

Clinical Perspectives of Stem and Progenitor Cells for Liver Regenerative Medicine



Because the liver is the site of many vital functions, impairment of only one protein within a complex metabolic pathway is usually highly deleterious. Such a condition is called inborn error of metabolism, and concerns many genetic diseases linked to a non-functional enzyme in the liver. Treatments, and long-term management, are currently not efficient enough, and patients would greatly benefit from an innovative therapy that meets this medical need. Orthotopic liver transplantation is the only radical treatment of severe defects, and/or end stage diseases. Cell therapy has been identified as the best alternative tool to overcome scarcity of organ donation. Several cell types are under investigation, and adult liver stem/progenitor cells represent an attractive cell source for liver regenerative medicine.

Medical Context for Inborn Errors of Metabolism

The liver is a key organ in regulation of body homeostasis, and performs many vital functions such as blood glucose homeostasis, plasma protein synthesis such as albumin or coagulation factors, endogenous and xenobiotic detoxification. Hence, impairment of any one of the multiple liver functions leads to a dramatic impact on health. The total worldwide incidence of both acute and chronic liver diseases sets these pathologies within the first 15 causes of death, according to the World Health Organization.

There are many important enzymes in the liver, and so a number of them can be deficient, each deficiency being associated to a specific disease. Each of these diseases is rare, and incidences can be as low as 1 in 1,000,000 births. However, altogether liver-based inborn errors of metabolism affect one child in 2,500 live births. This means that, within the European Community only, 2000 new cases every year are diagnosed.

Beside congenital liver diseases due to a gene-metabolic defect, acquired disease may also affect the liver, due to infectious, toxic or immune mechanisms. Acute liver diseases may lead rapidly to severe functional impairment, such as in fulminant hepatitis, whilst more chronic damage will cause progressive fibrosis and cirrhosis.

Within the panel of these genetic diseases, some are very severe and life-threatening, while others are milder in their clinical expression. Nonetheless, they all impede the general quality of life of both the patient and his or her family. For instance, patients suffering from urea cycle diseases cannot detoxify free ammonium resulting from protein catabolism. Free ammonium is highly toxic to the central nervous system, and these patients are at high risk of metabolic decompensation leading to irreversible neurological damage. Long-term management of patients suffering from urea cycle diseases is mainly based on low-protein diet and the use of ammonium scavengers. Patients often develop anorexia and naso-gastric feeding is required, and the risks of sudden hyperammonemia persist. Many patients still develop intellectual impairment due to the chronic ammonium intoxication.

This example is representative not only of the poor quality of life, but also the life-threatening nature of these conditions. For instance, a retrospective study performed in Italy showed that for the 1935 cases diagnosed with an inborn error of metabolism, only 118 reached adulthood.

Currently, the only radical treatment of end stage diseases is orthotopic liver transplantation (OLT) but this procedure is serious, irreversible and limited by organ shortage. Medical treatments and long-term management are not efficient enough, and patients would greatly benefit from an innovative therapy that meets this medical need.

Current Status of Liver Cell Transplantation

Liver cell transplantation (LCT) is the one of these emerging procedures. It consists of single or repeated infusions in the patient's liver circulation of allogenic mature hepatocytes isolated from healthy adult donors. The procedure has been well described, and >30 clinical attempts have been reported in the literature, although probably many more have been effectively performed.

The proof of concept has been established that is feasible to bring a missing function within a diseased liver by infusing a suspension of mature hepatocytes. The best results have been obtained in miscellaneous inborn errors of metabolism, such as Crigler Najjar syndrome (1), urea cycle defects, glycogen storage disease, clotting factor deficiencies and Refsum disease.

These clinical reports have demonstrated that LCT is safe and can restore metabolic liver function for up to 18 months post-infusion, or more. For instance, repeated doses of mature hepatocyte infusions in a child with urea cycle disorder and important secondary psychomotor retardation led to the presence of donor cells in liver biopsies up to eight months after the last infusion, restoring de novo activity of arginosuccinate lyase (2). In this clinical report, infused cells have contributed to restoring liver metabolism, as well as improving psychomotor development (2).

LCT is a fully reversible procedure, and is much less invasive than OLT because it avoids the risks related to native liver explantation, non-function of the graft or even long-term graft loss. In addition, LCT maintains the possibility of performing later OLT, which have been performed without complications in children having undergone previous LCT.

For genetic disease treatment, LCT is based on allogeneic cells, and patients receive immunosuppressive treatment following the infusion.

DRUG DISCOVERY / DEVELOPMENT & DELIVERY

However, several limitations in the use of mature hepatocytes prevent its wide application:

1. Organ shortage as for classical OLT, as only one or two patients can be treated by mature hepatocytes isolated from one donor.
2. Weak tolerance to cryopreservation. Cryopreservation of mature hepatocytes induces a severe impairment of cell adhesion, morphological changes of the mitochondria, loss of ATP production, alteration of mitochondrial respiratory chain enzymes, increased mitochondrial permeability and loss of membrane potential. Although some success has been reported with cryopreserved cells, these are clearly less efficient in the clinical experience, unless perhaps by selecting high quality cells from young donors, which is rarely achievable in routine transplantation.

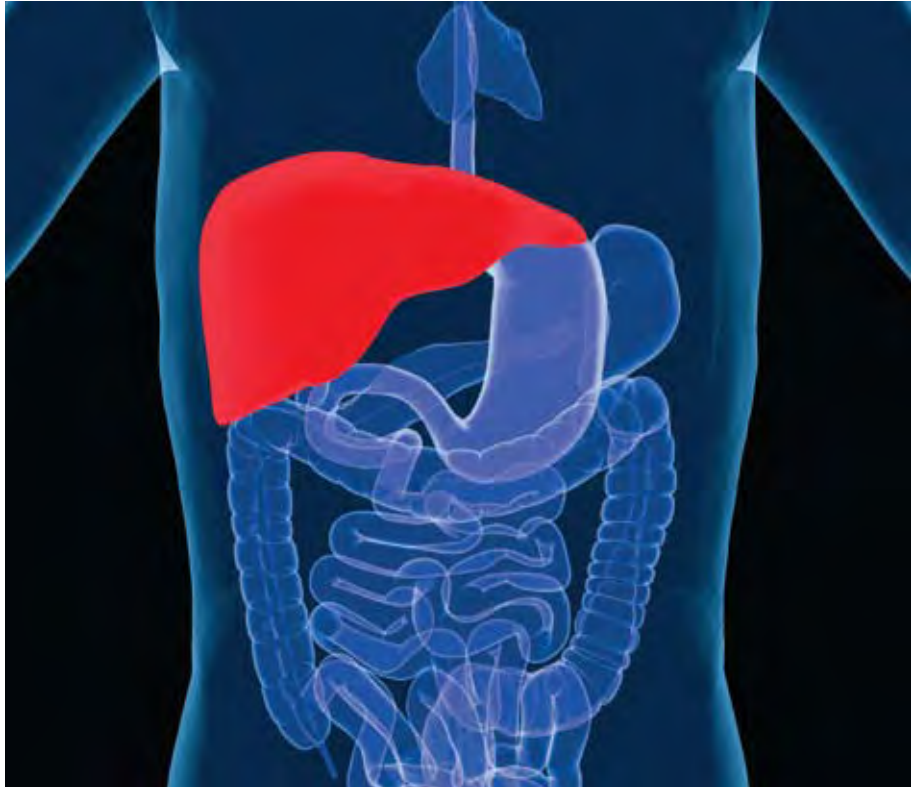
3. Possible bacterial contamination of fresh hepatocyte preparation, unknown at the time of infusion, as it is also observed for whole organs, partially addressed by the use of prophylactic antibiotherapy covering gram negative strains during the infusion procedure.

These storage limitations, adding to the organ shortage issue, lead to the consideration of other cell sources such as stem cells, as an alternative for liver cell therapy. Stem/progenitor cells are undifferentiated cells that demonstrate a high ability for self-renewal and importantly, potential for expansion in vitro. Adult tissue-derived stem cells are safer than embryonic, fetal or induced pluripotent cells, are closer to clinical applications, and do not raise any ethical issues. Stem cells have also a good resistance to cryopreservation. The prospect is that these cells can soon be used for cell therapy of the liver, after induced or spontaneous differentiation into functional hepatocytes.

Extrahepatic Sources of Stem/Progenitor cells

Bone Marrow and Hematopoietic Tissues

Adult bone marrow contains different cell populations, including mesenchymal stromal cells, endothelial cells, fibroblastic cells and hematopoietic cells. In different



studies, it has been postulated that these cells were able to improve hepatic function. In a mouse model of tyrosinemia, infusion of bone marrow from wild type mice was able to restore fumaryl acetoacetase activity in hepatocytes from the deficient mouse.

Beside direct functional activity of the transplanted cells, stem cells may also act through the release of cytokines or growth factors ("secretosomes") that modify the microenvironment and contribute to hepatocytes proliferation/function. This mechanism, suggested in other tissue repair mechanisms, has been suggested in a mouse model of toxic-induced fulminant hepatitis, but cannot explain de novo acquired metabolic function in models of inborn errors of liver metabolism.

Mesenchymal Stem Cells (MSCs)

MSCs were first described by Freidenstein and colleagues as plastic adherent fibroblastic cells with a high capacity for proliferation and differentiation into osteogenic lineage. MSCs have since then been isolated from various tissues such as skin, Wharton's Jelly (3), adipose tissues, amniotic membrane of the placenta, and also fetal tissues. In vitro studies have demonstrated the ability of MSCs from different origins to differentiate into hepatocyte-like cells when specific growth/differentiation factors are added in

the culture medium.

The adipose tissue is an easily accessible source of MSCs, and can be obtained from plastic chirurgic waste material (liposuctions and abdominal resections). These cells share similar phenotypic and immunological features with bone marrow-derived MSC, making them an attractive source for allogeneic transplantation. Their hepatic differentiation potential has been confirmed with acquisition of functional activities such as albumin production, glycogen storage, and drug-metabolising activities. Transplantation into nude mice with acute liver injury can restore liver functions. Predifferentiation before transplantation may improve engraftment and the cells subsequently display in these animals human proteins such as albumin and HepPar. Besides their high potential for differentiation, MSCs have been demonstrated to have immunosuppressive properties, being also able to reduce inflammation.

Intrahepatic Sources of Stem/Progenitor Cells

It seems logical to look for a candidate liver progenitor that would be already resident in the adult liver. Mature human hepatocytes play themselves an important role in liver tissue regeneration, and start to proliferate and repopulate the liver following an acute injury, under

stimulus from cytokines secreted by non-parenchymal cells. Besides regeneration from mature hepatocytes, different populations of stem/progenitor cells participate in the replacement of liver cell loss, including oval cells, small hepatocytes, liver epithelial cells and mesenchymal-like cells.

Oval cells are resident progenitor cells located in the bile ductules (canals of Hering), which are able to generate both hepatocytes and bile duct cells. They constitute the progenitor "niche" or "reservoir" of the liver. In response to injury, these cells proliferate and migrate to the liver parenchyma. However, these cells cannot be isolated and are not deliverable as cell suspensions, and are not therefore used in liver regenerative medicine.

Liver epithelial cells are derived from primary culture of human adult hepatic tissue. In vitro, they can be expanded and differentiated into both liver cell types including hepatocytes and biliary cells, and express mesenchymal and hematopoietic markers. They differ from oval cells by the absent expression of CD34 antigen and their polygonal shape morphology.

Small hepatocytes are isolated from the non-parenchymal fraction after a long culture period, and display a high potential for proliferation, but with a limited in vitro differentiation into mature hepatocytes.

ADHLSCs. The presence of mesenchymal cells, also named adult human hepatocytes stem/progenitor cells (ADHLSCs), was also identified in hepatocyte suspensions obtained after collagenase perfusion of the normal adult liver (4). These cells are isolated from a primary culture of hepatocytes, and demonstrate an important potential for proliferation, and a more advanced and complete morphological and functional differentiation into hepatocytes. In contrast with other MSCs, the cells have a preferential differentiation capacity into hepatocytes, and not into mature osteogenic and adipogenic cells, suggesting that these cells are already engaged in the hepatocytic lineage, being more progenitor than stem cells. Khuu and colleagues have demonstrated advanced liver metabolic activity of differentiated ADHLSC. Indeed, following in vitro differentiation, ADHLSC are able to metabolise ammonium, to conjugate bilirubin, and to express Phase I and Phase II enzymes responsible for metabolism of both exogen (i.e. drug) and endogen

(i.e. bilirubin) compounds. The cells are also more specifically hepatocytic, and not hepatobiliary precursors.

The ADHLSC ability to engraft, proliferate and differentiate into hepatocytes has been evaluated in animal models, and the results have confirmed their potential to be used as a liver cell-based therapy for the treatment of many liver diseases. Transplantation of undifferentiated ADHLSCs into rodents was followed by in vivo differentiation and synthesis of human albumin six weeks later, whilst the cells show a long-term engraftment potential.

Safety preclinical experiments also demonstrated the absence of tumorigenicity in vitro and in vivo (Scheers et al, unpublished data) in comparison with HepG2, a human liver carcinoma cell line.

Taken together, differentiated or not, ADHLSC could represent an excellent candidate for liver cell therapy.

Conclusion

Stem cell technology allows to the current consideration of regenerative medicine of the liver, targeting inborn errors of metabolism – a so far major unmet medical need – as well as some acquired diseases of the liver. The proof of concept that cell therapy is able to restore liver function has been obtained with mature hepatocyte transplantation, but efficacy is partial and suitable alternative sources of cells are needed to replace hepatocytes. Adult liver-derived progenitor cells have shown promising results. The optimal combination of in vitro expansion and hepatic engraftment ability makes this adult progenitor cell a truly competitive tool for liver regenerative medicine. Moreover, the progenitor cell commitment to the hepatic lineage, associated with their ability to be cultured in vitro, can offer the biopharmaceutical industry an interesting tool for pharmaco-toxicology screening of new drugs and lead compound optimisation ■

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