Review Article

The versatility of adipose derived stem cells in liver transplantation: a narrative review

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Background and Objective: Studies about stem cells in last twenty years have been increased exponentially. The several mechanism of actions which are related to immunomodulatory, regenerations and anti-inflammatory; beneficial effects in clinical and experimental studies have been shown. All these results are also the area of interest and research in liver transplantation. The aim of this review is to analyze the characteristics, functions of stem cells in different aspects of liver transplantation; and to find out possible therapeutic effects of stem cells in wearisome, complicated subjects of liver transplantation depend on the mechanism of actions.

Methods: A review of the literature was conducted on MEDLINE, PubMed. Studies were included in English language. Randomized controlled trial, meta analysis, review, case series, clinical and experimental study in stem cell were included. Controversial and complicated subjects of liver transplantation were analysed with the underlying mechanism which are also research of interest of stem cells.

Key Content and Findings: It was shown in several *in vivo* and *vitro* studies that adipose derived stem cells have anti-inflammatory, immunomodulatory and regenerative capacity. This characteristics have been shown beneficial effects in several transplantation studies. Administration route and amount of stem cells were analysed. Usage of bio-scaffold and perfusion machines with adipose derived stem cells were searched.

Conclusions: Anti-inflammatory, immunomodulatory and regenerative capacity of stem cells have been found both in experimental and clinical transplantation studies with beneficial results. The field of study about stem cells will increase with the development of genetic engineering and bio-scaffold technology.

Keywords: Stem cell; liver; transplantation; rejection

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Introduction

Liver is a vital organ that is producing several proteins, metabolising nutrients, secreting bile, removing toxins, and supporting immunity. Although the liver has the ability to repair itself by self-renewal, some liver diseases may lead to irreversible liver injury and result in liver failure. Liver transplantation is the best treatment option for liver failure. Unfortunately, nearly 2,000 patients die while waiting in the United States (1).

Scarcity of donor organs, high costs of the procedure, rejection risks, complications risks after procedures, side effects of immunosuppressive treatments, and posttransplantation infections are the main issues in liver transplantation (2,3). In order to overcome organ scarcity, marginal grafts can be selected according to expanded donor

criteria. Unfortunately, usage of marginal grafts has risks of posttransplantation dysfunction or primary nonfunction, posttransplantation bile complications (4). Because they're more susceptible to ischemia/reperfusion injury (IRI). Machine perfusion devices are new techniques that had been developed in order to modulate the inflammatory and immune response in marginal organ transplantation (5). Improvements in immunosuppressive agents accelerated the success of transplantation up to 1-year survival above 90%. Liver dendritic cells express low levels of co-stimulatory molecules and weakly stimulate T-cell response (6). The microenvironment is the key point in modulating liver dentric cells and is the mechanism for transplant tolerance (7). Despite the use of current agents, nearly half of the patients suffer from allograft rejection (8). Moreover, lifelong usage of immunosuppressive drugs brought new problems like adherence, infection and malignancy (9).

Mesenchymal stem cells (MSCs) are gaining importance because of their regenerative and immunomodulatory effects in several diseases (10,11). Bone marrow, umbilical cord blood, amniotic fluid, adipose tissue can be used for the source of MSCs (12). Adipose-derived stem cells (ADSCs) are the most frequently used MSCs because of their relative accessibility and widespread ethical acceptability. It has been shown that ADSCs have more advantages than other stem cells: (I) ubiquitous and can be relatively easily harvested in larger quantities; (II) more homogenous; (III) superior in maintaining stemness and resisting differentiation; (IV) high proliferative active; (V) better tolerance against apoptosis under hypoxic conditions; (VI) more active in autocrine production of some growth factors and immune modulators (13-15).

MSCs have been studied in several studies related to liver disease and liver transplantation. However, there are limited number of studies about ADSC in liver transplantation (16-22). Adipose derived stem cells can be therapeutic option for the main problems in liver transplantation.

Objective

The goal of our research is to identify the most recent and significant studies and meta-analysis regarding the stem cell characteristics and mechanism of actions. The controversial and complicated subjects of liver transplantation including ischemia and reperfusion injury, small for size, non-function grafts, bile complications, rejection, and De novo diseases were analysed with the underlying mechanism which are also research of interest of stem cells. We aimed to investigate the possible advantages of ADSCs in liver transplantation regarding the characteristics of ADSCs and main mechanisms in liver transplantation. We present this article in accordance with the Narrative Review reporting checklist (available at https://dmr.amegroups.org/article/view/10.21037/dmr-22-24/rc).

Methods

Systematic review of literature was performed by searching in MEDLINE and PubMed. Studies were included in English and Chinese languages. Randomized controlled trials, meta analyses, experimental and animal studies, reviews and case reports about stem cells from January 2001 to February 2022 were included (*Table 1*).

ADSCs

Adipose tissue functions as an endocrine organ throughout the body of mammalian species. The term ADSCs is used to describe the multipotent, plastic-adherent, cell type derived from adipose tissue (23). Due to the potential of ADSCs to differentiate into various cell types including neural cells, vascular endothelial cells, osteocytes, pancreatic β-cells, adipocytes, cardiomyocytes, hepatocytes; they hold promise for the future of regenerative medicine. MSCs are the adult multipotent stem cells that were originally isolated from bone marrow; however, with the increase in technological developments, ADSCs were harvested from the adipose tissue and classified also as MSCs. Although both derived from the MSC familia, bone marrow-derived MSCs and adipose-derived MSCs differentiate from one another in terms of differentiation potential, the rate of proliferation, and the factors secreted. Through the studies applied on nude mice, the proliferation rate of adipose-derived MSCs was determined to be higher when compared to the proliferation rate of bone marrow-derived MSCs. ADSCs have distinct surface markers such as $CD105^+$, $CD34^+$/c-kit^-, and $CD90^+$ Thy-1. Furthermore, ADSCs are advantageous in a way that they can be obtained abundant from adipose tissue by an invasive liposuction procedure that can be carried out with local anesthesia. ADSCs have better accessibility and isolation compared to bone marrow MSCs (BM-MSCs) (12). The easy access possibility of the technique results in a high yield of stem cells. ADSCs are superior to BM-MSCs and umbilical-MSCs in terms of colony frequency and show also higher proliferation

Table 1 The search strategy summary

Items	Specification
Date of search	January 2001–February 2022
Databases and other sources searched	MEDLINE, PubMed
Search terms used	Adipose derived stem cell, liver transplantation, ischemia reperfusion injury, small for size liver, primary non function liver, *de novo* hepatitis
Timeframe	September 2021–February 2022
Inclusion and exclusion criteria	Reviews, meta analyses, case reports, experimental studies, randomized controlled trials and only English, Chinese written studies. Most recent studies which include mechanism of action

capacity in hepatic lineage as compared to BM-MSCs (24).

Brown and white adipose tissues are the source of ADSCs. The white adipose tissue is mostly found in abdominal, hip, thigh; and high amount of stem cells have been recovered when compared from Brown adipose tissue (25). ADCSs can be obtained in huge amount during the liposuction which has low risk. Nearly 10 million ADSCs can be obtained from 300 mL of liposuction material. This high availability of human subcutenous ADCSs led the use of human ADCSs in non-human studies as the ADCSs are lack of major histocompatibility complex class II (MHC-II) expression and did not provoke alloreactivity (26). This might lead interspecies usage of ADCSs which are derived from liposuction materials. Although there is no presented complication in ADCS xenotransplantation; from the immunological point of view, allotransplantation looks a better option as seen in many studies. But ongoing studies showed ADSC plasticity depends on donor gender, anatomic harvesting location and age which are subject of future studies (27).

Ischemia and reperfusion injury

While deprivation of oxygen and nutrient to the cells by cessation of arterial blood flow is defined as ischemic injury, a sudden increase of the oxygen concentration after ischemia results in oxidative injury or reperfusion injury. Hypoxic conditions and oxidative stress lead to cellular oedema and disruption of cellular membranes. Dissipation of endoplasmic reticulum, mitochondrial membranes results in massive reactive oxygen species which detriment proteins, lipids, and nucleic acids (28). In the early phase of ischemic and reperfusion (I/R) injury, morphologic changes have resulted from activated Kupfer cells (29). Proinflammatory cytokines [such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β)] secretion exacerbate the injury by activation of endothelial adhesion molecules (30). Damage associated molecules activate the innate immune system via toll-like receptors (TLRs). This inflammation leads to a rise in antigen-specific lymphocytes and also a rise in antibody production. In the late phase of I/R injury, leukotrienes and proteases are responsible for hepatocyte destruction. Polymorphonuclear neutrophils play a pivotal role in the late phase. Proinflammatory macrophages (M1) are activated by TLR and produce several inflammatory factors like TNF-α, IL-1β, chemokine (C-C motif) ligand 2 (CCL2) and chemokine (C-X-C motif) ligand 1 (CXCL1) (20).

MSCs in inflammatory response

It was known that MSCs inhibit the immune response to generate a tolerogenic microenvironment by cell-cell interaction with Kupfer cells, hepatocytes, $CD4^+$ cells, and $CD8^+$ cells or by a paracrine mechanism with releasing cytokines (20). During I/R injury, apoptosis is generated by exogenous death receptor pathways, caspase families, and proapoptotic proteins like Bax, Fas. In the study of Jiao *et al.*, it was shown that ADSC secretes proteins like angiopoietin (ANG)-1, ANG-2, vascular endothelial growth factor (VEGF), and basic-fibroblast growth factor (b-FGF) which promote the expression of antiapoptotic protein B cell lymphoma-2 (Bcl-2) (31,32). The mechanism of ADSC in reducing apoptosis is related to antiapoptotic proteins and reducing the exogenous death receptor apoptosis pathway (31). After I/R, liver regeneration is mediated by cytokines and growth factors. Cytokines and growth factors like TNF-α, hepatocyte growth factor (HGF), and VEGF activate extracellular signal-regulated protein kinase (ERK) and c-Jun N-terminal kinases (JNK) and stimulate genes like c-fos and c-Jun. In the study of Seki *et al.*, it was shown that

ADSCs promote liver regeneration by producing several bioactive proteins via paracrine way (33). In the study of Sun *et al.*, autologous administration of ADSCs was evaluated in hepatic I/R in several aspects (34). In ADSC administration, oxidative stress and myeloperoxidase (MPO) expression are decreased; however anti-oxidative proteins like nicotinamide-adenine dinucleotide phosphate:quinone oxidoreductase 1 and heme oxygenase-1 are increased (34). Decreased proinflammatory cytokines like matrix metalloproteinase (MMP)-9, plasminogen activator inhibitor (PAI)-1, IL-1β, IL-6, and TNF-α are important stimulants for hepatic satellite cells (HSCs) and polymorphonuclear neutrophils (PMNs). The main indicator of HSC activation is α-smooth muscle actin (α-SMA) and is decreased in ADSC administration. Endothelin-1 is the key mediator for HSC contraction. Endothelin-1 is found to be decreased in ADSC administration. Endothelial cell markers like CD31 and the Willebrand factor were shown to be restorated in ADSC treatment. The possible mechanism in the increased apoptosis in I/R is related with increased expression of Bax, caspase 3, and cleaved poly (ADP-ribose) polymerase and cytochrome c from mitochondrial intermembranous space (35). Administration of ADSC suppresses the hepatic I/R induced apoptotic cell death.

Contraction of HSC and enhanced leukocyte-endothelial interaction by PMN disturbs hepatic perfusion. By suppressing the activation of HSC and PMNs, homogenous perfusion in hepatic macrovascular can be achieved. Microcirculatory derangement, inflammation, activation of HSCs and PMNs, oxidative stress and apoptosis are all improved by administration of ADSCs in the study of Sun *et al.* (34).

The mechanism of ADSC are not limited to immunomodulation of the recipient but also possible differentiation into injured tissue and change the microenvironment of the tissue. In the injured tissues, MSC secretes a broad range of growth factors like human growth factor, insulin-like growth factor-1, basic fibroblast growth factor and transforming growth factor. These secretions decrease hepatocellular necrosis and promote hepatic function and bile synthesis (36,37). Both the anti-inflammatory, and immunomodulatory effects of ADSC showed clinical improvements in recent experimental studies. In the study of Sasajima, administration of ADSCs in a model of cardiac death of swine, improved viability of grafts was achieved with better histological and biochemical results (38). While all donation after circulatory death (DCD) recipients died within 24 hours, 3 of 5 recipients in ADSC administration survived more than 7 days.

Administration routes for stem cells

There is no consensus on the optimum route of ADSC transplantation. Sang *et al*. demonstrated that portal administration of ADSC improves liver function, inhibits apoptosis, prolongs survival time, and quickly participates in swine model (39). However, this is not a comparative study. In the study of Cao *et al*., jugular and portal administration of MSC were compared in an acute liver failure model (40). Prolonged survival was achieved in administration through the portal vein when compared within administration through the jugular vein. Intraportal administration from portal vein also increased survival in the fulminant hepatic failure in pig model when compared with systemic administration (41). The major concern in the administration of MSC via portal vein is the risk of induced portal hypertension and obstruction of hepatic sinusoids. Nevertheless no such a kind of complication has occurred. This risk is probably much more related to the dose of stem cell. The amount of stem cells is changing between 2.5×10^5 and 1×10^7 in previous experimental models. No hepatic sinusoidal obstruction has been declared (37,42-44). But most preferred amount is 1×10^6 independent from the administration route. In human models, the amount of stem cells must be high enough to function but low enough for preventing the sinusoidal obstruction. In human studies the amount of stem cells have been varied between 0.5×10^6 and 4×10^8 cells/kg independent from the administration route in liver failure models (45-50). The previous studies were given in *Table 2*.

Small for size

An important issue in liver transplantation especially the living donor liver transplantation is the size mismatch of graft. And recipients may suffer from the post-operative graft dysfunction. Portal reflow results in shear stress which is a necessary trigger for regeneration of liver. However, excessive shear stress which is seen in small-for-size (SFS) grafts disturbs sinusoidal microcirculation (51). Huge amount of blood for small-for-size graft forms a kind of portal hypertension in intrahepatic portal system and leads to serious vascular endothelial damage. Vascular endothelial damage with excessive blood flow result in severe inflammatory response, more reactive oxygen species, increased susceptibility of liver cells to apoptosis, and increased risk for rejection (52). Therefore, the ischemia reperfusion injury could delay liver

Table 2 ADSCs in animal models

Species of ADSC	Dose	Model	Route	Mechanism	Effect	Refs.
Pig	1×10^6	Pig	Direct liver parenchyma	Decreased caspase activity Decreased p53, Bax, Fas, and Fasl mRNA Increase Bcl-2 levels	Decreased apoptosis	(28)
Pig	1×10^6	Pig	Direct liver parenchyma	Decreased TNF-α, IL-1β Increased VEGF, ANG-1, and ANG-2 levels Increased Ki-67 positive cells Increased expression of PCNA Decreased expression of SOCS3	Alleviates inflammation Promotes regeneration	(32)
Human	2×10^6	Wistar rats	Systemic	Increased mitotic index, anti-proliferative antigen levels Increased phospho-ERK1/2, phospho-JNK, phospho-p38 MAPK, c-fos, and c-Jun levels Increased expression of IL-6, VEGF, and c-Jun genes	Increased regeneration	(33)
Autologous	1.2×10^6	Fisher rats	Systemic	Decreased MPO levels, oxidative stress Increased anti-oxidative proteins Suppressed α-SMA expression Decreased endothelin-1, TNF-α Increased IL-10	Suppressed hepatic satellite cell activation	(34)
Human	1×10^6 and 2×10^6	Mice	Systemic	Decreased IL-6 Increased PCNA cells Decreased histologic injury	Promote regeneration	(36)
Swine	2×10^5 and 1×10^6	Rat	Portal vein	Increased bile secretion Decreased LDH level Less hepatocellular vacuolation	Improved function	(37)
Swine	1×10^7	Swine	Splenic vein	Less hepatocellular swelling Less TUNEL-positive cells	Increased survival	(38)

ADSCs, adipose-derived stem cells; Bax, Bcl-2-associated X; Bcl-2, B cell lymphoma-2; TNF-α, tumor necrosis factor-α; IL, interleukin; VEGF, vascular endothelial growth factor; ANG, angiopoietin; PCNA, proliferating cell nuclear antigen; SOC, suppressors of cytokine signaling; ERK, extracellular signal-regulated protein kinase; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MPO, myeloperoxidase; α-SMA, α-smooth muscle actin; LDH, lactate dehydrogenase; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.

regeneration and worsen liver injury small-for-size grafts. Prophylactic measures against SFS graft are represented by portosystemic shunts, splenectomy, splenic arterial ligation/embolization, somatostatin. All are being used in clinical practice but the main aim is to increase the liver regeneration (53,54).

The role of MSC in endothelial regeneration

VEGF signal proteins can be considered as a vascular endothelial mitogen. They play a crucial role in the survival of endothelial cells by promoting liver sinusoidal reconstruction, angiogenesis, and liver regeneration through

the stimulation of sinusoidal endothelial cells. VEGF is additionally the sole growth factor that has the ability to promote the proliferation of cultured sinusoidal endothelial cells *in vitro* and drive hepatocyte development as well as neovascularization *in vivo*; moreover, liver regeneration is an angiogenesis-associated process (55,56). Through the study conducted by Ma *et al.*, the secretion of VEGF by the implanted ADSCs had the potential to promote the recovery and regeneration of SFS grafts through protecting and stimulating regeneration of sinusoidal endothelial cells (57). This increased VEGF secretion is not only from the ADSCs but also from the macrophages which are stimulated by ADSCs. Macrophages in liver might be stimulated to release various proangiogenic and antiapoptotic factors by implanted ADSC via autocrine/paracrine way (57). Maintaining sinusoidal endothelial cell integrity by VEGF is the most prominent positive effect of stem cells that is shown in several studies (57,58). Improved sinusoidal microcirculatory have resulted in improved liver functions and graft survivals (57,59,60). The other possible mechanism of sinusoidal microcirculation improvement was related to the downregulation of endothelin receptor type A (ETAR) in ADSC implanted livers (57). Because blockage of ETAR in SFS showed attenuated microcirculatory disturbances, reduced hepatocellular damage in SFS grafts (61).

The role of MSC in hepatocyte regeneration

The regeneration ability of hepatocytes in SFS grafts is an another objective of stem cell therapy. Quiescent hepatocytes are primed to enter the cell cycle through the activation of transcription factors such as nuclear factor-kappa B (NFB), activator protein-1 (AP-1), signal transducer and activator of transcription 3 (STAT3), and CCAAT/enhancer binding protein (C/EBP), as well as the translation of immediate early genes (62). As a result, hepatocytes become receptive to growth factors such as HGF, epidermal growth factor (EGF), and transforming growth factor-α (TGF-α). Moreover, TNF-α and IL-6 molecules can be given as an example to the reactive oxygen species and cytokines that assist in the transcription factor activation and generate early signals for regeneration (63). In the study of Zhong *et al.*, an increase in the levels of proinflammatory cytokines proclaimed to be promoting an injury of small-for-size liver grafts (64). Nevertheless, regeneration-suppressing mechanisms could operate downstream of cytokine signaling and intracellular processes are affected as a result of cytokine signaling. Inhibition of JNK/c-Jun and CyD1 signaling is thought to be a cause of the failure of small-for-size liver graft regeneration (64). During development, JNK and c-Jun prevent hepatocytes from going through apoptosis. Through several stem cell researches that do not apply ADSCs, it was concluded that HGF, Bcl-2, Bcl-XL, IL-6, IL-10, IP-10, and CXCR2 mRNA expressions were upregulated, as were the activities of AP-1 and NF-kB; as well as enhanced levels of p-c-Jun, cyclin D1, and proliferating cell nuclear antigen (PCNA) (65). Furthermore, in SFS grafts, overexpression of MSC by HGF has been demonstrated to promote liver regeneration and minimize fibrosis (60,66). On hepatocytes, HGF exerts a significant cytoprotective action. Exogenous HGF, on the other hand, is promptly removed from circulation by the liver, with a half-life of less than 15 minutes. A reasonable solution to this problem would be to establish a gene transfer approach that would allow HGF proteins to be continuously expressed *in vivo* (67). As mentioned before, MSC has a prominent role in the reduction of systemic and local proinflammatory cytokine levels, including TNF-α, IL-6, monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-2 (MIP-2), and IL-1β; neutrophil infiltration; and Kupffer cell activation but also upregulation of anti-inflammatory cytokines, such as IL-10. These mechanisms are important to induce the liver regeneration. Anti-apoptotic properties of ADSC were shown by increased Bcl-2/bax ratio, reduced Bcl-2 associated agonist of cell death (BAD) expression and elevated proportion of phosphorylated BAD in SFS grafts (57). Anti-inflammatory and regenerative characteristics of ADSC were also shown by Gao *et al.* in SFS graft rat model (68). Both PCNA positive cells and liver weight were significantly increased in ADSC group (68). Also the rejection rate in ADSC group was less in SFS rats. This mechanism was explained by increased amount of Treg which has an important role in induction of immune tolerance to transplanted tissues (68).

In previous SFS graft model studies, it was shown that MSCs have the potential to inhibit the death of hepatocytes and stimulate liver regeneration via a paracrine mechanism, or directly differentiate into hepatocytes and repopulate the injured liver (59,60,65,66,69,70). However more studies are needed in ADSC models especially HGF gene transferred models. The other concern about MSCs in SFS graft is the time of MSC settlement and maturation in the injured liver. Because main hepatocyte regeneration is desired in 5 days after transplantation; however, MSC differentiation exceeds 5 days. In order to overcome this problem, condition

mediated MSC can be preferred (59). Some studies about enrichment of the ADSC culture media showed improved physiologic parameters in primary hepatocyte culture (71).

Primary non-function and delayed graft function

The number of patients waiting for the liver is increasing every day. For this reason more marginal organs/donors are used. Primary non-function (PNF) and delayed graft function (DGF) are the unwanted complication in marginal graft usage. Although several definitions have been made; PNF can be defined as graft non-function that ultimately progresses to graft loss, on the other hand most of DGFs are reversible (72). The incidence of PNF varies between 1.3–6.6%; the incidence of DGF varies between 5.2–36.3 (72). Risk factors for dysfunctioning grafts are all related to the increased sensibility of I/R injury of grafts (72). Although patients can recover from DGF, recovery involves high postoperative costs, long hospitalization, prolonged treatment with possible complications. This time can exceed 1 month. On the other hand there's no effective therapy for PNF except re-transplantation. Main target in treatment of DGF is to support hepatic regeneration and suppress oxidative stress. Whereas in PNF, main approach should be prophylactic precautions especially in high risk groups and re-transplantation. Stem cell treatment might be used in order to gain time in waiting for new transplantation. Although there is no published study about the effects of MSC on PNF or DGF, MSC can be used in treatment of DFG and in precautions of PNF via their anti-inflammatory and hepatic regenerative characteristics. As I/R injury is considered the main cause of PNF and DFG, we tried to summarise the protective roles of ADSC in I/R injury and liver failure.

MSCs in acute liver failure

Acute liver failure is a good model for evaluating the effect of ADSC. As seen in *Table 3*, there are several studies showing the protective effects of ADSC in liver failure models. Carbon tetrachloride (CCl_4) liver failure models is a toxic model. A chemical inducer, CCl_4 can be catalyzed by cytochrome P450 (CYP450) in liver tissue, resulting in the formation of free radicals and oxygen-active substances causing liver injury. In carbon tetrachloride models, alanine aminotransferase, aspartate aminotransferase, ammonia, were decreased and increased survival was achieved after ASC-derived hepatocyte transplantation (82,83). In CCl_4 models, the increased regenerative capacity of ADSC is a promising role for PNF and DFG. In acetaminophen liver failure model, the main mechanism is increased oxidative stress and detoriated liver regeneration. The mechanisms of action of ADSC in acetaminophen models are antioxidative role, suppression of inflammation, and enhancement of regeneration (73,84). Concanavalin A model of hepatic failure is occurred by activation of natural killer (NK) cells and T cells and secretion of TNF-α and interferon-δ (IFN-δ) (76). In the model of concavalin A liver failure model, ADSC showed antiinflammatory effects and protection of hepatocytes against necrosis. In the D-galactosamine liver failure model, the liver injury is mediated by release of pro-inflammatory mediator (78). In the study of Chen *et al.*, although most of the injected ADSC have stayed in spleen, the growth factors were significantly increased and resulted in improved liver function (78). As urgent treatment is needed in PNF and DGF, time for housing in liver and differentiation to hepatocytes of stem cells is a great obstacle. In order to overcome this problem, ADSC with heparin has been studied; shorter time and better results have been obtained (79,80). The superiority of ADSC over bone marrow derived stem cells has been studied by Zare *et al.* and found that ADSC are superior in CCl_4 liver failure model in achieving better liver function (81).

Beside ADSC, several MSC models have been studied in liver failure models. In these models, stem cells inhibited the hepatocyte necrosis and the vacuolar degeneration. As a result, improved survival, improved liver function tests have been achieved. The main mechanisms in this success is the anti-inflammatory action of stem cells. The anti-inflammatory action has been shown by decreasing the levels of liver isoprostanes, 8-OHG, and nitrite-nitrates; preserved glutathione (GSH) levels and superoxide dismutase; decreasing the levels of TNF-α, MCP-1, IL-1β, intercellular adhesion molecule (ICAM)-1, and phospho-JNK. Also anti-inflammatory action anti-apoptotic and regenerative mechanisms are shown by upregulating antiapoptotic Bcl-2 and downregulating proapoptotic Bcl-2-associated X (Bax) and TNF-α. All these mechanisms are also targeted for PNF and DGF. Usage of stem cells might gain time for retransplantation or regeneration.

MSCs in machine perfusion

Because of the shortage of donor organs, several marginal grafts are used. DCD organs or grafts with high donor risk indexes carry a high risk for PNF or DGF. Machine

Table 3 ADSCs in liver failure animal models

Author	Stem cell source	Liver failure model	Mechanism	Result
Salomone F *et al.* (73)	ADSC	Acetaminophen	Decreased levels of isoprostane	Enhance liver regeneration
			Decreased 8-OH	Protect against oxidative stress
			Decreased nitrite/nitrate	
			Increased glutathione	
			Increased hepatocyte TNF-α, MCP-1, IL-1β, ICAM-1 and phospho-JNK levels	
			Increased liver expression of cyclin D1 and PCNA	
Huang YJ *et al.* (74)	ADSC	Acetaminophen	Suppressed cP450	Antioxidative role
			Reduce toxic nitrotyrosine	
			Upregulation of NF-E2 related factor 2	
			Suppression of MAPK and inflammatory cytokines	
Banas A *et al.* (75)	ADSC	CCl_4	Increased IL-1Rα, IL-6, IL-8, G-CSF, GM-CSF, monocyte chemotactic protein 1, nerve growth factor, and hepatocyte growth factor	Trophic activity of ADSC
				Inhibit fibrosis, promote angiogenesis
Kubo N *et al.* (76)	ADSC	Concanavalin A	Improve in liver enzymes	Protection of hepatocytes
			Decreased serum TNF-α and IFN-δ	Anti-inflammatory effect
			Increased survival	
			Less necrotic hepatocytes	
Yan Y *et al.* (77)	ADSC	CCl_4	Improve ALT and AST	Anti-inflammatory role
			Improve hepatic glycogen synthesis	
Chen G *et al.* (78)	ADSC	D-galactosamine	High level of HGF, VEGF	Enhance regeneration by secretion of growth factors
			Improved liver functions and morphology	
Hwang Y *et al.* (79)	ADSC with heparin	Acetaminophen	Improved AST and ALT	Improved regeneration
			Increased HGF	Anti-inflammatory role
			Reduced levels of macrophage and CYP2E1	
Yukawa H *et al.* (80)	ADSC with heparin	CCl_4	Improved ALT, AST, and LDH	Protection of liver
Zare H *et al.* (81)	ADSC and BM-MSC	CCl_4	Improved ALT, AST	Protection of liver better than BM-MSC
			Improved liver histology	

ADSC, adipose derived stem cell; TNF-α, tumor necrosis factor-α; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; IL, interleukin; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; ICAM, intracellular adhesion molecule; JNK, c-Jun N-terminal kinases; PCNA, proliferating cell nuclear antigen; MAPK, mitogen-activated protein kinase; NF, nuclear factor; IFN, interferon; ALT, alanine aminotransferase; AST, aspartate aminotransferase; VEGF, vascular endothelial growth factor; HGF, hepatocyte growth factor; LDH, lactate dehydrogenase; BM-MSC, bone marrow mesenchymal stem cells; CCl_4, carbon tetrachloride.

perfusion devices have several advantages for storing donor livers. Normothermic machine perfusion (NMP) has considerably increased the quality of DCD donor livers, preventing ischemia-induced hepatocyte damage, promoting hepatic waste elimination, and removing inflammatory factors. Ferroptosis and reduced cell viability

are known to be caused by increased reactive oxygen species (ROS) and free Fe^{2+} in the I/R of hepatocytes. Intracellular damage is also caused by mitochondrial ROS. The combination of stem cells and NMP was effective in lowering oxidative stress, minimizing ferroptosis, and maintaining mitochondrial morphology (85). In addition to mitochondrial changes, the I/R after static cold perfusion demonstrates microcirculatory hyperinflammation caused by macrophage activation, microvascular leukocyte aggregation, disordered endothelin/nitric oxide (ET-1/NO) balance, arterial spasm, hepatic sinusoidal congestion, and microcirculation occlusion. Intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and von Willebrand factor (vWF) are products of inflammatory response and expressed on endothelial cells after I/R. Increased expression of these molecules are indicates the deterioration of hepatocyte congestion. Stem cells were shown to inhibit DCD liver macrophage activation, endothelial cell activation and ICAM-1 and VCAM-1 activation under NMP. As a result, stem cells in NMP systems significantly improved the ultrastructure of hepatocytes (86).

Another advantage of perfusion devices is existence of a chance to add several enzymes that increase the effects of stem cells. Heme oxygenase 1 (HO-1) is an important enzyme for protection of cells. Antioxidative and anti-apoptotic features of HO-1 lead decreased cell damage and increased survival. In the HO-1 modified stem cell models in NMP showed improved liver enzymes, decreased proinflammatory cytokines and decreased HMGB1 expression (87). As seen in the experimental studies, addition of stem cells to organ perfusion systems improves the graft function, bile secretion, pH self-regulation and survival of recipients.

Human liver stem cells (HLSCs) are multipotent stem cells derived from adult liver tissue that display hepatic markers such albumin, α-fetoprotein, and cytokeratins 8 and 18. HLSCs employ paracrine pathways to carry out their biological functions. Furthermore, human liver stem cell-derived extracellular vesicles (HLSC-EVs) are cell-secreted vesicles that can assist damaged tissues to recover. In fact, HLSC-EVs play a significant role in the communication between stem cells and adult cells by horizontally transferring lipids, proteins, and, most importantly, genetic information, which is effectively converted into proteins with a biological activity. In a DCD model with extended warm ischemia, HLSC-EV therapy during NMP proved to be feasible and efficient in minimizing injury. The other important advantage of HLSC-EVs is early exertion of their effects (88,89). Combination of stem cells and machine perfusion devices can decrease the risk of PNF and DFG and can be an option for protection against PNF in future studies.

Bile complications

Even though the occurrence of biliary problems following liver transplantation has been declining due to ongoing advancements in surgical techniques, they continue to be a major cause of morbidity and mortality. The common biliary problem after a liver transplant are strictures and leakage. Split grafts, concomitant vascular complications, extended ischemia, and reperfusion injury are all frequent causes of these complications.

Bile leaks

The rates of bile leaks after liver transplantation vary in 2–25% (90). Bile leaks mostly occur at the anastomotic site and are caused by ischemia and failed anastomotic healing due to immunosuppression of tension on the anastomosis. Several surgical techniques have been used and several local agent applications have been tried (91-94). Whether anatomic or nonanatomic, main issues in preventing the bile leaks are the prevention of ischemia and enhancing the regeneration of the anastomosis (95). As the most important feature of the stem cell therapy is regenerative capacity and angiogenesis, ADSC might be a treatment or preventive option for bile leaks.

The mechanism of biliary epithelial cell injury in ischemia can be best explained by depletion of intracellular adenosine triphosphate. As a result, biliary epithelial cells lose their connections and result in sloughing. Inadequate proliferation and regeneration is the main determinant in the pathogenesis of bile anastomosis. Peribiliary glands (PBG) are the main cells in the proliferation and the repair of biliary epithelium, because of having stem cells in PBGs (96). PBG are critically involved in the proliferation and regeneration of the biliary epithelium of donor bile ducts after transplantation. As a consequence, adequate preservation of the PBG has become a central objective in the development of improved donor liver preservation methods (97). The study of Tian *et al*. demonstrated that HO-1/BMMSCs enhanced PBG proliferation while also inhibited cell apoptosis (98). PBG cells exhibited high levels of VEGF after being treated with HO-1/BMMSCs, in addition to expressing VEGF receptors (98). Thus,

VEGF released by PBGs drive the proliferation of nearby vascular endothelial cells while also exhibiting autocrine and paracrine effects on PBG cells; enhancing their proliferation and triggering the shift from a static to an active phenotype.

The other targets for prevention of bile leaks are the local application of stem cell sheets on the biliary anastomosis, and usage of artificial bile ducts with stem cells in biliary anastomosis. The amniotic membranes acts as a reservoir of stem cells. Since amniotic stem cells have a pluripotent potential to differentiate into different cell types, they represent a potential candidate to provide biliary mucosal endothelial growth in duct reconstruction repair and subsequently less leakage probability. In the study of Ismail *et al.*, amniotic membranes are a good choice for biliary injury reconstruction (99). Extraluminal application of stem cells was studied and successful results were obtained in the study of Zhang *et al.* (100). Extraluminal application of an ADSC solution to biliary anastomoses was safe and did not lead to cholangitis, biliary obstruction, strictures, or leaks. It was also demonstrated that ADSCs applied extraluminally are able to engraft within the wall of the bile duct, prevent anastomotic fibrosis/inflammation, and promote neoangiogenesis of the biliary plexus (100). Existence of CD44 and CD31 stains in this study suggest that extraluminal applied ADSCs are engrafted and retained in a perivascular distribution within the bile duct and are accompanied by enhanced staining markers for neoangiogenesis. Application of stem cell sheets over biliary anastomosis is an another way of application of stem cells (101). Biliary anastomosis which was wrapped by ADSC sheets has resulted in increased vessels around anostomosis and lack of any leakage (101). This study showed the direct angiogenetic effects of ADSCs on biliary anastomosis.

For the repairing and functional reconstruction of damaged and missing tissues, biologic scaffold materials that consist of allogeneic or xenogeneic extracellular matrix are routinely used. The scaffold, which is a vital component in bile duct tissue engineering, should not only serve as a physical template for bile flow and loading bioactive substances; rather also function as a support for cell adhesion, proliferation, and differentiation, with a degradation rate that complements the new tissue formation rate. For this reason bio-scaffolds with stem cells have been tried for biliary anastomosis. Selection of ideal bio-scaffold gain much more importance rather than stem cells. In the study of Zhang *et al.* intraluminal vicryl woven mesh with ADSC lead to the breakdown of mesh and resulted in cholangitis (100). In previous studies, artificial bile ducts including poly (L-lactide-co-glycolide) (PLGA) have better results (102). PLGA layers in bio scaffold can effectively support cells, load other bioactive substances and accelerate the formation of new tissue; on the other hand rapid degradation of PLGA and formation of new tissue prevent the cholangitis. Bio scaffolds should also be biocompatible with MSCs (103). In the study of Li *et al.* coverage of BMSCs seeded bioscaffolds reached more than 90% (104). In the study of Zong *et al.*, bio scaffolds with MSC used in bile anastomosis have increased epithelialization in a shorter time than blank scaffolds (105). Beside enhanced proliferation during bile duct repair, MSCs suppress or reduce the excessive inflammatory response (105). There were no biliary leakage issues in long-term follow-up, and the neo-bile duct tissue resembled the native bile duct both morphologically and functionally (106). Even though various promising experimental studies have been conducted, bioscaffolds with stem cells still have limitations (107). Materials for artificial bile ducts should be enhanced in terms of biocompatibility, biomechanical compliance, and durability.

Bile stricture

After a liver transplant, biliary stricture is characterized as either anastomotic or non-anastomotic. Insufficient mucosa-mucosa anastomosis, local tissue ischemia, and the fibrotic nature of the recovery process are thought to be the pathogenesis of anastomotic biliary strictures. Early leakage is thought to be a risk factor for stricture development as well. Contradictorily, non-anatomic strictures are assumed to occur as a result of ischemia and immunological factors (95). Ischemia and immunological factors stimulate the inflammation and fibrosis in the anastomosis. Also hypertrophic changes are seen with inflammatory conditions like cholangitis, minor bile leakages, or stent placements (108). Enhancement of angiogenesis and immunomodulation capacity of stem cells can be the target for prevention of biliary strictures. Due to the anti-inflammatory effects and immunomodulatory capacity of ADSCs, ADSC sheets around bile anastomosis suppressed the hypertrophic changes (101). Hepatocyte growth factor and VEGF which are secreted from ADSCs are important trophic factors in wound healing (109). Anti-fibrogenic effects of HGF which were related to fibroblast growth factor-2 have been shown (110). As the ADSCs secrete HGF and VEFG, ADSCs can play anti-

fibrogenic, anti-inflammatory, angiogenetic factor in biliary anastomosis and prevent biliary strictures. In high risk biliary anastomosis, MSCs can be used in order to prevent complications.

Biliary strictures after liver transplantations are tried to be managed by Endoscopic retrograde cholangiopancreatography (ERCP) or percutaneous techniques. During these time-consuming treatments, several cholangitis attacks might be seen. Bile drainage problems with infections damage the hepatocytes and bile duct cells. In continuous cholestatic situation, the liver injury might be irreversible and lead to chronic cholestatic liver failure.

The primary mechanism during biliary stricture is the failure of bile salts excretion in cholestasis causes hydrophobic bile salts to be retained inside hepatocytes, generating apoptosis and necrosis (111). Inflammatory damage and oxidative stress are caused by an abnormal flux of bile acids and bilirubin in the liver, which results in the retention and buildup of toxic hydrophobic bile salts within hepatocytes. The process of fibrogenesis has been associated with oxidative stress. Cholestatic liver fibrosis is related to bile acid-induced oxidative stress and lipid peroxidation and is distinguished by an excessive buildup of extracellular matrix (ECM) proteins. By activating stellate cells, oxidative stress triggers liver fibrosis (112). Extracellular matrix alters and fibrosis increases as a result of stellate cell activation. During liver injury, it was noted that bone marrow recruited mesenchymal cells in the liver, which was thought to be initiating fibroblasts; however, research revealed that these cells had a slight influence on collagen production (113). For this reason, suppression of chronic inflammation, resolving fibrosis by inhibition of extracellular matrix production and deposition, enhancing hepatocyte proliferation are the target treatments in cholestatic diseases

In bile ligation models, systemic application of stem cells recovered the liver function, arhitectural changes and resolved fibrous damage, increased liver regeneration via increasing HGF and subsequent upregulation of MMP-2 mRNA and downregulation of CK-19 mRNA (114). While MMP-2 expression is a sign of ECM degradation, CK-19 is a predictor of stellate cell activation. ECM remodeling is the way of action of MMPs in hepatic regeneration. Anti-fibrogenic effects of MSC are observed in bile duct ligation. Also, the anti-oxidative effects of MSC in bile ligated models were shown in the same study by an increased level of glutathione (114). Drastic decrease in intracelular fatty acid transport were partially reversed by MSCs administration in bile duct models (115). Also CPT1A which catalyzes β-oxidation of free fatty acids in the mitochondrial outer membrane has been shown to be increased with administration of MSC in bile duct ligation model (115). By these findings, it was shown that MSCs had beneficial effect on cellular energy metabolism in bile duct ligation models.

Beside the experimental studies, beneficial effects of MSCs were experienced in humans with ischemic type biliary lesions after liver transplantation (116). In this study, 12 patients who were suffering from ischemic type biliary lesion underwent infusion of 1.0×10^6 MSCs/kg of MSCs in six doses on the 1, 2, 4, 8, 12 and 24 weeks after transplantation. The patients treated with MSC need fewer interventional therapies (33.3%) when compared control group (64.3%).

Biliary strictures after liver transplantation might result in severe cholestatic diseases in the long term period. During the treatment of biliary strictures, the liver injury must be prevented against progressing to liver failure. MSCs can be an option during this period.

Rejections

Although liver is regarded as immune-tolerant, rejection is a serious problem in patients after liver transplantation. Although immunosuppression therapies against immune cells protects the grafts and increase the survival, secondary infections and side effects of the immunosuppressive drugs are the unfavorable results. The MSCs express low levels of class II major histocompatibility complex and costimulatory molecules and also have immunoregulatory and immunosuppressive effects in various settings. These properties favor the usage of MSC after liver transplantation in order to develop tolerance or decrease the amount of immunosuppressive drugs.

MSCs in rejections

Several *in vitro* studies have shown the relationship between MSCs with immune cells via different mechanisms (*Table 4*). Although some conflict results were obtained, tolerogenic microenvironment is achieved by direct interactions of MSCs and the immune system. MSCs induce immunosuppression without rejection in several animal models. Among these models, ADSCs were used in rat and swine models and reduced rejection rates and enhanced liver function were obtained (20,68,138).

All sources of MSC, including bone marrow, adipose

Table 4 MSCs interactions with immune cells

Immune cells	Action	Comment	Reference
B-cells	Inhibit B-cell proliferation when MSCs are cultured under LPS, CpG, CD40L, IL-2, IL-4, IL-10	Effect of MSC on B-cells depend on dose, and culture of MSCs	(117-122)
	Inhibit B-cell differentiation and maturation by stimulating by anti-CD40 and anti-IL-4		
	Arrest B-cells in the G0/G1 phase		
	Suppress chemokine dependent migration by downregulating the CXXR4, CXCR5, and CCR7 receptors		
	Decreased Ig secretion by blocking Blimp-1 expression		
	Some authors showed stimulation of MSC on B-cell proliferation.		
	Low dose MSCs stimulate Ig production		
NK-cells	Inhibit NK cells activation and inhibit secretion of INF-δ and TNF-α from NK cells by mediation of LFA and ICAM1	Dose dependent	(123-126)
	Inhibit NK cell proliferation by PGE2, TGF-β, IDO	MSCs can only suppress non-activated NKs	
	Coculturing of MSC, NK cells and stimulator leads deactivation of NK cells	Coculturing is needed	
	Downregulate NK cells in very high concentrations		
DCs	Maintain DCs in immature stage, support tolerance when cocultured by upregulating PD-L1	Coculturing is needed	(127-131)
	Prevent maturation by direct interaction or by PGE2 and IL-6		
	Suppress inflammatory cytokine		
	Induce tolerogenic DCs by manipulating the molecular phenotype od DCs		
	DCs loose ability to activate T cells by promoting Th2 response, encouraging DCs to promote Treg generation or reducing activation capacity of $CD8^+$ T cells by activating Notch pathway		
T-cells	Suppress T cell activation by increasing Treg and Th2	Dose dependent	(16,132-135)
	Suppression of T cells by PD-1 pathway and secretion of TGF, HGF, IDO, PGE2, Insulin-like GF, HO, HLA-G5, C-C motif, CCL, IL-10 and galectin	Activated T cells needed for MSCs action	
	Promote T cell apoptosis	Mechanism differ between species	
Kupffer cells	Reprogram the phenotype of KCs through TNF-α and PGE2	Kupffer cell may destroy MSCs unless glycine treatment	(136,137)
	Modulate Kupffer cells activity to inhibit TNF-α	High success in PGE2 overexpression	

MSCs, mesenchymal stem cells; IL, interleukin; TNF-α, tumor necrosis factor-α; ICAM1, intercellular adhesion molecule 1; LPS, lipopolysaccharide; INF, interferon; LFA, lymphocyte function-associated antigen; KCs, Kupffer cells; GF, growth factor; CCL, chemokine (C-C motif) ligand; NK, natural killer; DCs, dendritic cells; PD-L1, programmed death-ligand 1; PD-1, programmed death 1; HLA, human leukocyte antigen; TGF, transforming growth factor; HGF, hepatocyte growth factor; PGE2, prostaglandin E2; HO, heme oxygenase; IDO, indoleamine 2,3-dioxygenase.

tissue, share similar therapeutic potentials (16). Pretransplant infusion of MSCs might lead to migration of MSC to the lungs and lost of function. On the other hand, posttransplantation infusion may lead to migration of MSCs to the liver but also may lead to graft dysfunction (139). The other possible problem is concurrent immunsuppression

treatment after liver transplantation. Calcineurin inhibitors are mostly used immunosuppression. In short term (<7 days) exposure to MSC, usage of the tacrolimus, mycophenolate or rapamycin does not result in unwanted results (140). But longer usage might result in MSC toxicity with tacrolimus and decreased MSC proliferation with mycophenolate and rapamycin. For this reason, it is important to evaluate the dosage of these drugs in MSC treatment.

Immunosuppressive period after liver transplantation not only suppresses the immune system of the recipient but also may influence the functions of MSCs . In order to overcome the limitations of MSCS therapy and to increase the success of immunosuppressive effects of MSC, drug combinations or coculturing methods have been used (141,142). The effects of tacrolimus on MSC were reversed by combined use of oxytocin (142). It was shown that ability of MSC to suppress natural killer cell activation seems to be enhanced with dexamethasone (143).

Interaction of MSCs with inflammatory cells

Different inflammatory conditions might affect the phenotype, gene expression and function of ADSCs (141). These changes are mediated by cell-cell or cell-membrane contact between MSCs and immune cells. In the absence of inflammation, lymphocytes survive longer by the effect of MSC. However in the existence of inflammatory conditions, interferon (IFN)-γ, TNF-α and IL-6, are much more secreted. And MSC have close relationship with these cytokines and show their effect by changing their immunomodulatory function (144-146). Up-regulation of the expression of indoleamine 2,3-dioxygenase (IDO), TGF-β and HGF were seen with the interaction of MSC and IFN-γ. And MSCs which are activated by IFN-γ show beneficial effect in the treatment of graft-versus-host disease (146). Coculture methods of MSCs with several factors have been shown to enhance immunosuppressive features of MSCs (147). Hepatocyte growth factor, IL-7, IL-10, and Foxp3 gene integration improved therapeutic performance of MSCs (148). Similarly, IL-35 gene integrated MSCs can suppress the activity of $CD4^+$ T cells *in vitro* (149).

MSCs in clinical studies

Although several experimental studies about ADSCs have been performed, insufficient clinical trials have been reported. Main reasons for these limitations can be listed as ethical problems, immune environment issues, source and pretreatment of MSCs, timing and dosage of MSCs infusion, safety concerns.

There are limited number of clinical studies about stem cells (*Table 5*) (16). With regard to recent studies, it was seen that (I) MSCs infusion does not have adverse effect; (II) early application of MSC after liver transplantation is advised because of long-term protolerogenic effects; (III) drug combinations with immunosuppressive drugs should be continued. The interactions of MSCs and immunosuppressive drugs should be studied. It was shown in a prospective kidney transplantation study that usage of MSCs might balance the dose of tacrolimus without accelerated rejection risk (155). Also MSCs combination with mycophenolic acid suppress the lymphocyte proliferstion more than CNIs, m-TOR inhibitors (156). Although several *in vitro* or clinical studies exist in kidney transplantation, further investigation is needed for combination regimens with MSCs. (IV) Despite not presented in liver studies, increased opportunistic infections in renal transplant cases with MSCs treatment have been presented (157). There is no sufficient study about the usage of MSCs in patients diagnosed with liver cancer in liver transplantation. But exosomes are involved in the communication between hepatocellular carcinoma (HCC) cells. MSCs can be transfected with exosome miRNA to inhibit HCC (158). ADSCs-transfected with miR-122 expression plasmid showed inhibition of hepatocellular cancer and enhancement of chemotherapy sensitivity (159). miR-122-transfected ADSC might be a new treatment choice for the patient with advanced HCC or recurrence HCC after liver transplantation.

MSCs in chronic rejection

In contrast acute rejection, pathogenesis of chronic rejection is related to vascular complications, antibodies, and cell-mediated pathways. In chronic rejection obliterative arteriopathy, interstitial inflammation, atrophy of parenchymal cells and interstitial fibrosis were seen. Due to liver injury, several pro-inflammatory cytokines like TGF-β, IL-4, IL-13, are secreted (160). These cytokines trigger the hepatic inflammation. Activated HSCs differentiate into myofibroblasts. Myofibroblasts induce scar formation by ECM and matrix proteins formation.

TGF-β1 which is secreted from macrophages stimulate the fibrogenic myofibroblasts. Activated myofibroblasts trigger the liver fibrosis. After co-culturing MSCs with

Table 5 MSCs in clinical liver transplantation studies

Study title	Transplant protocol	Sample size	Result	Follow up time
Infusion of Allogeneic Mesenchymal Stromal Cells After Liver Transplantation: A 5-Year Follow-Up (150)	(1.5 to 3)×10^6/kg third party unrelated MSCs given on the postoperative 3rd day	10	No adverse effect No difference between graft survival, patient survival, bile complication rate, and rejection rate High anti-HLA antibodies against MSC	85 months
First-in-Human Case Study: Multipotent Adult Progenitor Cells for Immunomodulation After Liver Transplantation (MiSOT) (47)	1.5×10^8 multipotent adult progenitor cells were given on day 0 and day 2	1	No adverse effect Increased frequency of Treg and reduced expression of HLA-DR on $CD4^+$ monocytes	219 days
A Pilot Study of Mesenchymal Stem Cell Therapy for Acute Liver Allograft Rejection (151)	1×10^6/kg UC-MSCs. Given after recurrent nonresponding rejections	14	No adverse effect Alleviate liver damage Downregulate $CD4^+$ T-cell activation Elevated levels of TGF-β and PGE2	12 weeks
Third-party bone marrow-derived mesenchymal stromal cell infusion before liver transplantation: A randomized controlled trial (152)	(1–2)×10^6 cells/kg third-party BMD MSC before liver transplantation	10	No adverse effect Increase in the percentage of Treg in the first 2-week No effect on serum tacrolimus levels	12 months
Safety and Tolerance of Donor-Derived MSCs in Pediatric Living-Donor Liver Transplantation: The MYSTEP1 Study (153)	Donor-derived MSCs ~1×10^6 cells/kg infusion on day 0 and on day 1–3	Still going on	Not published	24 months
Infusion of mesenchymal stromal cells after deceased liver transplantation: A phase I-II, open-label, clinical study (154)	(1.5–3)×10^6 cells/kg on day 3±2	10	No adverse effect No significant change in Treg counts, phenotypes No difference between control Immunosuppressive withdrawal was not achieved	12 months

MSCs, mesenchymal stem cells; TGF, transforming growth factor; PGE2, prostaglandin E2; HLA, human leukocyte antigen; DR, donor and recipient; UC, umbilical cord; BMD, bone marrow-derived.

colony-stimulating factor-1-induced macrophages, macrophages turn into anti-inflammatory M2 phenotype. Also, IL-4 and IL-10 which are synthesized by MSC stimulate the M2-type macrophages. Activation of M2-type macrophages leads alleviating liver fibrosis in rats (161). It was shown in the study of CCL4-treated mice liver that Treg expansion is promoted and suppression of Th17 cell is achieved by MSC treatment via the production of IDO. The end result is the attenuation of liver fibrosis. Delta-like 1 (Dlk1) protein is responsible for liver fibrogenesis by activation of HSC. MSCs prevent the liver from fibrosis by suppression of Dlk1 (162).

Chronic rejection is presented with cholestatic graft failure and graft loss. Although escalation of immunosuppressions can be tried, most of them underwent retransplantation. Main target in chronic rejection is to enhance the regeneration capacity of liver and provide an additional site of action in immune cascade (163). There is no presented study about the treatment of MSCs in chronic liver rejection. As mentioned before, MSCs can be therapeutic agents for the treatment of liver fibrosis because they can turn into hepatocytes and synthesize several trophic factors which function as immune-modulation.

MSCs in graft versus host disease

Graft versus host disease (GVHD) is a clinical situation in which recipient tissues are damaged because of the immune cells from the donor. Existence of high level of human leukocyte antigen (HLA) compatibility in both donor and recipient is the major risk factor for GVHD in allogeneic HSCT recipients. Infusion of third-party, HLA-unrelated, or related bone marrow donor MSCs can be a new treatment option for GVHD. MSCs show their effects in GVHD treatment by both suppressing the proliferation and the cytotoxic activity of immune cells. It's shown in several studies that infusion of MSCs in the treatment of GVHD is a safe and effective treatment (164-166). Interaction of MSCs with NK cells, monocytes, and regulatory T cells lead suppression of the immune response by inhibition of monocyte-derived dendritic cell (DC) differentiation and activation (132). Also changes in antigen-presenting cell maturation after MSCs interaction lead alteration of cytokines secreted from T cells, DCs, and NK cells. Anti-inflammatory Th2 cytokines are seen much more than the Th1 cytokines. These mechanisms explain the success of MSCs in prevention of graft rejection and treatment of GVHD. The success of MSC in GVHD depend on (I) engraftment capacity of MSCs to the inflammed site; (II) secretion of several molecules which show anti-inflammatory actions; (III) immunomodulatory effects on immune system cells (167). Survival benefits of MSC infusion is mainly depend on treating, not preventing GVHD (168).

De novo hepatitis

It is believed that loss of tolerance to autoantigens of hepatocytes is the main reason for autoimmune hepatitis. Increased number of Th17 cells and Treg cell related pathologies are blamed for the loss of this tolerance. The recurrence rate after liver transplantation is 1–3% in adults. Proposed mechanism beyond this loss of tolerance is the increased number of Th17 cells and defects in Treg cells. Mutations in CTLA4 which has important role in lymphocyte activation, may contribute to autoimmune-hepatitis (169). MSCs suppress lymphocytes by expressing and secreting CTLA4 and PD-L1 on their surfaces (170). Other factors that MSCs exert their immunosuppressive effects on lymphocytes are IDO, Inducible nitric oxide synthase (iNOS) and FasL (171). Peripheral self-tolerance is under the control of Treg cells. Treg cell differentiation is achieved by direct effect of MSC or via anti-inflammatory macrophages which are formed by MSCs stimulation (172). This process is mediated by secretion of TGF-β and prostaglandin E2 (PGE2), factors produced by MSCs. Moreover, PGE2 which is secreted by the influence of MSCs has important role in suppression of Th17 cells. This result in the tolerogenic influence of Th17 cells (173). In experimental models of autoimmune hepatitis, it was shown that serum ALT and AST levels decreased in BMSC-treated groups when compared to the model group. BMSC-treated group showed decreased lymphocyte infiltration and less intra-lobular inflammatory lesions and necrosis (174). Beside the experimental studies, there are two presented case reports in which autoimmune hepatitis was regressed by the administration of stem cells (175,176). Although there are several experimental researches supporting benefits of MSC treatment in AIH, more studies are needed for clinical usage.

Primary biliary cholangiopathy (PBC) is an autoimmune disease in which non-suppurative inflammation in the small interlobular bile ducts are seen. It is now well accepted that PBC can recur in the post-transplant setting in 10% to 40% of patients (177). In the PBC mouse model by Wang *et al.*, intravenous BM-MSCs (1×10^6 cells) treated rats showed improved liver histology, decreased AMA titers, and increased Tregs (178). Similar results were obtained in clinical studies also. In the study of Wang *et al.*, 7 patients with PBS received 0.5×10^6 cells/kg body weights three times at 4-week intervals (49). After 48 weeks follow up significant clinical improvement is achieved. In an another study, 10 patients who have UDCS resistant PBC received $(3–5)\times10^5$ cells/kg body weight MSCs intravenously (179). It is seen in the peripheral blood mononuclear cells of patients that Treg is increased even 6 months. And the quality of life of patients are shown to be increased. In order to show the beneficial effects of MSC treatment in PBC, randomized designed studies are required.

Primary sclerosing cholangitis (PSC) is a progressive cholestatic liver disease. The recurrence rate of PSC after LDLT nearly 50% after 10 years (180). Immune dysregulation is the main pathology in PSC. It was seen in PSC mouse models that the $CD8^+$ T cells and NK cells showed much more cytotoxicity in the existence of increased levels of IFN-γ. Similarly decreased amount of IFN-γ might suppress inflammatory macrophages, decreased hepatocyte death and as a result attenuate liver fibrosis (181). It was seen in PSC cases that, increased number of M1-type macrophages were enrolled in peribiliary region by cholangiocytes via the CCR2/CCL2 axis. Biliary injury and

fibrosis can be prevented by exhaustion of CCR2 (182). In the PSC animal model, biliary hyperplasia was ameliorated by injection of human amnion-derived MSCs. Also MSCs injection decreased the CK19 expression and led less necrotic lesions (183). Also, peribiliary fibrosis markers such as α-SMA, TGF-β, type I collagen, MMP-2, MMP-9, and TIMP-1 were found to be decreased after ADSCs therapy (183). As PSC is presented by immune system activation, immunoregulatory characters of MSCs might be beneficial in PSC.

Conclusions

Anti-inflammatory, immunomodulatory and regenerative capacity of stem cells have been found both in experimental and clinical transplantation studies with beneficial results. The field of study about stem cells will increase with the development of genetic engineering and bio-scaffold technology.

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Footnote

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