhi* 2011; **19**: 480-484 [PMID: 21518513]

95 **Zhang W**, Ge W, Li C, You S, Liao L, Han Q, Deng W, Zhao RC. Effects of mesenchymal stem cells on differentiation, maturation, and function of human monocyte-derived dendritic cells. *Stem Cells Dev* 2004; **13**: 263-271 [PMID: 15186722 DOI: 10.1089/154732804323099190]

96 **Spaggiari GM**, Capobianco A, Abdelrazik H, Becchetti F, Mingari MC, Moretta L. Mesenchymal stem cells inhibit natural killer-cell proliferation, cytotoxicity, and cytokine production: role of indoleamine 2,3-dioxygenase and prostaglandin E2. *Blood* 2008; **111**: 1327-1333 [PMID: 17951526 DOI: 10.1182/blood-2007-02-074997]

97 **Aggarwal S**, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood* 2005; **105**: 1815-1822 [PMID: 15494428 DOI: 10.1182/blood-2004-04-1559]

98 **Meisel R**, Zibert A, Laryea M, Göbel U, Däubener W, Dilloo D. Human bone marrow stromal cells inhibit allogeneic T-cell responses by indoleamine 2,3-dioxygenase-mediated tryptophan degradation. *Blood* 2004; **103**: 4619-4621 [PMID: 15001472 DOI: 10.1182/blood-2003-11-3909]

99 **Corcione A**, Benvenuto F, Ferretti E, Giunti D, Cappiello V, Cazzanti F, Risso M, Gualandi F, Mancardi GL, Pistoia V, Uccelli A. Human mesenchymal stem cells modulate B-cell functions. *Blood* 2006; **107**: 367-372 [PMID: 16141348 DOI: 10.1182/blood-2005-07-2657]

100 **Ezquer F**, Ezquer M, Contador D, Ricca M, Simon V, Conget P. The antidiabetic effect of mesenchymal stem cells is unrelated to their transdifferentiation potential but to their capability to restore Th1/Th2 balance and to modify the pancreatic microenvironment. *Stem Cells* 2012; **30**: 1664-1674 [PMID: 22644660 DOI: 10.1002/stem.1132]

101 **Le Blanc K**, Tammik C, Rosendahl K, Zetterberg E, Ringdén O. HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. *Exp Hematol* 2003; **31**: 890-896 [PMID: 14550804]

102 **Gao J**, Dennis JE, Muzic RF, Lundberg M, Caplan AI. The dynamic in vivo distribution of bone marrow-derived mesenchymal stem cells after infusion. *Cells Tissues Organs* 2001; **169**: 12-20 [PMID: 11340257]

103 **Lee RH**, Pulin AA, Seo MJ, Kota DJ, Ylostalo J, Larson BL, Semprun-Prieto L, Delafontaine P, Prockop DJ. Intravenous hMSCs improve myocardial infarction in mice because cells embolized in lung are activated to secrete the anti-inflammatory protein TSG-6. *Cell Stem Cell* 2009; **5**: 54-63 [PMID: 19570514 DOI: 10.1016/j.stem.2009.05.003]

104 **Schrepfer S**, Deuse T, Reichenspurner H, Fischbein MP, Robbins RC, Pelletier MP. Stem cell transplantation: the lung barrier. *Transplant Proc* 2007; **39**: 573-576 [PMID: 17362785 DOI: 10.1016/j.transproceed.2006.12.019]

105 **van Poll D**, Parekkadan B, Cho CH, Berthiaume F, Nahmias Y, Tilles AW, Yarmush ML. Mesenchymal stem cell-derived molecules directly modulate hepatocellular death and regeneration in vitro and in vivo. *Hepatology* 2008; **47**: 1634-1643 [PMID: 18395843 DOI: 10.1002/hep.22236]

106 **Tetta C**, Ghigo E, Silengo L, Deregibus MC, Camussi G. Extracellular vesicles as an emerging mechanism of cell-to-cell communication. *Endocrine* 2013; **44**: 11-19 [PMID: 23203002 DOI: 10.1007/s12020-012-9839-0]

107 **Camussi G**, Deregibus MC, Cantaluppi V. Role of stem-cell-derived microvesicles in the paracrine action of stem cells. *Biochem Soc Trans* 2013; **41**: 283-287 [PMID: 23356298 DOI: 10.1042/BST20120192]

108 **Herrera MB**, Fonsato V, Gatti S, Deregibus MC, Sordi A, Cantarella D, Calogero R, Bussolati B, Tetta C, Camussi G. Human liver stem cell-derived microvesicles accelerate hepatic regeneration in hepatectomized rats. *J Cell Mol Med* 2010; **14**: 1605-1618 [PMID: 19650833 DOI: 10.1111/j.1582-4934.2009.00860.x]

109 **Li T**, Yan Y, Wang B, Qian H, Zhang X, Shen L, Wang M, Zhou Y, Zhu W, Li W, Xu W. Exosomes derived from human umbilical cord mesenchymal stem cells alleviate liver fibrosis. *Stem Cells Dev* 2013; **22**: 845-854 [PMID: 23002959 DOI: 10.1089/scd.2012.0395]

110 **Ezquer M**, Ezquer F, Ricca M, Allers C, Conget P. Intravenous administration of multipotent stromal cells prevents the onset of non-alcoholic steatohepatitis in obese mice with metabolic syndrome. *J Hepatol* 2011; **55**: 1112-1120 [PMID: 21356258 DOI: 10.1016/j.jhep.2011.02.020]

111 **Seki A**, Sakai Y, Komura T, Nasti A, Yoshida K, Higashimoto M, Honda M, Usui S, Takamura M, Takamura T, Ochiya T, Furuichi K, Wada T, Kaneko S. Adipose tissue-derived stem cells as a regenerative therapy for a mouse steatohepatitis-induced cirrhosis model. *Hepatology* 2013; **58**: 1133-1142 [PMID: 23686813 DOI: 10.1002/hep.26470]

112 **Zhang Z**, Lin H, Shi M, Xu R, Fu J, Lv J, Chen L, Lv S, Li Y, Yu S, Geng H, Jin L, Lau GK, Wang FS. Human umbilical cord mesenchymal stem cells improve liver function and ascites in decompensated liver cirrhosis patients. *J Gastroenterol Hepatol* 2012; **27** Suppl 2: 112-120 [PMID: 22320928 DOI: 10.1111/j.1440-1746.2011.07024.x]

113 **Forbes SJ**, Russo FP, Rey V, Burra P, Rugge M, Wright NA, Alison MR. A significant proportion of myofibroblasts are of bone marrow origin in human liver fibrosis. *Gastroenterology* 2004; **126**: 955-963 [PMID: 15057733]

114 **di Bonzo LV**, Ferrero I, Cravanzola C, Mareschi K, Rustichell D, Novo E, Sanavio F, Cannito S, Zamara E, Bertero M, Davit A, Francica S, Novelli F, Colombatto S, Fagioli F, Parola M. Human mesenchymal stem cells as a two-edged sword in hepatic regenerative medicine: engraftment and hepatocyte differentiation versus profibrogenic potential. *Gut* 2008; **57**: 223-231 [PMID: 17639088 DOI: 10.1136/gut.2006.111617]

115 **Baertschiger RM**, Serre-Beinier V, Morel P, Bosco D, Peyrou M, Clément S, Sgroi A, Kaelin A, Buhler LH, Gonelle-Gispert C. Fibrogenic potential of human multipotent mesenchymal stromal cells in injured liver. *PLoS One* 2009; **4**: e6657 [PMID: 19684854 DOI: 10.1371/journal.pone.0006657]

116 **Carvalho AB**, Quintanilha LF, Dias JV, Paredes BD, Mannheimer EG, Carvalho FG, Asensi KD, Gutfilen B, Fonseca LM, Resende CM, Rezende GF, Takiya CM, de Carvalho AC, Goldenberg RC. Bone marrow multipotent mesenchymal stromal cells do not reduce fibrosis or improve function in a rat model of severe chronic liver injury. *Stem Cells* 2008; **26**: 1307-1314 [PMID: 18308943 DOI: 10.1634/stemcells.2007-0941]

117 **Kim S**, Kim HS, Lee E, Kim HO. In vivo hepatic differentiation potential of human cord blood-derived mesenchymal stem cells. *Int J Mol Med* 2011; **27**: 701-706 [PMID: 21347513 DOI: 10.3892/ijmm.2011.627]

118 **Arteel GE**. Animal models of alcoholic liver disease. *Dig Dis* 2010; **28**: 729-736 [PMID: 21525757 DOI: 10.1159/000324280]

119 **Mathews S**, Xu M, Wang H, Bertola A, Gao B. Animals models of gastrointestinal and liver diseases. Animal models of alcohol-induced liver disease: pathophysiology, translational relevance, and challenges. *Am J Physiol Gastrointest Liver Physiol* 2014; **306**: G819-G823 [PMID: 24699333 DOI: 10.1152/ajpgi.00041.2014]

120 **Starkel P**, Leclercq IA. Animal models for the study of hepatic fibrosis. *Best Pract Res Clin Gastroenterol* 2011; **25**: 319-333 [PMID: 21497748 DOI: 10.1016/j.bpg.2011.02.004]

121 **Tanimoto H**, Terai S, Taro T, Murata Y, Fujisawa K, Yamamoto N, Sakaida I. Improvement of liver fibrosis by infusion of cultured cells derived from human bone marrow. *Cell Tissue Res* 2013; **354**: 717-728 [PMID: 24104560 DOI: 10.1007/s00441-013-1727-2]

122 **Parekkadan B**, van Poll D, Suganuma K, Carter EA, Berthiaume F, Tilles AW, Yarmush ML. Mesenchymal stem cell-derived molecules reverse fulminant hepatic failure. *PLoS One* 2007; **2**: e941 [PMID: 17895982 DOI: 10.1371/journal.pone.0000941]

123 **Han Z**, Jing Y, Zhang S, Liu Y, Shi Y, Wei L. The role of immunosuppression of mesenchymal stem cells in tissue repair and tumor growth. *Cell Biosci* 2012; **2**: 8 [PMID: 22390479 DOI: 10.1186/2045-3701-2-8]

124 **Lin N**, Hu K, Chen S, Xie S, Tang Z, Lin J, Xu R. Nerve growth factor-mediated paracrine regulation of hepatic stellate cells by multipotent mesenchymal stromal cells. *Life Sci* 2009; **85**: 291-295 [PMID: 19559033 DOI: 10.1016/j.lfs.2009.06.007]

125 **Parekkadan B**, van Poll D, Megeed Z, Kobayashi N, Tilles AW, Berthiaume F, Yarmush ML. Immunomodulation of activated hepatic stellate cells by mesenchymal stem cells. *Biochem Biophys Res Commun* 2007; **363**: 247-252 [PMID: 17869217 DOI: 10.1016/j.bbrc.2007.05.150]

126 **Zhang D**, Jiang M, Miao D. Transplanted human amniotic membrane-derived mesenchymal stem cells ameliorate carbon tetrachloride-induced liver cirrhosis in mouse. *PLoS One* 2011; **6**: e16789 [PMID: 21326862 DOI: 10.1371/journal.pone.0016789]

127 **Fiore EJ**, Bayo JM, Garcia MG, Malvicini M, Lloyd R, Piccioni F, Rizzo M, Peixoto E, Sola MB, Atorrasagasti C, Alaniz L, Camilletti MA, Enguita M, Prieto J, Aquino JB, Mazzolini G. Mesenchymal stromal cells engineered to produce IGF-I by recombinant adenovirus ameliorate liver fibrosis in mice. *Stem Cells Dev* 2015; **24**: 791-801 [PMID: 25315017 DOI: 10.1089/scd.2014.0174]

128 **Higashiyama R**, Inagaki Y, Hong YY, Kushida M, Nakao S, Niioka M, Watanabe T, Okano H, Matsuzaki Y, Shiota G, Okazaki I. Bone marrow-derived cells express matrix metalloproteinases and contribute to regression of liver fibrosis in mice. *Hepatology* 2007; **45**: 213-222 [PMID: 17187438 DOI: 10.1002/hep.21477]

129 **Chang YJ**, Liu JW, Lin PC, Sun LY, Peng CW, Luo GH, Chen TM, Lee RP, Lin SZ, Harn HJ, Chiou TW. Mesenchymal stem cells facilitate recovery from chemically induced liver damage and decrease liver fibrosis. *Life Sci* 2009; **85**: 517-525 [PMID: 19686763 DOI: 10.1016/j.lfs.2009.08.003]

130 **Chagoya de Sánchez V**, Martínez-Pérez L, Hernández-Muñoz R, Velasco-Loyden G. Recovery of the Cell Cycle Inhibition in CCl(4)-Induced Cirrhosis by the Adenosine Derivative IFC-305. *Int J Hepatol* 2012; **2012**: 212530 [PMID: 23056951 DOI: 10.1155/2012/212530]

131 **Wang Y**, Lian F, Li J, Fan W, Xu H, Yang X, Liang L, Chen W, Yang J. Adipose derived mesenchymal stem cells transplantation via portal vein improves microcirculation and ameliorates liver fibrosis induced by CCl4 in rats. *J Transl Med* 2012; **10**: 133 [PMID: 22735033 DOI: 10.1186/1479-5876-10-133]

132 **Le Blanc K**, Götherström C, Ringdén O, Hassan M, McMahon R, Horwitz E, Anneren G, Axelsson O, Nunn J, Ewald U, Nordén-Lindeberg S, Jansson M, Dalton A, Aström E, Westgren M. Fetal mesenchymal stem-cell engraftment in bone after in utero transplantation in a patient with severe osteogenesis imperfecta. *Transplantation* 2005; **79**: 1607-1614 [PMID: 15940052]

133 **Fouillard L**, Francois S, Bouchet S, Bensidhoum M, Elm'selmi A, Chapel A. Innovative cell therapy in the treatment of serious adverse events related to both chemo-radiotherapy protocol and acute myeloid leukemia syndrome: the infusion of mesenchymal stem cells post-treatment reduces hematopoietic toxicity and promotes hematopoietic reconstitution. *Curr Pharm Biotechnol* 2013; **14**: 842-848 [PMID: 24372262]

134 **Le Blanc K**, Rasmusson I, Sundberg B, Götherström C, Hassan M, Uzunel M, Ringdén O. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. *Lancet* 2004; **363**: 1439-1441 [PMID: 15121408]

135 **Lee ST**, Jang JH, Cheong JW, Kim JS, Maemg HY, Hahn JS, Ko YW, Min YH. Treatment of high-risk acute myelogenous leukaemia by myeloablative chemoradiotherapy followed by co-infusion of T cell-depleted haematopoietic stem cells and culture-expanded marrow mesenchymal stem cells from a related donor with one fully mismatched human leucocyte antigen haplotype. *Br J Haematol* 2002; **118**: 1128-1131 [PMID: 12199796]

136 **Ortiz LA**, Gambelli F, McBride C, Gaupp D, Baddoo M, Kaminski N, Phinney DG. Mesenchymal stem cell engraftment in lung is enhanced in response to bleomycin exposure and ameliorates its fibrotic effects. *Proc Natl Acad Sci U S A* 2003; **100**: 8407-8411 [PMID: 12815096 DOI: 10.1073/pnas.1432929100]

137 **Gnecchi M**, Danieli P, Cervio E. Mesenchymal stem cell therapy for heart disease. *Vascul Pharmacol* 2012; **57**: 48-55 [PMID: 22521741 DOI: 10.1016/j.vph.2012.04.002]

138 **Amin MA**, Sabry D, Rashed LA, Aref WM, el-Ghobary MA, Farhan MS, Fouad HA, Youssef YA. Short-term evaluation of autologous transplantation of bone marrow-derived mesenchymal stem cells in patients with cirrhosis: Egyptian study. *Clin Transplant* 2013; **27**: 607-612 [PMID: 23923970 DOI: 10.1111/ctr.12179]

139 **El-Ansary M**, Abdel-Aziz I, Mogawer S, Abdel-Hamid S, Hammam O, Teaema S, Wahdan M. Phase II trial: undifferentiated versus differentiated autologous mesenchymal stem cells transplantation in Egyptian patients with HCV induced liver cirrhosis. *Stem Cell Rev* 2012; **8**: 972-981 [PMID: 21989829 DOI: 10.1007/s12015-011-9322-y]

140 **Jang YO**, Kim YJ, Baik SK, Kim MY, Eom YW, Cho MY, Park HJ, Park SY, Kim BR, Kim JW, Soo Kim H, Kwon SO, Choi EH, Kim YM. Histological improvement following administration of autologous bone marrow-derived mesenchymal stem cells for alcoholic cirrhosis: a pilot study. *Liver Int* 2014; **34**: 33-41 [PMID: 23782511 DOI: 10.1111/liv.12218]

141 **Kharaziha P**, Hellström PM, Noorinayer B, Farzaneh F, Aghajani K, Jafari F, Telkabadi M, Atashi A, Honardoost M, Zali MR, Soleimani M. Improvement of liver function in liver cirrhosis patients after autologous mesenchymal stem cell injection: a phase I-II clinical trial. *Eur J Gastroenterol Hepatol* 2009; **21**: 1199-1205 [PMID: 19455046 DOI: 10.1097/MEG.0b013e32832a1f6c]

142 **Mohamadnejad M**, Alimoghaddam K, Mohyeddin-Bonab M, Bagheri M, Bashtar M, Ghanaati H, Baharvand H, Ghavamzadeh A, Malekzadeh R. Phase 1 trial of autologous bone marrow mesenchymal stem cell transplantation in patients with decompensated liver cirrhosis. *Arch Iran Med* 2007; **10**: 459-466 [PMID: 17903050]

143 **Peng L**, Xie DY, Lin BL, Liu J, Zhu HP, Xie C, Zheng YB, Gao ZL. Autologous bone marrow mesenchymal stem cell transplantation in liver failure patients caused by hepatitis B: short-term and long-term outcomes. *Hepatology* 2011; **54**: 820-828 [PMID: 21608000 DOI: 10.1002/hep.24434]

144 **Shi M**, Zhang Z, Xu R, Lin H, Fu J, Zou Z, Zhang A, Shi J, Chen L, Lv S, He W, Geng H, Jin L, Liu Z, Wang FS. Human mesenchymal stem cell transfusion is safe and improves liver function in acute-on-chronic liver failure patients. *Stem Cells Transl Med* 2012; **1**: 725-731 [PMID: 23197664 DOI: 10.5966/sctm.2012-0034]

145 **Wang L**, Li J, Liu H, Li Y, Fu J, Sun Y, Xu R, Lin H, Wang S, Lv S, Chen L, Zou Z, Li B, Shi M, Zhang Z, Wang FS. Pilot study of umbilical cord-derived mesenchymal stem cell transfusion in patients with primary biliary cirrhosis. *J Gastroenterol Hepatol* 2013; **28** Suppl 1: 85-92 [PMID: 23855301 DOI: 10.1111/jgh.12029]

146 **Cui Q**, Wang GJ, Balian G. Steroid-induced adipogenesis in a pluripotential cell line from bone marrow. *J Bone Joint Surg Am* 1997; **79**: 1054-1063 [PMID: 9234882]

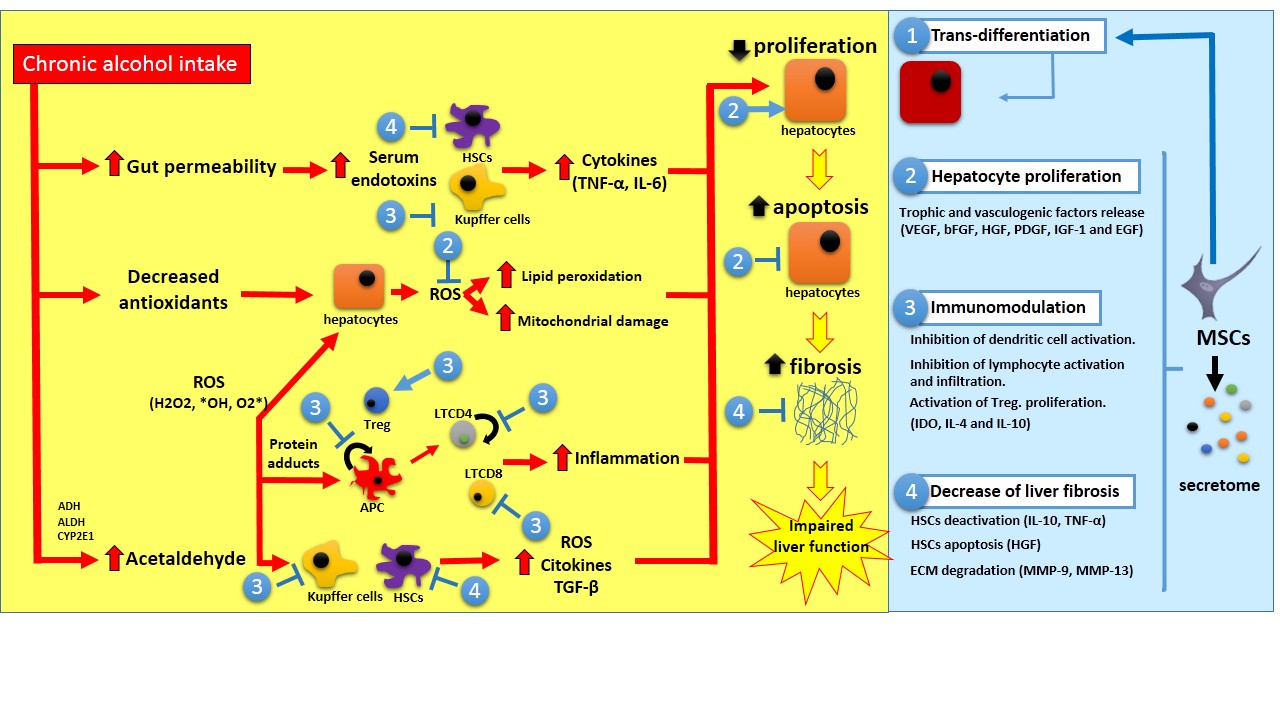
147 **Cuiffo BG**, Karnoub AE. Mesenchymal stem cells in tumor development: emerging roles and concepts. *Cell Adh Migr* 2012; **6**: 220-230 [PMID: 22863739 DOI: 10.4161/cam.20875]

148 **Kunter U**, Rong S, Boor P, Eitner F, Müller-Newen G, Djuric Z, van Roeyen CR, Konieczny A, Ostendorf T, Villa L, Milovanceva-Popovska M, Kerjaschki D, Floege J. Mesenchymal stem cells prevent progressive experimental renal failure but maldifferentiate into glomerular adipocytes. *J Am Soc Nephrol* 2007; **18**: 1754-1764 [PMID: 17460140 DOI: 10.1681/ASN.2007010044]

149 **Breitbach M**, Bostani T, Roell W, Xia Y, Dewald O, Nygren JM, Fries JW, Tiemann K, Bohlen H, Hescheler J, Welz A, Bloch W, Jacobsen SE, Fleischmann BK. Potential risks of bone marrow cell transplantation into infarcted hearts. *Blood* 2007; **110**: 1362-1369 [PMID: 17483296 DOI: 10.1182/blood-2006-12-063412]

150 **Epperly MW**, Guo H, Gretton JE, Greenberger JS. Bone marrow origin of myofibroblasts in irradiation pulmonary fibrosis. *Am J Respir Cell Mol Biol* 2003; **29**: 213-224 [PMID: 12649121 DOI: 10.1165/rcmb.2002-0069OC]

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**Figure 1 Pathogenesis of alcoholic liver disease and possible interventions of mesenchymal stem cells**. Ethanol promotes the translocation of LPS from the gastrointestinal lumen to the portal vein. In Kupffer cells and in HSCs LPS increase the expression of multiple pro-inflammatory cytokines reducing liver regeneration. Chronic alcohol exposure, reduces the intracellular concentration of antioxidants with subsequent mitochondrial dysfunction, leading to hepatocyte apoptosis. Acetaldehyde is highly toxic to hepatocytes because it binds to proteins forming adducts that promote glutathione depletion, lipid peroxidation and mitochondrial damage. Additionally, these adducts act as antigens that activate the adaptive immune response, leading to lymphocyte recruitment to the liver. HSCs can be activated by damaged hepatocytes and activated Kupffer cells through various pro-fibrogenic mediators, resulting in ECM accumulation and fibrosis. The interventions of MSCs include: (1) tras-differentiation into parenchymal cells; (2) induction of endogenous regeneration (*i.e.,* stimulation of hepatocyte proliferation, inhibition of hepatocyte apoptosis and improvement of the impaired endogenous regeneration); (3) modulation of inflammation (*i.e.,* inhibition of antigen-presenting cells –APC- maturation, proliferation, activation and/or T cell priming activity; reduction of lymphocyte proliferation and stimulation of Treg proliferation); and (4) decrease of liver fibrosis (*i.e.,* inhibition of HSCs proliferation, stimulation of HSCs apoptosis and induction of ECM degradation). → and  represent stimulation and inhibition, respectively.

**Table 1 Proposed cellular and molecular mechanisms that could contribute to hepatic protection by mesenchymal stem cells in alcoholic liver disease**

|  |
| --- |
| **MSCs in liver inflammation** |
| * Inhibit the proliferation of CD8 cytotoxic T lymphocytes and increase the relative rate of CD4 Th2 lymphocytes[97,100]. |
| * Inhibit the maturation of monocytes into dendritic cells[94]. |
| * Inhibit the secretion of TNF-α, INF-γ and IL-12 by dendritic cells and increase the secretion of IL-10 by these cells reducing the pro-inflammatory potential[95]. |
| * Suppress the proliferation, cytolytic activity and cytokines secretions of the NK cells[96]. |
| * Express indoleamine 2,3-dioxygenase upon INF-γ stimulation, leading to tryptophan depletion and the inhibition of T cell proliferation[98]. |
| **MSCs in liver fibrosis** |
| * Reduce the proliferation of HSCs and the synthesis of collagen type I through the secretion of TNF-α[125]. |
| * Induce HSCs apoptosis[124]. |
| * Express matrix metalloproteinase-9 that degrades the extracellular matrix[128,129]. |
| **MSCs in liver regeneration** |
| * Secrete trophic factors like, HGF, EGF and IGF-1 that promote hepatocyte proliferation and function during liver regeneration[68,128,130]. |

**Table 2 Preclinical studies using mesenchymal stem cells or their derivatives to treat liver injury**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Animal model | Liver injury induction/kind of liver injury | MSCs administration route | Number and source of transplanted MSCs | Therapeutic effect | Proposed mechanisms | Ref. |
| Rat | Allylalcohol (i.p. administration)/chronic damage | Intrahepatic | 1 × 106 MSCs from human BM | Hepatocyte regeneration | Hepatocyte differentiation without evidence of cell fusion | [76] |
| Mice | Low-level of radiation/minimal, hepatic damage | Tail vein | 2 × 104 MSCs from mice BM | Hepatocyte regeneration | Hepatocyte differentiation | [78] |
| Mice | Chronic exposure to high fat diet/NASH | Tail vein | 0.5 × 106 MSCs from mice BM | Prevention of NASH onset  Preclusion of the inflammatory process | Paracrine promotion of hepatic proliferation  Increase in the fatty-acid oxidation enzymes expression | [110] |
| Mice | Chronic exposure to atherogenic diet/NASH | Splenic capsule | 0.1 × 106 MSCs from mice adipose tissue | Restoration of albumin expression in hepatic parenchymal cells  Amelioration of fibrosis  Suppression of persistent hepatic inflammation | Modulation of inflammation  Increase in mmps expression | [111] |
| Mice | CCL4 (i.p. administration)/liver fibrosis | Spleen | 0.5 × 106 MSCs from human amniotic membrane | Reduction of liver fibrosis  Improvement of hepatic function | Inactivation of hscs  Reduction in hepatocyte apoptosis  Promotion of liver regeneration  Differentiation in hepatocyte like cells | [126] |
| Mice | CCL4 (i.p. administration)/liver fibrosis | Tail vein | 0.5 × 106 MSCs from human BM | Reduction of liver fibrosis | Induction of MMP-9 expression  Reduction in TGF-β expression | [121] |
| Rat | D-galactosamine (i.p. administration)/fulminant hepatic failure | Penile vein | Conditioned medium from human BM MSCs | Reduction in the mortality rate  Reduction in panlobular leukocyte infiltrates  Reduction in hepatocellular death | Modulation of the immune response  trophic factors release (*i.e.,* Vegf, hgf, and igf-bp) | [105,122] |
| Mice | CCL4 (i.p. administration)/liver fibrosis | Intrahepatic | Exosomes derived from human umbilical cord MSCs | Recovery of serum aspartate aminotransferase activity  Decrease in collagen type I and III, TGF-β1 level | Not determined | [109] |

BM: Bone marrow; HGF: Hepatocyte growth factor; HSCs: Hepatic stellate cells; IGF-BP: Insulin growth factor binding protein; i.p.: Intraperitoneal; MMP: Matrix metalloproteinase; MSCs: Mesenchymal stem cells; NASH: Nonalcoholic steatohepatitis; TGF-β: Transforming growth factor; VEGF: Vascular endothelial growth factor.