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Concise Review

Recent Advances in Mesenchymal Stem/Stromal Cell-Based Therapy for Alcohol-Associated Liver Disease and Non-alcoholic Fatty Liver Disease

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Abstract

Alcohol-associated liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD) represent pathological conditions that include many distinct stages, potentially leading to the final stage of cirrhotic liver. To date, liver transplantation is the sole successful treatment with concomitant limitations related to donor organ shortage and the need of life-long immunosuppressive therapy. Recently, cell-based therapies for ALD and NAFLD have been proposed with mesenchymal stem/stromal cells (MSCs) as promising effectors. MSC therapeutic applications offer hepatoprotection, regulation of the inflammatory process and angiogenesis particularly in ALD and NAFLD pre-clinical disease models. Recent studies suggested that hepatospecific MSC-based therapies could benefit liver diseases by restoring liver function and decreasing inflammation and fibrosis. Similarly to solid-organ transplantation, limitations in MSC approaches include donor availability exacerbated by high number of cells and cell trapping into lungs. Herein, based on recent advances, we discuss the use of MSCs as a therapeutic approach for ALD and NAFLD and we provide the available information for the establishment of a framework toward a potential clinical application.

Key words: mesenchymal stem/stromal cells; alcohol-associated liver disease; alcoholic steatohepatitis; non-alcoholic fatty liver disease; non-alcoholic steatohepatitis; fatty liver; cell therapy

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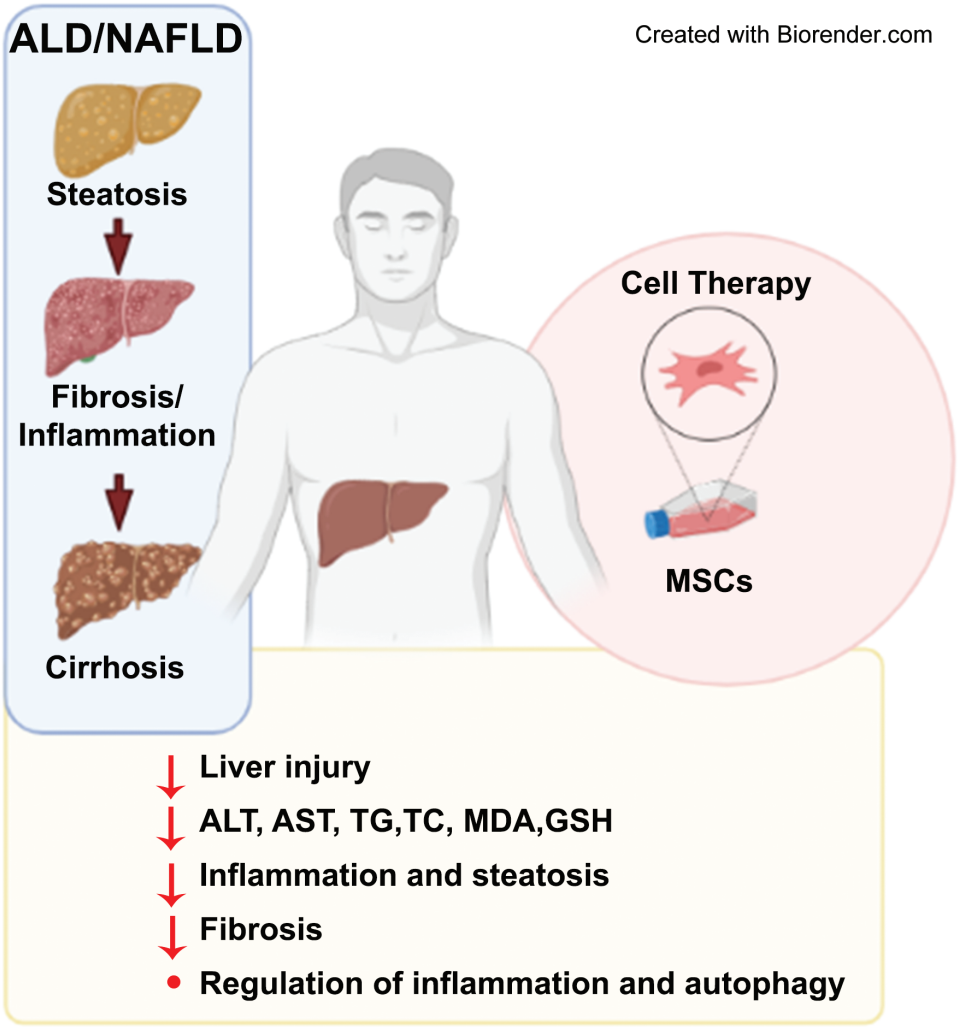
Graphical Abstract



Significance Statement

Mesenchymal stem/stromal cell (MSC) therapy aspires to provide proof of concept for an innovative, non-invasive, less immunogenic, and less-toxic alternative strategy for the management of alcohol-associated liver disease (ALD) and non-alcoholic-fatty liver disease (NAFLD). MSC-based therapy offers hepatoprotection, regulation of the inflammatory process and angiogenesis in ALD and NAFLD preclinical disease models. However, further studies are needed to highlight their important mechanistic effect on the reparative function of progenitor cells in the liver.

Introduction

Liver represents one of the most important organs in humans, consisting of hepatocytes, biliary epithelial cells (cholangiocytes), stellate cells, Kupffer cells, and liver sinusoidal endothelial cells.[1] Each cell type fulfills a specific role that keeps the organ functional. Therefore, liver participates in the maintenance of various functions, such as metabolism, immunity and detoxification and exhibits a regenerative potential by activation of endogenous hepatic progenitors and/or adult hepatocytes.[1-4]

Pathological conditions that may result from different factors, such as alcohol abuse or viral infection, could alternate and inhibit liver's regeneration. In the case of extensive damage, referred as liver cirrhosis, there is increased accumulation of extracellular matrix (ECM) components and extended fibrosis.[5]

Consequently, liver transplantation (LT) represents the gold-standard therapy, with concomitant limitations related to donor shortage and the need of life-long immunosuppressive therapy.[6,7] However, compared to other medical modalities, liver transplantation is recommended, with survival rate of 80%-85%.[3,8]

Therefore, research focuses on alternative therapeutic applications including cell transplantation, mainly by the use of stem or progenitor cells.[9-12] To this end, Mesenchymal stem/stromal cells (MSCs) and liver stem cells (LCs) represent cell types with great proliferation and differentiation potential. Induced pluripotent stem cells (iPSCs) and embryonic

stem cells (ESCs) can also be used in similar cell therapeutic modalities, however, with limitation related to the risk of tumourogenesis.[13,14]

MSCs consist an alternative progenitor cell type, which by differentiation or secretion of trophic factors can alleviate cirrhosis and acute liver disease symptoms.[6,15] MSCs are routinely isolated from bone marrow (BM-MSCs), adipose tissue, and dental pulp. While they can be sourced from fetal sources such as umbilical cord (UC-MSCs), amniotic fluid (AF-MSCs), and placenta (p-MSCs).[14,16] MSCs are less immunogenic and can differentiate into adipocytes, chondrocytes, or osteocytes and depending of their source of origin, into hepatocyte like cells or myocytes. They also secrete trophic bioactive factors and extracellular vesicles (EVs), including microvesicles and exosomes that prevent the apoptosis of parenchymal cells and lead to a therapeutic effect in many diseases, such as myocardial infarction, acute kidney injury, and acute liver failure.[16-18]

The present review focuses on the use of MSC-based therapy for liver diseases, recording the available preclinical and clinical studies. A summary of the pathological signatures related to alcohol-associated liver disease (ALD) and the non-alcoholic fatty liver disease (NAFLD) as well as the available cell therapeutic modalities based on the use of MSCs are also given.

Mesenchymal Stem/Stromal Cells for Liver Therapy

MSCs are well characterized, have a high proliferation and differentiation potential, and exhibit tropism to damaged tissue as well as immunosuppressive properties, which make them an attractive cell type for allogeneic or autologous transplantation.[11,15] More importantly, MSCs through the secretion of trophic factors and EVs can induce anti-apoptotic and pro-proliferative features and activate the proliferation of the host hepatocytes.[6] The increased number of hepatocytes, and the activation of the endogenous progenitor cell population and immune cells, could facilitate more efficiently liver regeneration.[17,19]

Furthermore, the dysregulation of lipid metabolism, originated from a high fat diet, leads to excess lipid load, inflammation and tissue deterioration. MSC treatment mediates these symptoms, by increasing protein levels involved in triglyceride synthesis, and by decreasing lipid levels.[20]

Fibrosis is improved after MSC administration, mainly due to their paracrine effects.[20,21] It could also be indirectly decreased, by matrix metalloproteinases (MMPs), highly expressed by MSCs that degrade and re-model the fibrotic matrix.[6,22] Moreover, after MSC treatment, the polarity of macrophages can adopt an anti-inflammatory phenotype.[19] Also, the ability of MSCs to reduce T-cell activation and induce regulatory T cells and anti-inflammatory macrophages could inhibit HSCs and decrease fibrogenesis.[17,20]

An example of MSCs effect on fibrosis was described in a CCl_4-intoxicated mouse model. Mice were injected with CCl_4 to induce cirrhosis over a 12-week period, twice weekly. At 8 weeks, MSCs or induced bone marrow-derived macrophages (id-BMMs), or a combination of both was administered to the animal models. Coculture of BM-MSCs with id-BMMs can synergistically improve liver fibrosis in mice (Fig. 1, Supplementary Fig. S1). Thus, MSCs trophic factors induce id-BMMs to adopt an M2 phenotype by increasing the levels of markers such as the one found in inflammatory zone (FIZZ-1) or matrix metallopeptidase 13 (MMP-13). M2 phenotype suppresses inflammation and fibrosis and boosts phagocytosis. Secondly, a higher number of id-BMMs, when administered with MSCs, migrated to the fibrotic area of liver, which indicates that id-BMMs affect directly the phenotype of liver fibrosis and induce liver regeneration, whereas MSCs may play an indirect role affecting macrophage's action. This multimodality therapy enhances host macrophages and neutrophils migration to the fibrotic area.[21]

Recently, administration of exosomes derived from MSCs from different sources (BM, AD, UC) in acute liver injury animal models lead to reduction of liver inflammation, oxidative stress and necrosis. They also promote liver regeneration and they present antiapoptotic properties and higher expression of autophagy genes. Finally, exosome treatment results in a reduction in the levels of transaminases.[14]

More recently, engineered MSCs represent a promising tool for the treatment of liver diseases. Research focuses on the modification of some key-proteins that participate in many important cellular procedures such as cell survival, apoptosis, inflammation, regeneration. Interleukin-1β (IL-1β), B-cell lymphoma 2 (Bcl-2), hepatocyte growth factor (HGF), or protein kinase B (PKB or AKT) represents examples of genes that are mainly modified either with the use of viral vectors or the technology of CRISPR-Cas9.[23]

Pathological and Molecular Signatures Related to ALD and NAFLD

ALD and the NAFLD represent the most common pathological conditions for the liver. According to World Health Organization (WHO), alcohol consumption is the seventh risk factor for early death with more than 3 million deaths worldwide per year. One in 4 people is an ongoing alcohol drinker, with the 27% at an early age. Particularly, the majority represents Europeans between the ages of 15 and 19.[24,25]

ALD includes a wide spectrum of diseases that vary from asymptomatic early ALD (fatty liver or steatosis) to severe forms like steatohepatitis, advanced ALD (alcoholic hepatitis-AH, alcoholic cirrhosis-AC) and at a final stage to hepatocellular carcinoma (HCC).[26] At the same time, NAFLD is a multisystem malignancy affecting the liver and can progress from simple steatosis characterized by lipid accumulation, to a more severe liver disease called non-alcoholic steatohepatitis (NASH), with concomitant features like, hepatocyte damage, leukocyte infiltration, and fibrosis.[27-29] Eventually, cirrhosis could be developed as a result of co-existent scarring with a rearrangement of organ circulation and failure of liver function, with the possibility of leading to HCC.[18,20,30]

Alcohol-Associated Liver Disease

ALD is mainly caused by alcohol over-consumption and can develop a spectrum of pathological changes in the liver, including steatosis, inflammation, and cirrhosis.

ALD patients are mostly asymptomatic; thus, the diagnosis can be accomplished by the high levels of hepatic enzymes such as gamma-glutamyltransferase (GGT) and aspartate aminotransferase (AST). Also, sonography or computed tomography or magnetic resonance imaging (MRI) could also be used for diagnosis, with MRI to be the most precise methodology to estimate the fat accumulation in liver.[31] Downregulation of peroxisome proliferator-activated receptor

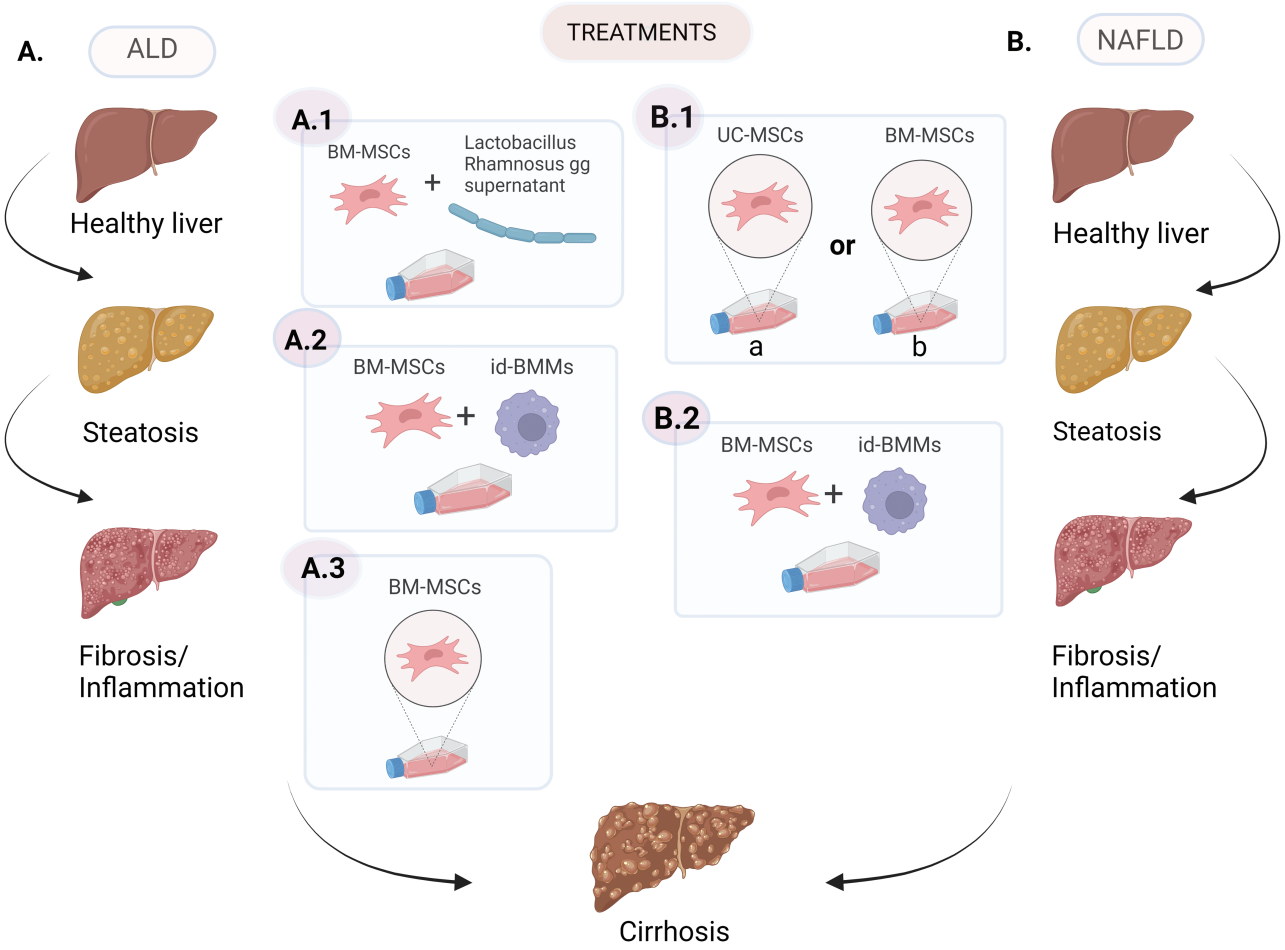


Figure 1. Treatment of alcohol-associated liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD) with use of MSCs. (**A**) ALD progression and potential MSC-based treatments. (**A.1**) Administration of both BM-MSCs and Lactobacillus Rhamnosus gg supernatant or (**A.2**) administration of BM-MSCs and id-BMMs can improve the fibrotic phenotype in ALD or (**A.3**) Administration of BM-MSCs. (**B**) NAFLD progression and potential MSC-based treatments. (**B.1**) Administration of BM-MSCs or UC-MSCs and (**B.2**) administration of BM-MSCs and id-BMMs can improve the fibrotic phenotype in NAFLD (Created with Biorender.com).

alpha (PPAR-α), 5′ adenosine monophosphate-activated protein kinase (AMPK) and upregulation of acetyl-CoA carboxylase (ACC) result in elevated levels of fat production, indicating a possible mechanism of the disease.[26]

Alcoholic Hepatitis

Histological observations in asymptomatic patients or with mild clinical symptoms AH patients are related to increased numbers of neutrophils and enhanced steatosis and fibrosis.[31] Currently, the only therapy for AH is a combination of alcohol detoxification, balanced diet, and the treatment with food supplements and corticosteroid.[31] Corticosteroid therapy, however, can cause side effects related to the increased risk of sepsis and gastrointestinal hemorrhage.[8]

Alcoholic Cirrhosis

Patients with AC are diagnosed after having lost part of liver function, mainly detected by biochemical tests and the presence of other abnormal clinical symptoms, in parallel with an alcohol consumption history. The progression of the disease is strongly dependent on patient's willingness to refrain from drinking; alternatively, AC will mainly lapse into AH.[31]

Etiology of Alcohol-Associated Liver Disease

In 2012, in the US, the mortality rate of ALD was estimated to 5.5 per 100 000 people. During 2008-2016, the number of young patients, between the age of 25-34, who suffered from AC was significantly increased.[31] The cause of ALD has been associated with alcohol abuse. Specifically, ethanol stimulates the overexpression of Cytochrome P450 Family 2 Subfamily E Member 1 (CYP2E1) and the downregulation of aldehyde dehydrogenase (ALDH), which leads to acetaldehyde aggregation. Due to accumulation of acetaldehyde and high levels of ethanol, molecules such as adiponectin, signal transducer and activator of transcription 3 (STAT3), AMPK and PPARα are negatively regulated.[25,26] This leads to phospholipid peroxidation and lipid-free radical production. Furthermore, this causes upregulation of the early growth response protein 1 (Egr-1) and the adiponectin and acetyl-CoA carboxylase (ACC). Secondly, oxidative stress and the production of reactive oxygen species (ROS) formed from ethanol hepatotoxicity may also result in ALD. ROS can bind to both proteins, altering their structure and function and to DNA, creating genetic mutations. In addition, alcohol exposure is correlated with high levels of endotoxins in intestines and liver in the form of lipopolysaccharides (LPS). LPS can interact with

Table 1. Cell therapy for alcohol-associated liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD).

Disease	Stage of disease	In vitro	In vivo	Clinical trials	Cell source	Dosage per mouse	Administration route	Outcome
ALD	ASH/fibrotic liver	–	Mouse model	–	BM-MSCs + LGGs	LGGs 10^9 CFU	Intravenous	1. Alleviation of liver damage 2. Regulation of inflammation and autophagy through the NF-kB and mTOR signaling pathways. 3. Reduced both serum ALT and AST levels
	AH	–	Mouse model	–	BM-MSCs	5×10^6	Intraperitoneal	1. Decrease of liver injury 2. Decrease of ALT, AST, TG,TC, MDA,GSH 3.Decrease of inflammation and steatosis
	AC	–	–	+	BM-MSCs	5×10^7	Intravenous	1. Decrease of ALT, AST, GGT,ALP 2. Alleviation of liver fibrosis
NAFLD	NASH/fibrotic liver		Mouse model	–	UC-MSCs	1×10^6	Intravenous	1. Amelioration of lipid storage 2. Decrease of TC, TG, LDL-C 3. Restore glucose homeostasis
	NASH/fibrotic liver	–	Mouse model	–	UC-MSCs	1×10^6	Intravenous	1. Change of microbiome and metabolome disorders in NASH model 2. Alleviation of hepatic steatosis and inflammation
	NASH/fibrotic liver	–	Mouse model	–	BM-MSCs	0.9-1×10^6	Intrasplenic	1. Decrease of hepatic lipid storage/increased expression of lipid utilization genes 2. Amelioration of inflammation/liver homeostasis 3. Mitochondria transfer from injected MSCs to hepatocytes

toll-like receptors (TLRs) and promote the production of a number of pro-inflammatory cytokines, such as interleukin 6 (IL-6), tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1) resulting in extensive liver inflammation. Induction of proliferation of hepatic stellate cells (HSCs) that augment the deposition of collagen in the ECM can be proved beneficial. In addition, patatin-like phospholipase domain containing-3 (PNPLA3), that is expressed mainly in liver adipocytes and regulates the metabolism of lipids, is primarily responsible for the progression of the disease. A mutation in the PNPLA3 gene (C.444 C > G p.Ile148Met) results in fat aggregation and inflammation in the liver. Genes such as TM6SF2, MBOAT7, and HSD17B13 can also be involved in the progression of ALD. Environmental factors, such as obesity, can also influence ALD phenotype mainly due to a change in ethanol lipid solubility and in the secretion of pro-inflammatory cytokines, resulting in alcoholic steatosis (AS).[25,26]

Diagnosis of Alcohol-Associated Liver Disease

For ALD diagnosis, liver biopsy is conducted to determine the disease stage progression and speculate the prognosis. Under this condition, there is a high risk of bleeding, infection and a possible damage to other organs nearby while at the same time patients suffer from mild to severe pain. There is also 0.01% possibility of patient's death.[32] Therefore, non-invasive methods could be applied, such as measurements of the levels of liver enzymes [aspartate aminotransferase (AST), alanine aminotransferase (ALT), GGT or carbohydrate-deficient transferrin (CDT)].[33] Some metabolites of ethanol, such as ethyl sulfate (EtS) in urine, fatty acid ethyl esters (FAEEs) in hair, and phosphatidylethanol (Peth) in serum, may be also assessed for diagnosis. Transient elastography (TE) represents another non-invasive method by which the level of cirrhosis could be estimated. Ultrasonography has been also applied for the detection of AS.[26] Moreover, clinical tests can determine timely and accurately whether AH is related to histological changes.[31] However, the use of noninvasive tests, such as the measurement of caspase-cleaved keratin 18 (K18) epitopes M30 and M65 serum levels, can be assessed in parallel with the clinical symptoms.[8]

Cell Therapy

MSC-based therapy and ALD

MSCs represent a potential cellular treatment for ALD patients through differentiation, modulation of the immune response, inhibition of liver fibrosis and tissue regeneration. MSC differentiation into hepatic cells can reverse the loss of hepatocytes due to alcohol consumption, induce the expression of hepatocyte markers [cytokeratin 18 (CK18), cytokeratin 19 (CK19), cytochrome P450 3A4 (CYP3A4)], and increase storage of glycogen and secretion of albumin. The MSC-based treatment also results in activation of dendritic cells, lymphocytes, and Tregs.[3] Finally, MSCs produce trophic factors, such as VEGF, EGF, and IGF-1, that could mediate tissue repair[7]

MSC-based therapy and ASH

A monotherapy with BM-MSCs has not been proved as efficient as a combined therapy with BM-MSCs and

Lactobacillus rhamnosus GG culture supernatant (LGGs; Table 1, Fig. 1, Supplementary Fig. S1).[34] ASH exhibits both signs of inflammation and autophagy with activation of PI3K/NF-kB or PI3K/mtOR signaling pathways. Furthermore, unbalance in gut microbiome leads to increased fat accretion in the liver. Therefore, chronic-binge alcoholic male mouse model C57BL/6 were treated with LGGs (10^9 CFU/mouse) added in their ethanol diet, resulting in an intestinal barrier reinforcement and reestablishment of a balanced immune response. Consequently, a combined therapy of BM-MSCs and LGGs for 7 weeks via tail vein was proposed. Liver damage was decreased with the underlying mechanism not yet understood. Specifically, it was observed a reduction in both ALT and AST serum levels. However, this multimodality therapy repressed NF-kB phosphorylation[34] and inhibited the phosphorylation of mTOR, which subsequently decreases the autophagy, and can result in the TFH and NKT-cell balance.[34]

MSC-based therapy and AH

MSC-based therapy has been also applied for AH. To simulate the AH phenotype, C57BL/6 N mice had free access to a chronic-binge ethanol diet for 10 days.[35] BM-MSCs were then administered intraperitoneally (5×10^6 cells/mouse) and resulted in improvement of the liver damage by decreasing the levels of AST and ALT (Table 1). Administration of BM-MSCs, resulted in decreased malondialdehyde (MDA) levels, and increased glutathione (GSH) levels. MDA was produced due to oxidative damage and lipid peroxidation, whereas GSH had a protective role against oxidative stress. This MSC-based therapy diminished the number of neutrophils and macrophages. Previous studies showed that MSCs also secrete TNF-stimulated gene-6 (TSG-6) factor which plays an inhibitory role in the mitogen-activated protein kinase (MAPKs) and nuclear factor (NF)-κB pathways, both involved in ALD.[35]

MSC-based therapy and AC

A cellular MSC-based therapy has been administered in 72 patients diagnosed with AC and had abstained from alcohol for at least half a year. Specifically, autologous BM-MSCs (5×10^7) were injected intravenously once. This resulted in improvement of the histological parameters, amelioration of fibrotic phenotype and at decrease in the total Child-Pugh. Finally, the levels of specific biochemical and serological markers (ALT, AST, GGT, ALP) were almost restored to normal (Table 1, Fig. 1).[36]

Non-Alcoholic Fatty Liver Disease and Non-Alcoholic Steatohepatitis

One in 4 people worldwide suffers from NAFLD. It is characterized by diffused fatty infiltration and can progress to NASH with intense signs of hepatic fibrosis which is inclined to develop cryptogenic cirrhosis and hepatic failure. However, both the mechanisms of NAFLD and NASH are not entirely understood.[37]

The progression of NAFLD leads to NASH, and finally to cirrhosis and hepatocellular carcinoma. It is also strongly linked to metabolic disorders like diabetes type 2.[6] Numerous factors could contribute to NASH, such as consumption of a high-fat diet (HFD), genetic factors, oxidative stress, secretion of high levels of cytokines, upregulation of TLRs, and inflammation originated by gut-derived factors (ie, LPS).[17,27] Main symptoms of NASH are obesity, insulin resistance, and liver steatosis.[17] Histological signs include fat-laden hepatocytes, inflammation, and apoptosis, while deposition of collagen fiber, indicates the progress of the disease.[20] The pathogenesis of NASH is mediated by the innate and adaptive immune system. Pro-inflammatory cytokines, such as TNF-α, IL1, and IL6, contribute to the development of fibrosis and cirrhosis, while they could regulate the expression of carbohydrate and lipid metabolism enzymes.[6,38]

Etiology of NAFLD/NASH

The pathogenesis of NAFLD is affected by lifestyle factors, such as lack of physical activity and poor nutrition and by genetic determinants and innate immunity.[28,37] Genetic polymorphisms PNPLA3, membrane bound *O*-acyl transferase (MBOAT), Transmembrane 6 superfamily member 2 (TM6SF2), and glucokinase regulatory protein (GCKR)] and epigenetic signatures, especially histone modifications, are associated with the evolution of NAFLD. The deacetylase Sirtuin-1 (SIRT1) modulates hepatic energy metabolism through deacetylation of metabolic regulators and therefore its overexpression or downregulation regulates fatty liver disease.[28,39]

Diagnosis of NAFLD/NASH

Liver biopsy is also the gold standard for the diagnosis of NAFLD since is the only method to clearly identify the severity of the disease. However, it has some major complications that have been mentioned previously and therefore other clinical tests should also be performed. Through blood tests, the levels of serum transaminases can be estimated, with the necessity of additional tests, since 80% of patients with NAFLD present normal levels of these liver enzymes. MRI can produce more accurate and sensitive images of hepatic fat deposition. Moreover, TE and controlled attenuated parameter (CAP) represent also ultrasound-based screening tools for detecting liver stiffness and fat liver accumulation.[40]

Cell Therapy

MSC-Based Cell Therapy and NASH

MSC-based therapy has been recently applied in mice models with the phenotype of NASH. Specifically male immunodeficient mice models Pfp/Rag2$^{-/-}$ received a highly fatty diet and 21 weeks after were intrasplenically administered with $0.9\text{-}1 \times 10^6$ human BM-MSCs. This therapeutic scheme decreased 3 times the deposition of fat in liver compared to the control animals. Human mitochondria were detected in the mouse liver after cell administration. Those mitochondria derived from the donor cells contribute to the lipid breakdown and to 25% decrease of the levels of triglycerides. Tissue inflammation and fibrosis were ameliorated, while tissue homeostasis was achieved (Table 1, Fig. 1).[20,41]

Another in vivo study showed that male mice 28-week-old male C57BL/6 db/db mice fed with high-fat diet after intravenous administration of 1×10^6 human UC-MSCs restored glucose homeostasis and liver function. Decreased levels of alanine transferases, TC, TG, LDL-C, and amelioration of lipid storage were detected (Table 1, Fig. 1).[42]

Moreover, in 6- to 8-week-old C57BL/6 mice fed with methionine/choline deficient diet (MCD) and were administered

intravenously 1×10^6 human UC-MSCs. Reductions in bacterial populations responsible for increased inflammation and damaging of the integrity of the intestinal mucosa and increase of those that protect the homeostasis of intestinal environment were observed (Table 1, Fig. 1). Furthermore, hepatic steatosis and inflammation were alleviated.[27]

Conclusion and Challenges Related to Mesenchymal Stem/Stromal-Based Therapy

MSC-based therapy is applicable to various therapeutic modalities with promising outcomes[20,34] and represents a promising therapeutic approach for ALD and NAFLD with concomitant limitations depending on techniques of isolation, preservation, dosage, route of administration, donor age, source of cells, passage, culture conditions, or cost[20,34] Firstly, administration of cryopreserved cells is considered safe up to a percentage of 60%; although the efficacy is debatable mainly due to cryopreservation storage that could affect the quality of cells. Thawing can also stress cells and affect their viability and function. Thus, MSC administration can cause instant blood mediated inflammatory reaction (IBMIR), a cascade of innate immune system reactions that finally lead to thromboinflammation and cell loss. The administration of immediately post thawing cells can also modify the biodistribution and the migration of cells in different tissues as this methodology diminish some cell adhesion proteins. However, the survival rate of cells can be increased with the use of cell coating, hydrogels and biomaterials.[43] Furthermore, MSCs (BM, AT, PT) from different sources exhibit heterogeneity in the expression of some membrane proteins, such as the tissue factor [(TF)/CD142 coagulation factor III/thromboplastin)]. This protein represents a marker for MSC hematocompatibility that mainly activate the extrinsic coagualation pathway and promote the IBMIR. Specifically, AT-MSCs and PT-MSCs exhibited higher expression levels of TF/CD142 and decreased hematocompatibility.[44,45] However, MSC products are not routinely tested for TF/CD142 expression which is related to variability in effectiveness and to a risk for patient safety.[44] Regarding the route of administration, there are no specific guidelines, and clinicians attempt to optimize the appropriate administration conditions depending on patient's history and MSC avaliability.[44] Nevertheless, the intravenous infusion of MSCs normally results in their accumulation into the lungs.[46-49] Furthermore, MSC-based therapy has not been proved an appropriate one for patients with preexisting tumor malignancies, since the factors they secrete (ie, TGF-β, HGF, and/or epidermal growth factor) may support tumor growth.[12,47-50]

Despite the limitations discussed herein, MSC therapy aspires to provide proof of concept for an innovative, noninvasive, less immunogenic, and less-toxic alternative strategy for the management of ALD and NAFLD. Further studies are needed to highlight their important mechanistic effect on the reparative function of progenitor cells in the liver.

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Author Contributions

F.K., A.S.: literature review, manuscript writing, and final approval of manuscript. M.G.R.: conception and design, financial support, manuscript editing, and final approval of manuscript.

Conflict of Interest

The authors declared no potential conflicts of interest.

Data Availability

No new data were generated or analyzed in support of this research.

Supplementary Material

Supplementary material is available at *Stem Cells Translational Medicine* online.

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