Editorial

JOURNAL
OF HEPATOLOGY

New cell-based approaches for liver disorders: From experimental to validated clinical treatment

Roberto Gramignoli*

DOI of original article: https://doi.org/10.1016/j.jhep.2022.08.037

The year 1992 is widely considered "year zero" for liver cell-based treatments in humans. It was December 1992 when the first clinical report was published, describing an autologous cell-based approach for 10 individuals with end-stage cirrhosis in Japan.[1] It was still 1992 when Drs Strom and Fisher used allogeneic hepatocytes to rescue a patient with fulminant hepatitis for the first time.[2] This early success led to the development of similar treatments for both acute and congenital liver disorders in subsequent years.[3] Drs Strom and Fisher's seminal transplants paved the way for further clinical studies and for the compassionate use of such treatments by several groups and transplant centers around the globe. The major indications remain treatment for patients with acquired or inherited liver disease, where the long-term acceptance and metabolic support offered by allogenic proficient cells can correct metabolic defects. Additionally, cell therapies have gained recognition as important supportive treatments to rescue patients with fulminant hepatitis or acute liver failure, an alternative to solid organ transplantation, or temporary support for patients waiting for a matched donor.

Today, thirty years later, hepatocyte transplantation has been offered to no more than 150 patients. Several roadblocks have limited the widespread application of hepatocyte transplantation, and it took several years to solve most of them.[4] The obstacles of significance included the lack of clinical-grade reagents and procedure; the limited number and quality of donor tissues (as a cell source); short- and long-term storage of cells prior to transplant; tracking or monitoring cells after transplantation; preconditioning treatments to enhance engraftment and proliferation of donor cells. Although major accomplishments have been made, there are still hurdles limiting the widespread application of hepatocyte transplantation, or cellular therapies in general, for liver disorders.

Apart from the initial success, the past decades have witnessed several attempts with sometimes modest and short-term effects, where allogeneic cells have been administered or implanted. The reported differences in the level of corrections or outcomes have been largely imputed to quality in donor cells. The isolation procedure to generate primary hepatocytes has been optimized according to Good Manufacturing Practice (GMP) requirements, and the final cell product has been extensively studied to generate hepato-specific release criteria to validate the final product before infusion.[5] But the limited availability of primary hepatocytes for clinical approaches is still recognized as a major burden. Livers rejected from organ transplantation have represented the primary source of hepatocytes for a long time. But the yield and quality of human hepatocytes isolated from rejected organs is severely hampered by prolonged ischemia and elevated micro-/macro-steatosis. Furthermore, hepatocytes isolated from such tissues exhibit reduced hepatic functions, and are sometimes insufficient in number to reach an adequate cell dose for a single adult patient.[6,7] Alternatively, explanted organs from patients undergoing orthotopic liver transplantation have been investigated and validated.[8] The livers of many individuals with inborn errors of metabolism are morphologically and biochemically normal, except for the impaired function that characterizes that disease, and these livers rarely suffer from ischemia or steatosis. Importantly, the cells isolated from explanted livers and infused into patients are not sufficient in number to transfer the donor's disease. But explanted livers are not an abundant source of hepatocytes either and are often insufficient to meet clinical needs. Thus, prenatal (fetal) and neonatal donors have been investigated.[9,10] Unfortunately, such young or progenitor cells, despite undoubted resistance to cryogenic preservation and poor immunological profile, are limited in number and introduce another important risk in cellular therapy: donor

* Corresponding author. Address: Department of Laboratory Medicine, Division of Pathology, Karolinska Institutet, Stockholm, Sweden.
E-mail address: roberto.gramignoli@ki.se (W. alazawi).
Received 13 October 2022; received in revised form 17 October 2022; accepted 17 October 2022; available online xxx
https://doi.org/10.1016/j.jhep.2022.10.017

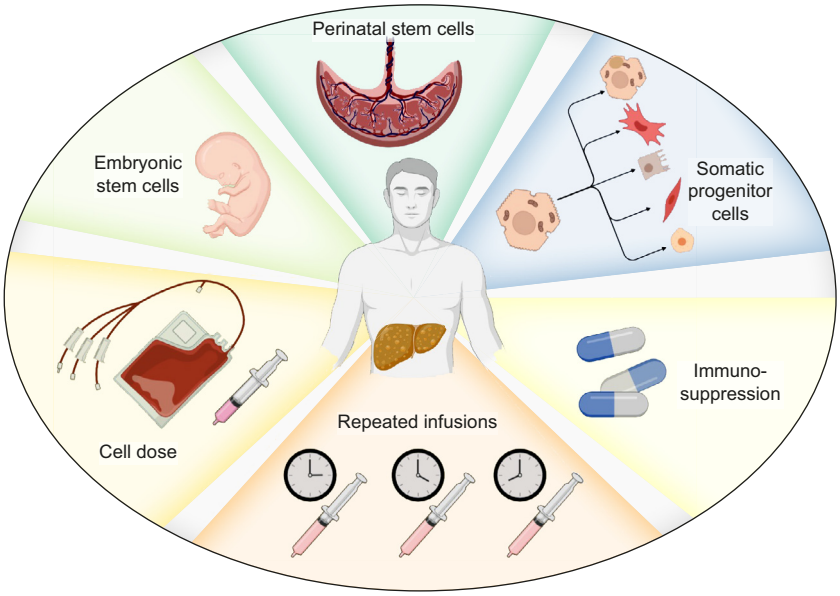

Fig. 1. Stem cell sources and treatment considerations. Liver cell strategies, including different mature or progenitor cell sources, repeated cell transplantations, cell dose, and immunosuppressive regimen in support of allogenic treatments.

cells may not be able to mature into functional hepatocytes and supply the recipient with the required synthetic or enzymatic activities. Similar considerations need to be kept in mind when hepatic or ectopic progenitor/stem cells are proposed as alternatives to mature liver cells.

Cell transplantation is a platform technology in rapid and continuous development. The generation of stem cell-derived hepatocyte-like cells could potentially offer an almost unlimited cell source that could be quickly incorporated into existing procedures if cell products are proven safe and functional (in terms of hepatic secretive and metabolic pathways). Not all the proposed progenitor cells or multipotent stem cells described so far have been eligible and efficient in managing (or curing) patients on the waiting list. Over the past years, several groups and companies have proposed stem cells as an alternative solution, bounded by genetic and epigenetic instabilities and, more importantly, limited in hepatic maturation level (Fig. 1).[11] While all the stem and progenitor cells deserve to be studied, progenitors derived from foregut endoderm may represent the first line of investigation. Such cells may be reasonably equipped with molecules and transporters, as well as phenotypically closer to liver parenchymal cells (or hypothetically able to reach full maturation in a shorter time). Cardinale and co-authors have large experience in biliary tract analysis and progenitor cell extraction,[12] and in the present work, they exploited such expertise to investigate another tissue characterized by foregut endoderm origin: duodenal submucosal glands.[13]

The authors developed a method to isolate EpCAM-positive cells and coupled isolation with immunological analysis to determine if such progenitor cells may be able to acquire a hepatocyte-like phenotype. The extrahepatic progenitor cells isolated by Cardinale and coworkers have been described as possessing some level of homogeneity (determined by static surface markers) and inherited capacity to progress toward hepatic maturation. Interestingly, keratan sulfate-associated antigens (tumor rejection antigen [TRA] 1-60) have been found expressed on such progenitor cells. Notably, TRA-1-60 and TRA-1-81 have been largely described on pluripotent stem cells, such as embryonic or induced pluripotent cells, but also on perinatal stem cells.[14] However, the mechanism of action for such surface-bound molecules is still debated and their relevance in hepatic maturation is unclear. What is attractive is the ability of such duodenal submucosal gland-derived cells to offer support in reversing chronic (but not acute) liver injury. Correctly, the authors correlated such ability to potential paracrine mediators rather than direct maturation into a functional hepatic phenotype. Recent evidence suggests that the maturation of progenitor cells into a tissue-specific phenotype is not always required or strictly necessary. Paracrine effects, based on anti-inflammatory, extracellular matrix remodeling, anti-apoptotic and pro-angiogenic properties**,** may be sufficient to restore normal architecture and function in fibrotic tissues.

Bridging new cell-based approaches into clinical practice depends on relevant preclinical validation. The use of relevant experimental models is of irreplaceable importance to evaluate the efficacy and safety of the product. And 15 years between the first preclinical test and the first-in-human hepatocyte transplantation affirm such experimental steps as crucial. The authors tested human duodenal progenitor cells in an experimental model of fatty liver, observing support in liver regeneration.

Another critical factor, where preclinical analysis is instrumental to validate new products, is cell engraftment. Engraftment into a preclinical model has served as a valid predictor of the extravasation capacity and parenchymal integration characteristics of donor cells. Once in the portal blood, human epithelial cells, such as adult or pediatric hepatocytes, characterized by quite a large volume (approximately 20-40 μm in diameter), have been described to embolize into the terminal radicles of hepatic veins or arteries, resulting in transient portal hypertension and mild ischemia-reperfusion injury.[15] Such an effect on the

microvasculature stimulates fenestration enlargement by the endothelial cells but also activates tissue-resident macrophages to perform a rapid and efficient phagocytosis of residual cells or cellular elements in the circulatory system. As part of the safety and preclinical validation, cell distribution and engraftment evaluation for the donor cells is commonly required. Indeed, several stem cells have been quite unsuccessful in engrafting into the liver parenchyma when injected through the blood system. Hepatocytes delivered via the portal vein efficiently engraft in the liver or are cleaved by Kupffer cells, while smaller cells pass through the organ and relocate to extrahepatic compartments.[16] Hence, it is critical to evaluate donor cell engraftment to determine the cell dose to be administered to the recipient.

Clinical outcome is directly correlated to the administered cell dose. In clinical practice, such cell dose is conveniently calculated and commonly adjusted according to the patient's size. During the past decades, doses ranging between 10^6 and 10^7 viable cells per kg of a patient's weight have been used. Low cell dose has been used in patients affected by fulminant hepatitis, as well as end-stage cirrhosis. A great distinction, one or two log differences, needs to be made when inborn errors of metabolism need to be treated and pathophysiological effects need to be reversed. A liver mosaicism, where approximately 5-10% of the total parenchyma is replaced by proficient cells, is required to correct a metabolic imbalance. Considering an average liver organ contains approximately 1.5-2 billion hepatocytes, 8 to 15 billion proficient cells need to be implanted to reverse metabolic defects such as urea cycle defects or Crigler-Najjar (two of the major indications for allogenic hepatocyte transplantation, so far). Cardinale *et al.* extracted two to five hundred million duodenal progenitor cells from each donor. Such cell yield may be considered sufficient to infuse a patient with an acute or chronic disorder, but several donors will eventually be needed to grant long-term correction to a patient with a metabolic disorder. A burden shared with any other progenitor/stem cell sources, as mentioned. Notably, cell infusions are not limited to one or two procedures as commonly experienced with solid organ transplantation. The implantation of a large organ or tissue is an invasive procedure, requiring massive surgery, compared to cell infusion where donor cells are released into the circulatory torrent in close proximity to the target organ. Decades of hepatocyte transplantations have clearly shown that multiple cell infusions are not only possible, but preferable. Billions of donor(s) cells can be infused over a number of hours, days, weeks, or even months to achieve a physiological level of missing enzymes and reverse an inborn error of metabolism (Fig. 1). But as with any other cell therapies, compliance with Regulatory Agencies is required, including restrictions on the number of donors per recipient. Guidelines for GMP have been published and the vast majority of modern cell-based therapies (in particular allogeneic treatments) need to fulfill such criteria and respond to specific standard requirements. Liver cell-based therapies, where allogeneic progenitor or stem cells are infused into recipients, are classified as Advanced Therapy Medicinal Products. As an innovative medical treatment, human cells may offer groundbreaking new opportunities for the treatment of diseases and injuries that are largely incurable today. However, such products need to respond to strict and specific release requirements. In published reports, cell viability is the sole evaluation for cell quality before infusion. Cell viability both before and after cell manipulation (*i.e.*, isolation, selection, or cryogenic preservation) reflects cell integrity and characteristic, but common viability tests have little to no correlation with hepatic metabolic capabilities. Hepatic maturation and metabolism are critical parameters to assess liver therapies. During the past years, release criteria and functional tests have been developed and optimized to specifically evaluate donor liver or progenitor cells before infusion in patients.[16,17] As a result, cellular products can be qualified and customized to better match the recipient's needs. Progenitors and stem cells are no exception, and their path to reach full maturity and recognition as new treatments will require *in vitro* and *in vivo* validations. We are very much looking forward to observing the progression in the described new source of hepatic progenitors generated by duodenal submucosal glands. These progenitor cells and other stemness sources may carve out a place as corrective cell therapies, or represent an adjunct product in support of more conventional treatments. In the medical community, we all contemplate similar new opportunities that can be brought to clinical practice, upon complete and rigorous validation analyses.

Affiliations

Department of Laboratory Medicine, Division of Pathology, Karolinska Institutet, Stockholm, Sweden

Financial support

The author did not receive any financial support.

Conflict of interest

The author declares no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure form for further details.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2022.10.017.

References

[1] Mito M, Kusano M, Kawaura Y. Hepatocyte transplantation in man. Transpl Proc 1992;24(6):3052–3053.

[2] Strom SC, Fisher RA, Thompson MT, Sanyal AJ, Cole PE, Ham JM, et al. Hepatocyte transplantation as a bridge to orthotopic liver transplantation in terminal liver failure. Transplantation 1997;63(4):559–569.

[3] Gramignoli R, Vosough M, Kannisto K, Srinivasan RC, Strom SC. Clinical hepatocyte transplantation: practical limits and possible solutions. Eur Surg Res 2015;54(3–4):162–177.

[4] Gramignoli R, Tahan V, Dorko K, Venkataramanan R, Fox IJ, Ellis EC, et al. Rapid and sensitive assessment of human hepatocyte functions. Cel Transpl 2014;23(12):1545–1556.
[5] Tolosa L, Bonora-Centelles A, Teresa Donato M, Pareja E, Negro A, Lopez S, et al. Steatotic liver: a suitable source for the isolation of hepatic progenitor cells. Liver Int : official J Int Assoc Study Liver 2011;31:1231–1238.
[6] Gramignoli R, Green ML, Tahan V, Dorko K, Skvorak KJ, Marongiu F, et al. Development and application of purified tissue dissociation enzyme mixtures for human hepatocyte isolation. Cel Transpl 2012;21(6):1245–1260.
[7] Gramignoli R, Tahan V, Dorko K, Skvorak KJ, Hansel MC, Zhao W, et al. New potential cell source for hepatocyte transplantation: discarded livers from metabolic disease liver transplants. Stem Cel Res 2013;11:563–573.
[8] Giancotti A, Monti M, Nevi L, Safarikia S, D'Ambrosio V, Brunelli R, et al. Functions and the emerging role of the foetal liver into regenerative medicine. Cells 2019;8(8):914.
[9] Bluhme E, Henckel E, Gramignoli R, Kjellin T, Hammarstedt C, Nowak G, et al. Procurement and evaluation of hepatocytes for transplantation from neonatal donors after circulatory death. Cel Transpl 2022;31:9636897211069900.
[10] Zabulica M, Srinivasan RC, Vosough M, Hammarstedt C, Wu T, Gramignoli R, et al. Guide to the assessment of mature liver gene expression in stem cell-derived hepatocytes. Stem Cell Dev 2019;28(14):907–919.
[11] Turner R, Lozoya O, Wang Y, Cardinale V, Gaudio E, Alpini G, et al. Human hepatic stem cell and maturational liver lineage biology. Hepatology 2011;53(3):1035–1045.
[12] Cooper S, Bennett W, Andrade J, Reubinoff BE, Thomson J, Pera MF. Biochemical properties of a keratan sulphate/chondroitin sulphate proteoglycan expressed in primate pluripotent stem cells. J Anat 2002;200(Pt 3):259–265.
[13] Cardinale V, Carpino G, Overi D, Safarikia S, Zhang W, Kanke M, et al. Human duodenal submucosal glands contain a defined stem/progenitor subpopulation with liver-specific regenerative potential. J Hepatol 2022;78(1). https://doi.org/10.1016/j.jhep.2022.08.037.
[14] Gupta S, Rajvanshi P, Lee CD. Integration of transplanted hepatocytes into host liver plates demonstrated with dipeptidyl peptidase iv-deficient rats. Proc Natl Acad Sci United States America 1995;92:5860–5864.
[15] Bohnen NI, Charron M, Reyes J, Rubinstein W, Strom SC, Swanson D, et al. Use of indium-111-labeled hepatocytes to determine the biodistribution of transplanted hepatocytes through portal vein infusion. Clin Nucl Med 2000;25:447–450.
[16] Gramignoli R, Tahan V, Dorko K, Venkataramanan R, Fox IJ, Ellis EC, et al. Rapid-and-sensitive assessment of human hepatocyte functions. Cel Transplant 2014;23(12):1545–1556.
[17] Bonora-Centelles A, Donato MT, Lahoz A, Pareja E, Mir J, Castell JV, et al. Functional characterization of hepatocytes for cell transplantation: customized cell preparation for each receptor. Cel Transplant 2010;19:21–28.