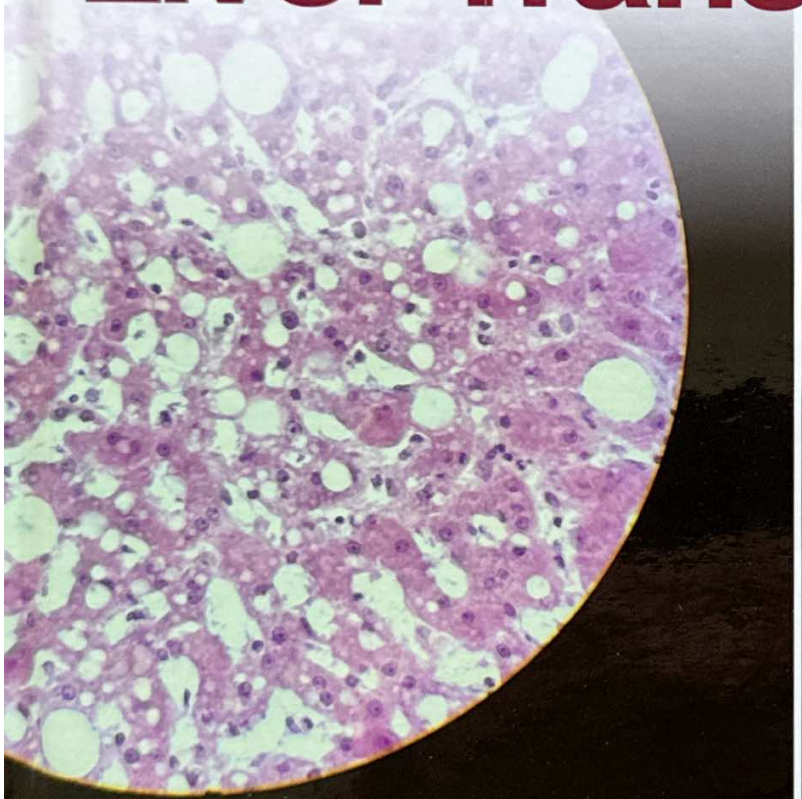
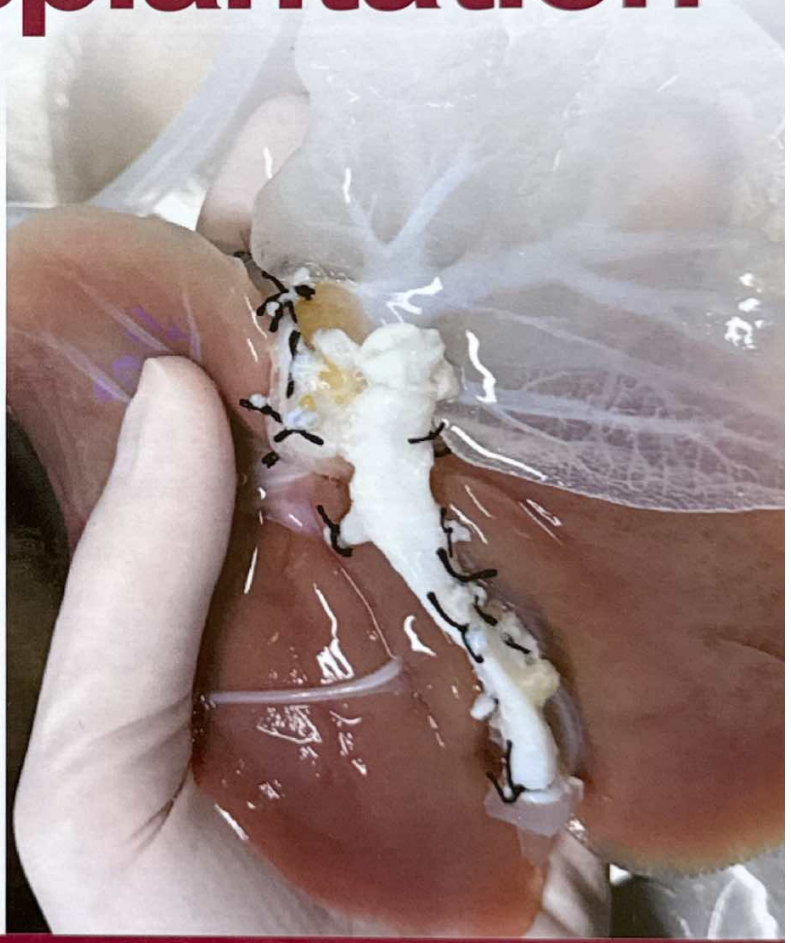
Regenerative and Transplant Medicine Series

Regenerative Hepatology and Liver Transplantation





Volume 2

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Volume Editors: Paulo Martins and Pedro M. Baptista

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Paulo Martins
Transplant Institute, Department of Surgery, University of Oklahoma, Oklahoma City, OK, United States

Pedro M. Baptista
Aragón Health Research Institute (IIS Aragón), Zaragoza, Spain;
Biomedical Engineering Department, Universidad Carlos III de Madrid, Madrid, Spain;
CIBER Enfermedades Hepáticas y Digestivas (CIBEREHD), Madrid, Spain;
Fundación ARAID, Zaragoza, Spain

Series Editor

Giuseppe Orlando
Department of Surgery, Section of Transplantation, Wake Forest Institute for Regenerative Medicine, Atrium Health Wake Forest Baptist, Wake Forest School of Medicine, Winston-Salem, NC, United States

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Chapter 13

Mesenchymal stem cell transplantation

M. Najimi, G. de Bodt, G. Jannone and E.M. Sokal

Laboratory of Pediatric Hepatology and Cell Therapy, Institut de Recherche Expérimentale et Clinique (IREC), UCLouvain, Brussels, Belgium

Introduction

The liver is the central organ with high-level and highly complex activities able to significantly influence the functions of distant organs like the brain and gastrointestinal tract [1]. Indeed, the liver detects in a fine-tuning manner the blood concentrations of many critical molecules and adapts their availability depending on the body's needs [2]. It also plays the role of immunoprotection against foreign bodies and their elimination to avoid major alterations of the hepatic parenchyma [3]. If this should happen, the liver would rapidly activate its regeneration to recover the lost mass by stimulating the proliferation of remnant hepatocytes while continuing to control the execution of the vital functions [4]. The liver parenchyma is composed of several cell types that are perfectly organized at the spatial level and communicate in a very specific manner. Mature hepatocytes represent the major cell population by number and mass and occupy over 80% of the liver parenchyma. These cells accomplish most of the metabolic functions of the mature adult liver. Furthermore, hepatocytes represent the main cell type that is dynamically involved in regenerating the damaged liver after ischemia or acute inflammation. The replicative activity of remnant hepatocytes is the foremost mechanism of action by which liver parenchymal tissue is restored. In humans, significant highly regenerative activity of the liver was measured during the first 2 weeks posttransplantation, followed by a complete recovery at 2 months.

When the ability of hepatocytes to restore the hepatic mass is significantly hampered, due to different etiological factors, structural changes may happen that chronically alter the function of the liver. Liver diseases are related to a significantly increased mortality rate worldwide. If conservative treatments are ineffective, orthotopic liver transplantation remains the widely accepted and gold-standard treatment (many other liver defects are still untreatable). However, the significantly increasing donor scarcity is limiting the wide access to grafts for patients in need. Therefore, several therapeutic approaches have been designed to substitute orthotopic liver transplantation or at least to stabilize the patients during the waiting time for a graft.

Isolated hepatocytes represent the first-generation cell type that has been applied to demonstrate the proof of concept of liver cell therapy

Liver cell transplantation has been proposed to provide the missing functions via the transplanted cells. Such intervention is less invasive as preserving the native recipient's liver, may be applied repeatedly without unwanted effects, and is characterized by a lesser hospitalization duration and thus lower morbidity risks. Restoration of the functional defects, which may be partial, can be measured early posttransplantation and/or after cell engraftment into the recipient's liver parenchyma. The proof of concept of liver cell transplantation was initially demonstrated by using isolated hepatocytes according to the two-step collagenase perfusion technique previously described by Seglen on rat hepatocytes [5].

Such demonstration was confirmed at the preclinical level after showing the ability of transplanted hepatocytes to integrate into the recipient's liver and to provide the missing enzyme activities, which has been documented to significantly support the functions of diseased liver and improve the transplanted animal survival [6,7]. In humans, liver cells may be isolated from whole livers unsuitable for transplantation or normal liver tissue from resection margins. The isolation process has been adapted from the two-step collagenase perfusion protocol previously applied to rat hepatocytes [5]. Such updated protocol allowed the recovery of high yield, and good viability, as well as preserved the integrity and function of the isolated human hepatocytes [8]. Infusion of isolated autologous hepatocytes was initially applied on cirrhotic patients

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with no beneficial effects documented [9]. Thereafter, isolated hepatocytes have been efficiently transplanted in patients with metabolic defects showing clear clinical benefits. This effect has been associated with several parameters, including the single-gene defect parameter to follow, as well as the dose of cells allowing their safe application.

Our team at UCLouvain-Brussels was able to isolate hepatoyctes compliant with the good manufacturing practices (GMP), from more than 220 human livers, with a recovery yield ranging from 17 to 20 million hepatocytes/g of liver tissue. The first pediatric transplantation in Europe was performed with hepatocytes that were isolated from two donors in the USA (Chicago Northern University, P Whitington & H Soriano) and transferred by airplane to Belgium (UCLouvain, Cliniques Universitaires Saint Luc). Two billion isolated hepatocytes [both freshly isolated (2 successive infusions on the first day) and cryopreserved/thawed cells (6 times over 3 days)] were transplanted into a 4-year-old girl with Refsum disease [10]. Subsequently, to hepatocyte transplantation, clearance of toxic metabolites was demonstrated and was correlated to cell engraftment, an effect that has been continued up to 18 months posttransplantation [10]. The same positive effect was documented in a urea cycle disease patient suffering from argininosuccinate lyase deficiency with secondary psychomotor retardation. Both freshly isolated and cryopreserved hepatocytes have been transplanted and have markedly diminished her ammonia and arginosuccinate levels up to 18 months postinfusion [11]. Interestingly, such an effect was associated with an amazing improvement in her psychomotor development and clinical status.

Isolated hepatocytes were further transplanted at Cliniques Universitaires Saint Luc in 15 patients with inborn errors in liver metabolism [12]. The efficacy of liver cell transplantation remains dependent on the etiology of the disease. Nevertheless, no safety issues were noticed in all patients infused, including those who underwent orthotopic liver transplantation.

Unfortunately, as widely accepted by researchers in the field, the features of freshly isolated hepatocytes are poorly preserved after cryopreservation/thawing. Indeed, mitochondrial damage due to the cryopreservation/thawing procedure has been shown to be a primary reason, with disruption of the mitochondrial respiratory chain and subsequent energy breakdown of thawed hepatocytes [13]. The mechanisms behind are still not yet well understood. Culturing isolated hepatocytes in 2D culture conditions is also accompanied with a rapid dedifferentiation and loss of their ability to proliferate. Both limitations, noticed postculture and postcryopreservation, significantly limit the schedulable use of hepatocytes, as well as the organization of their transportation from one center to the other when needed.

Isolated hepatocyte suspensions can be infused via a catheter surgically or radiologically inserted into the portal vein or in one of its branches in the adult or into the umbilical vein of inborn infants. Evaluation of the viability, sterility, and metabolic functionality of both freshly isolated and cryopreserved/thawed hepatocytes allow the selection of the optimal cell suspensions to be transplanted. With respect to the optimal dose to be infused, 200–400 million hepatocytes/kg body weight (30–100 million cells/kg body weight per session), is the preferable number of transplanted cells with the aim to theoretically achieve 5%–10% of the recipient's liver mass and hopefully measure/detect a clinical, biochemical effect in patients with metabolic defects. For acquired diseases, lesser doses seem to be needed based on the paracrine features and mechanism(s) of action involved. In that context, peripheral infusion is the preferable site for cell delivery. To assess the efficacy of liver cell transplantation, serum, urine, or liver samples should be recovered at different times following cell transplantation and depending on the etiology of the liver disease targeted. The aim is to measure protein activity associated with the presence of viable donor cells and/or to detect their expression in the recipient liver tissue. Those data should normally be associated with in situ engraftment of donor cells in the recipient liver tissue, which may be observed several weeks postcell transplantation. The presence of donor hepatocytes can also be investigated by using molecular biology approaches displaying different sensitivity detection levels on low quantities of recipient tissues.

Although liver cell transplantation is generally considered safe, some parameters including blood flow must be carefully monitored to avoid complications like portal vein thrombosis and bleeding [12,14]. Furthermore, based on clinical data from different centers developing liver cell transplantation worldwide, the accessibility to donor's livers to isolate hepatocytes remains a limiting factor for OLT. The quality of the liver grafts and the subsequently isolated hepatocyte suspensions are not yet well standardized between clinical centers for a further strong correlation with liver cell transplantation efficiency. The difficulty of translating animal data to the clinical level is another limiting factor to deeply understand the mechanisms of action related to cell engraftment, as well as to the optimization of cell infusion.

Stem cells display attractive features that have been exploited for the development of liver regenerative medicine

Lessons from clinical trials that evaluated the usefulness of isolated hepatocytes to treat metabolic liver diseases, highlighted the mandatory requirement of wider availability and optimized supply chain for the candidate cells to be developed for liver regenerative medicine. Indeed, the optimal cell candidate should be regularly available notably by displaying a

significant proliferation potential to bypass the close dependency on liver donors- mature metabolic functions and ideally, immunomodulatory properties for self-tolerance in an allogeneic setting. Both properties should not be altered by cryopreservation and in vitro culture conditions. Finally, the optimal cell type to be used for liver regenerative medicine should also exhibit immunosuppressive properties to hopefully avoid rejection subsequently to transplantation. Stem cells, which display many of the above cited features, have gained much interest as second-generation cell therapy products. Indeed, stem cells exhibit interesting alternative features as compared to isolated adult hepatocytes such as self-renewal potential, resistance to cryopreservation, as well as immunomodulation. The rationale of such strategic therapeutic approach is perfectly aligned with findings that demonstrate the potential of stem cells to maintain and repair adult tissues after birth. Within a given tissue, like blood and intestine, stem cells play critical roles, as the in situ differentiated mature cells are not able to self-renew and have only a short lifespan. Accordingly, stem cells from those tissues have an extensive ability to proliferate and generate daughter cells that will be differentiated according to the tissue's cellular needs towards one of several cell populations [15]. Stem cells have been described in situ, isolated and/or maintained in vitro, and can be divided into a variety of cell types. The mostly and widely studied stem cells are mesenchymal stem cells (MSCs) that can be isolated from both adult and fetal tissues, embryonic stem cells (ESCs), derived from the inner cell mass of the blastocyst [16,17], induced pluripotent stem cells (iPSCs), hematopoietic stem cells, hepatic stem cells and so forth. Those cell types exhibit different and specific expansion and differentiation potentials. For instance, ESCs are pluripotent and thus able to generate all adult differentiated cells. Most of those above cited stem cell types have been studied and assessed for their ability to treat many liver diseases. For instance, many of those stem cells have shown different therapeutic effects on liver fibrosis, based on their ability to inhibit the activation and proliferation of hepatic stellate cells (HSCs), to promote the apoptosis of activated HSCs, the proliferation of recipient hepatocytes as well as their differentiation into hepatocyte-like cells with the aim to replace damaged and dead hepatocytes.

Mesenchymal stem cells

MSCs are currently considered to be the most widely applied cell type in advanced therapies due to their specific immunomodulatory, antiinflammatory and regenerative properties. MSCs, initially described as spindle-shaped fibroblast-like cells able to adhere to plastic and of colony formation, display an interesting differentiation potential into various tissue lineages [18,19]. MSCs exist in almost all tissues and are commonly isolated from bone marrow, umbilical cord, placenta, amniotic fluid, endometrial polyps, and adipose tissue [20–22], but also from solid tissue such as the liver [23,24]. After tissue dissociation and seeding, MSCs adhere to the plastic substrate and exhibit high self-renewal ability when cultured in vitro in a serum-supplemented medium [25]. When incubated in specific culture conditions, MSCs also display significant intrinsic potential to differentiate into several cell types of mesodermal and non mesodermal lineages [26–29]. So far, there are no markers that allow for specific characterization or discrimination of MSCs. Still, the guidelines proposed by the ISCT in 2006 are widely used to describe the human MSCs, including in vitro positive expression of a subset of surface markers CD73, CD90, CD105, CD166, CD29, and CD44 [19,30] and negative expression of CD34, CD45, CD14, CD11b, CD79a, CD10, and HLA-DR [31]. Recent data reveal that the immunomodulatory properties of MSCs are associated with their expression of some cytokine, chemokine and toll-like receptors and their ability to secrete potent bioactive molecules [32–37]. Furthermore, MSCs have shown a tremendous capacity to migrate towards sites of tissue injury and tumor microenvironments. Organ-specific MSCs were shown to home in their native tissue [38]. All those interesting features support their preclinical and clinical development for liver regenerative medicine. The first human MSCs were isolated from bone marrow and their transplantation in the clinic was so far associated with an excellent safety profile according to the different clinical trials conducted to date. Based on literature data, the origin of the tissue, age of the donor, cryo-conservation, culture conditions and duration are factors that influence the therapeutic properties of MSCs in vitro [39–42]. The cell dosage and infusion are also critical in vivo factors [43–45]. This means that a well-controlled and standardized process should certainly improve the drug product and its therapeutic effect.

Mesenchymal stem cells involvement in tissue repair and modulation of inflammation, the new proposed mode of action of

Despite their ability to migrate and to integrate in tissues, the long-term engraftment level remains very low [46]. However, these observations are not aligned with the therapeutic effects that have been documented in both preclinical and clinical studies. Other studies have demonstrated that the conditioned media of MSCs have similar properties and efficacy as the cells themselves. This confirms that their in situ differentiation into mature liver cells is not a main mechanism of action, rather it highlights the potential paracrine effects that MSCs could exert upon transplantation and interaction with recipient

resident cells. Such effects could promote the release of potent bioactive molecules and/or the recruitment of specific cell populations.

MSCs actively react to stress or injury in the same manner that adaptive and innate immune system cells respond to pathogen exposure or apoptosis [47]. MSCs have been shown to interact with a myriad of adaptive immune cells, including T and B cells, as well as dendritic cells [48]. In the innate immune system, macrophages play a pivotal role in initiating and controlling inflammation, and MSCs were said to influence macrophage functions and thereby modulate inflammation [49]. Previous studies demonstrated the active interaction of MSCs with components of the innate immune system and that, through these interactions, they reported to show both anti- and proinflammatory effects [49,50].

Convincing evidence now reveals that several molecular mechanisms are behind the modulation of inflammation by MSCs. Firstly, MSCs activate the expression of interleukin (IL)-1 receptor antagonists. Secondly, tumor necrosis factor-α (TNF-α) and other proinflammatory cytokines from resident macrophages activate MSCs to secrete the multifunctional antiinflammatory protein Tumor necrosis factor-inducible gene 6 (TSG-6). This protein reduces nuclear factor-κB (NF-κB) signaling in the resident macrophages and thereby modulates the cascade of proinflammatory cytokines. Thirdly, lipopolysaccharide, TNF-α, nitric oxide, and possibly other proinflammatory markers from injured tissues and macrophages activate MSCs to secrete prostaglandin E2 (PGE2), which converts macrophages into the phenotype that secretes IL-10 [49]. MSCs also provide antiinflammatory effects through the activation of antireactive oxygen species protein stanniocalcin-1 expression [49]. In contrast, inflammation is emerging as an important regulator of stem cells and plays an intricate role in health and disease [51].

Mesenchymal stem cells for the treatment of acquired liver diseases

Liver regeneration in humans frequently arises after ischemia or inflammation, while in other conditions such as age, cirrhosis, and steatosis, this ability is significantly impaired [52,53]. In such a configuration, hepatocytes were shown to dedifferentiate and to display high plasticity with the acquisition of stem/progenitor cell phenotypes [53–55]. Preclinical studies in animal models have been extensively used to better understand the different mechanisms of action and fate of transplanted cells. Those investigations have allowed a deep understanding of the behavior of transplanted cells in the diseased liver environment, as well as the optimization of several parameters related to cell transplantation, including the route of infusion, the dosage, and the schedule of analysis posttransplantation. Preclinical animal models have clearly shown that transplanted cells are able to migrate from the injection site to home and engraft in the recipient's liver, to function in situ and, in some cases, to improve the survival in animal models of human diseases. In some settings, transplanted cells have been shown to proliferate postdifferentiation in situ and, in some cases, to participate in liver regeneration when hepatectomy was applied [56,57]. Those features have been demonstrated in several animal models in which liver diseases were mimicked both structurally and functionally after surgical intervention. Treatment of animals with hepatotoxicants and animals genetically modified for specific genes has also been widely used.

MSCs transplantation can promote angiogenesis, regeneration, remodeling, immune cell activation or suppression, and cellular recruitment [47,58]. When supplied exogenously, MSCs have the capacity to migrate and home to sites of injury, primarily inflamed or broken blood vessels [47,59]. Upon reaching the damaged tissue, MSCs exhibit therapeutic effects by cell replacement and cell empowerment [60]. Nevertheless, the ability of MSCs to differentiate and replace damaged resident cells was not shown to correlate with the efficiency of cell engraftment and replacement. Indeed, MSCs modulate the local immune responses and empower resident cells to facilitate tissue repair [61,62]. There is an intricate array of soluble mediators generated by MSCs in a milieu-specific manner, almost "sensing" the requirement of the environment [35].

Several studies indicate that MSCs transplantation may provide beneficial effects in the process of wound healing and tissue regeneration. In the dermatological wound context, human umbilical cord MSCs surface seeding implantation has been positively tested against irradiation-induced skin ulcers. The implanted MSCs have been reported to promote neovascularization and reepithelization, and improve the healing of irradiation-induced skin ulcers [63]. MSCs have been shown to correct delayed wound healing in diabetic mice by promoting epithelialization and augmenting angiogenesis and granulation tissue formation [64].

Adipose tissue-derived MSCs (AT-MSCs) transplantation has been shown to promote liver regeneration after hepatic ischemia-reperfusion and subsequent hepatectomy in rats. Interestingly, regeneration-associated proteins in the liver were significantly increased in the AT-MSCs' transplanted group. There was a significant improvement in liver regeneration noticed within 2 days of AT-MSCs' transplantation [65]. Studies have shown that MSCs transplantation could replace the injured cells and repopulate the damaged tissue, and thus transplanted MSCs assist the survival or proliferation of endogenous cells by direct contact or immunomodulation and cell death [66].

Tumor necrosis factor-inducible gene 6 protein (TSG-6), one of the cytokines released by human MSCs, has an antiinflammatory effect and alleviates several pathological conditions [58]. In a carbon tetrachloride-induced hepatotoxic mice model, human MSCs-TSG-6-CM treatment was shown to significantly increase the proinflammatory and profibrogenic markers and to promote liver regeneration [58]. In an animal model of acute liver failure (ALF), transplantation of bone marrow derived-MSCs derived conditioned medium has been shown to improve tissue repair thereby liver function through IL-10 secretion. Furthermore, activation of the signal transducer and activator of transcription 3 (STAT3) signaling pathway by MSCs in the liver was also correlated to antiinflammatory and wound healing properties [67]. Studies have shown that downregulation of heme oxygenase-1 (HO-1) is associated with exacerbation of inflammatory response by TNF-α and NF-κB. Therefore, in an ALF model, transplantation of bone marrow-derived MSCs mitigates the TNF-α and NF-κB activation, polymorphonuclear neutrophil infiltration and function via induction of heme oxygenase (HO) −1 [68]. Intravenous infusion of bone marrow-derived MSCs reduced the levels of major pro-inflammatory cytokines such as TNF-a, IFN-c, and IL-4 in both serum and the liver, but had no effect on the expression of the inducible form of nitric oxide synthase, IL-2, and IL-10 and suppress the activity of intrahepatic natural killer T cells [69]. Bone marrow-derived MSCs transplantation was shown to mitigate the ALF associated with inflammatory cytokines such as IL- 1β, IL-6, and TNF-α [68].

However, few large animal models have been well investigated to address specific questions such as infusion repeatability. Nevertheless, all this accumulated knowledge from animal studies has prompted the clinical development of MSCs for liver cell transplantation, particularly when orthotopic liver transplantation cannot be applied. Furthermore, as compared to other stem cells being tested in liver defects' configuration, MSCs look to be the appropriate cell therapy product thanks to their ability to target both tissue regeneration and inflammation, deeply connected processes of liver regeneration. Although several modes of action are proposed, it looks like the most convincing one is their paracrine activity and their ability to secrete a broad panel of potent bioactive molecules that act at the immunomodulatory, immunosuppressive, regenerative, angiogenic, and antibacterial levels.

Clinical trials using mesenchymal stem cells

MSCs are considered an ideal cell source for transplantation. To meet clinical requirements, these cells should be manufactured under large-scale culture conditions under GMP while preserving both their initial features (expression profile, plasticity, and immunomodulatory properties) and their therapeutic activities. Hence, millions of manufactured MSCs are mandatory to conduct clinical trials based on very critical factors including the targeted defect, the body weight of the patient, as well as the frequency of infusions. Most large-scale cultures of MSCs were conducted by using fetal bovine serum, which helps the MSCs to adhere and proliferate. Large-scale manufacturing of MSCs may be performed in static culture systems, like the multilayered flask, or in dynamic cell culture systems, like bioreactors. Both bioprocessing systems may achieve an expansion ratio of 20-fold and above without affecting cell quality [70]. Besides the initial ISCT criteria that should be investigated on the recovered MSCs, additional assays should be applied on large-scale manufactured MSCs, including the detection of oncogenic cytogenetic abnormalities. Those assays on the stability and quality of large-scale manufactured MSCs should support their clinical development and hopefully overwhelm the shortage of donor grafts.

The safety and feasibility of MSC-based therapies have been demonstrated in clinical trials for many diseases, including graft-versus-host disease, heart diseases, osteoarthritis, bone and cartilage injuries, diabetes and its complications, cancer, spinal cord injury, multiple sclerosis, liver cirrhosis, acute decompensation, acute-on-chronic-liver failure, respiratory disorders, Crohn's disease, autoimmune diseases, and others [35,71−77]. Notably, as of February 2025, 1733 clinical trials with MSCs were conducted against a myriad of diseases (http://www.clinicaltrials.gov), further confirming their promising therapeutic potential. Physicians and scientists are currently investigating the translatability of the MSCs' interesting abilities in clinical situations, including their differentiation, antifibrotic and immunomodulatory properties already demonstrated in preclinical models of liver diseases. At Saint-Luc Hospital in Brussels, GMP-liked ADHLSC suspensions were manufactured in accredited tissue banks and transplanted as allogeneic products in three first-in-man human studies [38,78]. From these trials, the safety of their transplantation has been then demonstrated, with preliminary signs of efficacy and engraftment.

Clinical trials using MSCs in liver disease started as early as 2005 and since then, 85 trials have been documented (www.clinicaltrials.gov). Most of those studies are phase 1 and/or phase 2 trials, involving a limited number of patients and focusing on the establishment of the safety of MSC therapy in liver diseases, as well as the demonstration of the proof of concept in these settings. Very few of these trials have reported their results but did not document any major adverse effects. Accordingly, it was for administrative or redundancy reasons that 5 of the 85 trials were terminated. It should be

highlighted that the different trials that were conducted had substantial variability in their designs. Firstly, the type of MSCs used greatly varies, with 36% of the trials using Umbilical Cord-derived and/or Bone Marrow-derived MSCs, 10% using Adipose Tissue-derived MSCs, while Menstrual Blood-derived, Dental Pulp-derived, Skin-derived, and adult human liver-derived MSCs were used in few studies. Unfortunately, 12% of the trials did not specify which type of MSCs was used. Secondly, the MSCs route of administration varied mainly between the peripheral vein (45% of studies), hepatic artery (30% of studies) and portal vein (10% of studies). The regimen of administration of the cells also widely differed, ranging from a single dose injection to serial injections over the span of multiple months. Future studies are mandatory to establish the ideal route and regimen of administration of MSCs so that the comparability of their results and the overlapping conclusions that can be drawn may not be hampered by these discrepancies. Afterward, trials encompassing a higher number of patients will be needed to evaluate the clinical potency of MSCs in liver diseases.

At UCLouvain, our team has obtained a mesenchymal-like cell population subsequently to human liver cell isolation and primary culture of the parenchymal fraction. Those cells exhibit stem/progenitor features [23], and display an hepatogenic differentiation potential, as well as immunomodulatory and immunosuppressive properties [23,24,79–82]. After extensive investigations, the cell product, and the technology of its obtention have been successfully transferred for industrial development and clinical evaluation. Those cells (HepaStem) were approved as medicinal products according to the EU regulation on advanced therapies and got the clinical trial authorization to be tested in 20 pediatric patients with metabolic liver diseases (Crigler-Najjar syndrome and urea cycle disorders) in a first clinical phase I/II trial in Europe. The analysis of the safety profile of HepaStem revealed that the infusion is well tolerated with no serious adverse events or side effects [83]. Furthermore, recovered data also revealed an increased de novo urea synthesis in patients with urea cycle defects, mostly after 6 months following HepaStem infusion. In Crigler Najjar patients, decreased bilirubin levels were observed in some patients [83]. Thereafter, demonstrating the ability of HepaStem to home into the liver following peripheral injection [76], as well as the in vitro and in vivo immunosuppressive, regenerative and antifibrotic properties [61,84–86] have supported the development of this cell product for the treatment of immune-related liver diseases. Recently, 24 cirrhotic patients with Acute-on-chronic liver failure (ACLF) or with acute decompensation at risk of developing ACLF were transplanted. It is a phase II study whose primary objective is the evaluation of the safety of HepaStem at day 28, while the assessment of safety and preliminary efficacy at month 3 is the designed secondary objectives. As for the previous clinical trial targeting metabolic liver disease patients, the safety profile of HepaStem was confirmed [71]. With the limitations that the study was not controlled, transplanted patients have shown preliminary signs of reduced systemic inflammation, improvement of liver functions and encouraging survival rate.

Based on the updated preclinical data on the new mechanism of action of MSCs, a shift has been noticed regarding the rationale behind the use of MSCs in liver diseases, and thus, the subset of diseases in which they are investigated is also evolving. Initially, MSCs ability to proliferate and differentiate into hepatocyte-like cells has been exploited to target liver diseases via a restoration of the functional liver mass lost throughout chronic liver diseases. However, recent evidence tends to show that a large part of the effects of MSCs come from their paracrine secretion and immunomodulatory properties. Accordingly, a rise of trials investigating MSCs in liver diseases with a prevalent immunologic component, such as autoimmune hepatitis, primary sclerosing cholangitis or ACLF is noticed. This evolution of paradigm also opens the possibility of assessing the efficacy of MSCs secretion products, particularly their extra-cellular vesicles at the clinical levels.

Conclusion

Thanks to the accumulated preclinical evidence on their usefulness in targeting liver diseases, MSCs are currently under extensive investigation to evaluate their efficacy and potential use in liver regenerative medicine. Information that will be recovered from the currently conducted clinical trials should provide valuable insights to efficiently adapt the MSC treatment to the type of liver defect based on the needed properties of the MSCs, their tissue of origin, their mechanism of action, the ideal dosage, and the frequency of its application. Liver regenerative medicine has also resolved other issues including optimal upscaling culture conditions, efficient transportation worldwide, as well as the assessment of the efficacy posttransplantation and the in vivo survival of infused cells.

Updated strategies to improve the healing effect of MSCs will also be designed and tested for instance the preconditioning of the cells, their genetic amelioration, and the deep exploitation of their secretome including the nonsoluble fraction containing extracellular vesicles.

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