Hepatocyte Transplantation

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Hepatocyte transplantation (HTx) has been developed for use in liver-based metabolic disorders and in acute liver failure. Worldwide, there are around 80 patients that have been transplanted with hepatocytes. Almost all reported studies prove feasibility and safety of the procedure with short- to medium-term success. Availability of good quality hepatocytes (HCs) is the main limiting factor, and therefore alternative sources of cells such as stem cells are being investigated. Other limiting factors include cell engraftment, survival, and function of transplanted cells. It remains to be seen if progress in HTx research can overcome these hurdles leading to the wider use of the technique as an alternative to liver transplantation in the future. (J Clin Exp Hepatol 2011;1:109–114)

Liver transplantation (LTx) is the only well-established treatment for both acute and chronic end-stage liver diseases and liver-based metabolic conditions such as ornithine transcarbamylase (OTC) deficiency. However, shortage of donor organs available for transplantation remains a major problem, with deaths occurring while awaiting LTx. Hence, alternative treatments such as hepatocyte transplantation (HTx) may delay whole organ replacement or obviate the need altogether for orthotopic LTx.

Hepatocyte transplantation is being developed as an alternative treatment for the management of acute liver failure (ALF) and liver-based metabolic disorders.[1–8] Compared with LTx, HTx has several advantages: (a) being less invasive, with less morbidity and mortality, (b) the native liver remains in situ allowing the possibility of regeneration over time and recovery in the setting of ALF, or at least buying the patient time until a suitable organ is available for transplantation.

Pre-clinical Studies

Mito and colleagues (1979) demonstrated that ectopically transplanted rat hepatocytes (HCs) survived outside the liver.[9] Pre-clinical HTx studies in vivo had started in the late 1970s, and showed that the technique was feasible and safe, with some studies reporting success in animals (reviewed in Weber et al 2009).[10] There are published animal model studies of HTx which includes the Gunn rat for Crigler–Najjar syndrome type I, Nagase analbuminemic rats for hypoalbuminemia, Spf-ash mice for OTC deficiency, dogs for hyperuricosemia, and others. These studies showed that it is possible to achieve medium to long-term improvements in their biochemical abnormalities. In the case of ALF, there are rodent models including D-galactosamine, 90% hepatectomy, and liver ischemic injury (Table 1). These studies also showed improvements in the condition and survival of the animals; however, there are questions that remain unanswered as results in animal models are not always transferable to man.

Clinical Studies

There are a number of centers worldwide that have reported their experience of clinical HTx. None of these studies demonstrated a complete cure; however, many of them reported improvement in phenotype from severe to moderate

Table 1 Examples of animal models corresponding to human liver conditions.

Human liver problem	Animal model	References
Crigler–Najjar type 1	Gunn rat	11
Familial hypercholesterolemia type 1	Watanabe rabbit	12
Hereditary tyrosinemia type 1	Fah$^{-/-}$ mouse	13
Progressive familial intrahepatic cholestasis	Mdr2$^{-/-}$ mouse	14
Wilson's disease	Long–Evans Cinnamon rat	15
X-linked severe combined immunodeficiency	Rag2$^{-/-}$ gamma(c)$^{-/-}$ mouse	16

Fah: fumarylacetoacetate hydrolase; Mdr2: multidrug resistance protein 2; Rag2: recombination activation gene 2.

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Abbreviations: ALF: acute liver failure; ApoB: apolipoprotein B; EGTA: ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetra-acetic acid; FVII: factor VII deficiency; GMP: good manufacturing practice; HAS: human serum albumin; HC: hepatocytes; HTx: hepatocyte transplantation; LDL: low density lipoprotein; LTx: liver transplantation; MRI: magnetic resonance imaging; OTC: ornithine transcarbamylase
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or mild. The indications included (a) liver-based metabolic disorders such as OTC deficiency, arginine succinyl lyase deficiency, citrullinemia, Crigler–Najjar syndrome type I, glycogen storage disease 1a and 1b, Refsum's disease, factor VII deficiency (FVII), progressive familial intrahepatic cholestasis type II, and hypercholesterolemia; (b) ALF, and a few reports on chronic liver disease (Tables 2 and 3).

Hepatocytes Isolation

Hepatocytes are isolated from donor liver tissues unused/rejected for transplantation (mainly due to being steatotic) using a collagenase perfusion technique (Figures 1 and 2).[41] The procedure must be carried out under strict sterile conditions, i.e. under Good Manufacturing Practice (GMP) standards in a clean room. Major vessels of liver tissue are cannulated, and the cannulae are secured in a place using suitable sutures. The tissue is then perfused with warm (37°C) buffer solutions. The first solution contains Ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetra-acetic acid (EGTA) to chelate calcium ions to break up desmosomes between the HCs, which makes it easier for collagenase to digest the tissue. The second solution is a plain buffer to wash out the EGTA as collagenase requires calcium ions in order to function optimally. The third solution contains collagenase which digests the tissue. Once the tissue digestion is complete, the HCs are released in a crude cell suspension. The HCs are then purified by repeated wash steps and slow speed centrifugation. The wash buffer solution is used ice cold and supplemented with human serum albumin (HAS) which will inhibit collagenase action and prevent tissue over-digestion, i.e. prevent killing of the released HCs. The cell number and viability are then estimated using a standard trypan blue test. It must be noted that the quality of the isolated HCs will depend on the quality of the liver tissue from which they were isolated.

It is best to transplant HCs fresh, but if no patient is waiting, these HCs can be cryopreserved using optimized protocols,[24] though there will be some loss of HC metabolic function on thawing.

Table 2 Summary of hepatocyte transplantation clinical studies in liver-based metabolic diseases.

Liver disease	No. of patients	Overall outcome	References
Ornithne transcarbamylase deficiency	5	Ranged from some stabilization to decrease in ammonia and detection of urea synthesis	4, 6, 8, 17, 18
Arginine succinyl lyase deficiency	1	Decrease in ammonia, and detection of transplanted cells using fluorescent *in situ* hybridization	19
Citrullinemia	1	Decrease in ammonia	20
Crigler–Najjar type I	8	30–50% decrease in bilirubin up to 7 months	21–27
Glycogen storage disease 1a	1	Better fasting, and decrease in triglyceride for up to 18 months	28
Glycogen storage disease 1b	1	Improvements in glucose levels, with detectable normal enzyme level on biopsy	29
Refsum disease	1	Partial clearance of bile salts, with improvement in development to 18 months	30
Factor VII deficiency	3	Drop in recombinant FVII requirement to 20% of pre OLT levels	7
Progressive familial intrahepatic cholestasis type II	2	No benefit; OLT at 5 and 14 months post cell transplantation	Unpublished data
Hypercholesterolemia	5	Ranged from no effect to 20% decrease in cholesterol level, low density lipoprotein (LDL), and apolipoprotein B (ApoB)	31

OLT: orthotopic liver transplant.

Table 3 Summary of hepatocyte transplantation clinical studies in acute and acute on chronic liver failure.

Liver disease	No. of patients	Overall outcome	References
Acute liver failure			
Drug	17	Ranged from no effect to full recovery without OLT	1–3, 20, 22
Viral	10	Ranged from no effect to full recovery without OLT	1–3, 18, 20, 33, 34
Miscellaneous	14	Ranged from no effect to full recovery without OLT	3, 20, 32, 34–37
Acute on chronic liver failure			
Alpha 1 antitrypsin deficiency	2	OLT 2 and 4 days	3, 4
Viral	1	ICH bleed; died	4
Alcohol	5	3 alive, and 2 died	1, 3
Miscellaneous	6	Decreased bilirubin in 1; 2 died; 2 OLT	3, 38–40

OLT: orthotopic liver transplant; ICH: intracranial hemorrhage.

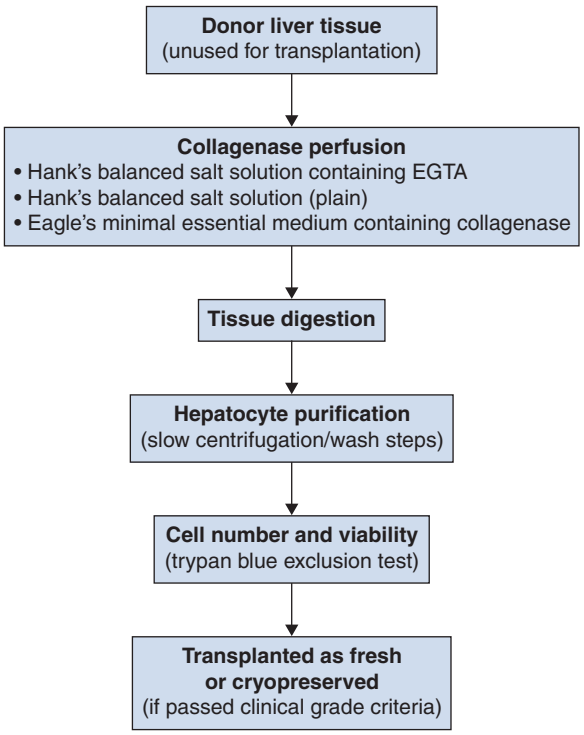

Figure 1 Main stages for hepatocytes isolation and handling. EGTA: Ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetra-acetic acid.

Preparation of Hepatocytes for Clinical Use

Every HTx center has set criteria for releasing the HCs for clinical use. From our experience at the King's College Hospital, London, the criteria for releasing clinical grade HCs must be (a) blood group ABO compatible, (b) have cell viability of ≥60%, (c) negative viral screening as for whole organ transplantation, and (d) micro-organism free on Gram stain immediately prior to administration. Up to 2.4×10^6 cells/g native liver may be transplanted, with a maximum total number of HCs of 5×10^8 per infusion. The goal is to transplant a total mass of cells equivalent to 10% of the recipient's liver mass or 1×10^8 HCs/Kg body weight. The cell preparation composition is HCs (1×10^7/mL) in a transplant medium (usually M199), HAS (3%, v/v), and heparin (1 U/mL).

Route of Administration of Hepatocytes

Animal models for HTx suggested that there are several possible sites suitable for cell transplantation; however, in humans, the preferred route for metabolic indications is intraportal. Portal circulation can be accessed by the direct portal vein puncture, the operative insertion of a central venous catheter in portal venous tributaries, or the umbilical vein in neonates. The portal pressure should be measured after every infusion (infusion time 5–10 min), and subsequent infusions to continue as long as the portal pressure stays below a persistent increase of 12 mmHg.

Possible Complications

There are a few complications that may be observed which include transient portal hypertension, sepsis, embolization to pulmonary capillary beds, and hemodynamic instability. There is also the possibility of portal plugging; however, the resulting ischemia could be minimized by limiting the number of cells per infusion, slow speed of infusion, and the addition of heparin to the cell preparation. Immunosuppression is also required, which is a similar regimen to that used in LTx.

Fate of Transplanted Cells

One of the challenges is monitoring and tracking cells following transplantation in man. The fate of these cells is dependent on their interaction with the local environment. Animal studies showed that it is possible to track transplanted HCs. However, these cells were labeled with, for example, green fluorescent protein.[42,43] New techniques for labeling cells are emerging using superparamagnetc iron oxide particles (contrast agent) in vitro, followed by transplantation; then cells can be tracked/monitored in vivo using magnetic resonance imaging (MRI). Clinical grade MRI contrast agents can be used[44]; however, the MRI images obtained will show both the transplanted labeled cells and other cells in the recipient liver that have taken up the contrast agent after release from dying transplanted cells.

Transient sinusoidal "blockage" in the liver occurs immediately after cell transplantation. A large number of these HCs will die as a result of hypoxia and congestion, while the remainder will be attacked by the immune system. Therefore, immunosuppression is considered essential. The immunosuppression regimen includes corticosteroids and calcineurin inhibitors.[45] Based on the islet transplantation protocol, a new approach is being evaluated where monoclonal antibodies like daclizumab (an IL-2 receptor monoclonal antibody) with low dose tacrolimus and sirolimus are also in vogue.[46] Monitoring rejection is difficult. Currently, the missing enzyme activity product changes are used as crude markers such as elevation of bilirubin in Crigler–Najjar syndrome type I and detoxification of ammonia by conversion into urea.

Other Cell Sources

As mentioned above, the quality of donor liver tissues offered for HC isolation continues to be poor, and hence the need for alternative sources of good quality HCs from stem-cell-based techniques. Stem/progenitor cells have many advantages including ease of expandability in vitro and in vivo. They can be autologous cells, and therefore avoid the need to use immunosuppression, and also it is believed that they are less immunogenic.[11,47] Table 4 summarizes the possible sources of stem/progenitor cells, and their advantages and disadvantages.

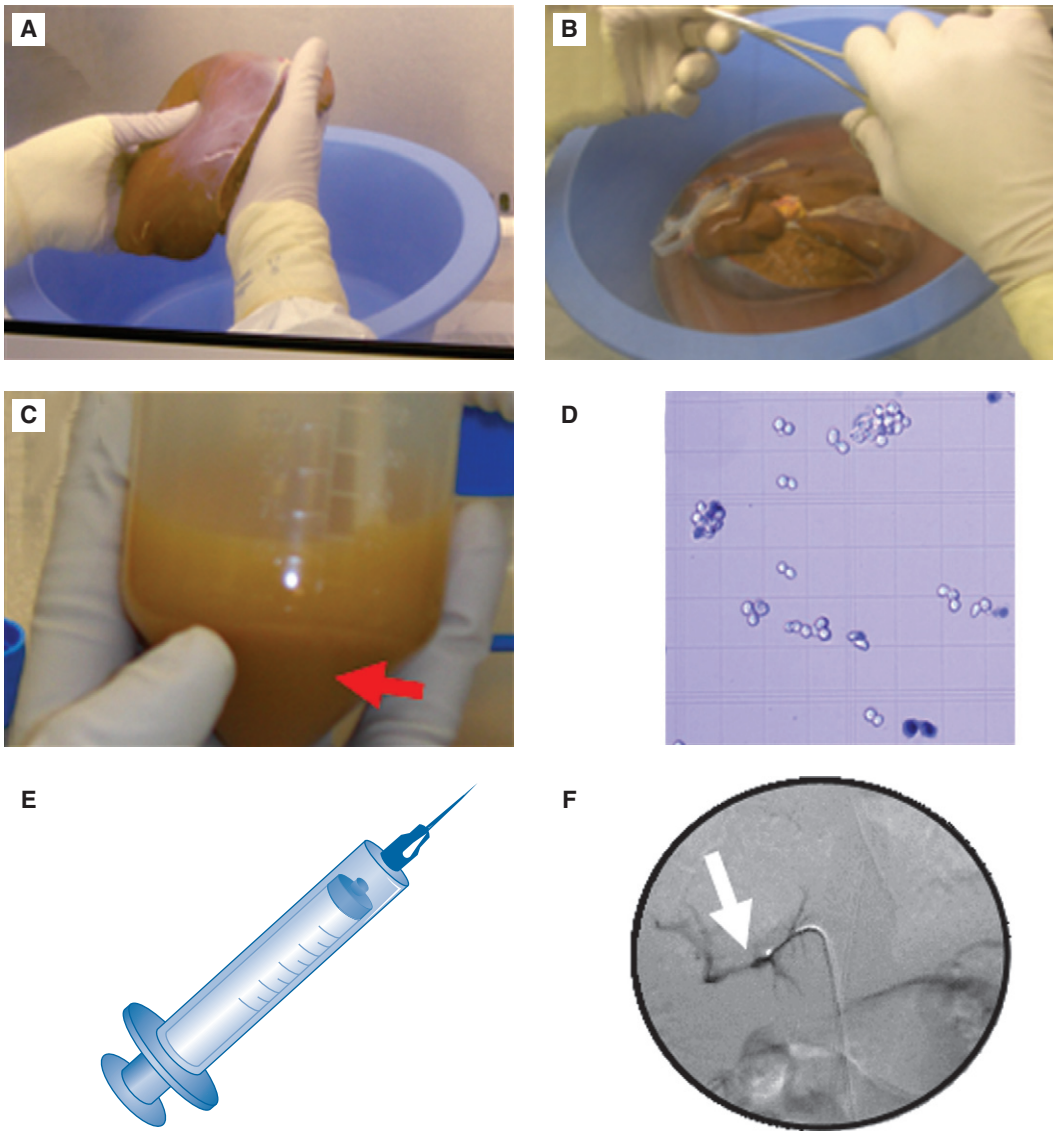

Figure 2 Isolation and preparation hepatocytes for transplantation. (A) Right lobe of the donor liver to be processed; (B) cannulation of major vessels on the cut surface of the tissue; (C) following tissue collagenase digestion, hepatocytes are pelleted (indicated by a red arrow) by centrifugation; (D) cell viability is checked with trypan blue, dead cells will stain blue; (E) cell suspension is ready to be infused; (F) an example of cells being infused in the right part of the liver using a long catheter through the umbilical vein (white arrow indicates end of catheter in liver).

Table 4 Stem cell sources of hepatocyte-like cells: advantages and disadvantages.

Stem cells	Advantages	Disadvantages
Embryonic	• Availability • Expandability • Less immunogenic	• Possibly tumorigenic • Hepatocyte-like functions (incomplete functions)
Fetal	• Committed cells (organ specific) • Proliferative potential	• Difficult to obtain • Hepatocyte-like functions (incomplete functions)
Hematopoietic	• Availability • Function demonstrated in animals	• Possible fusion and nuclear damage • Possibly tumorigenic
Mesenchymal	• Availability • Expandability • Less immunogenic • Can be autologous	• Incomplete differentiation • Fibrogenic potential
Liver-specific progenitor cells	• Availability • Function demonstrated in animals	• Possible fusion and nuclear damage • Possibly tumorigenic

CONCLUSION

In summary, HTx is becoming established as an alternative technique for treatment of certain liver diseases. Improvements in the availability of suitable cells are needed before wider application of liver cell therapy.

CONFLICTS OF INTEREST

No conflict of interest was declared.

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