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Hepatocyte transplantation: past efforts, current technology, and future expansion of therapeutic potential

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Abstract

Hepatic cell transplantation (HCT) continues to garner interest as an alternative to orthotopic liver transplantation and the attendant donor shortage. When compared with solid organ transplantation, advantages of cell transplantation include the potential to treat more patients with a considerably less invasive procedure, the ability to utilize organs otherwise unsuitable for transplant, and leaving the native organ *in situ* with the potential for regeneration. While studies date back to the early 1960s, advancement of clinical application has been slow due in part to limitations of suitable tissue supplies and reproducible robust techniques. Compared with orthotopic liver transplantation, there are fewer absolute contraindications for donor selection. And, current techniques used to harvest, isolate, store, and even transfuse cells vary little between institutions. Significant variation is seen due to a lack of consensus with maintenance therapy. Although the ideal recipient has not been clearly identified, the most significant results have been demonstrated with correction of congenital metabolic liver disorders, with a few trials examining its utility in cirrhotics and more recently acute liver failure. The most exciting new topic of discussion examines techniques to improve engraftment, with many such as ischemic preconditioning and nonselective partial embolization (microbead therapy), while not yet used in HCT study, showing promise in solid organ research. Advancements in HCT, although slow in progress, have great potential in the ability to alleviate the burden faced in solid organ transplantation and possibly become a long-term viable option, beyond that of a bridge or salvage therapy.

Keywords

Hepatocyte transplantation; Liver disease; Cell engraftment; Liver preconditioning; Congenital metabolic disease

Introduction

Hepatic cell transplantation (HCT) continues to garner interest as a potential alternative to liver organ transplantation and the attendant donor shortage. When compared with solid organ transplantation, advantages of cell transplantation include the potential to treat more patients with a considerably less invasive procedure, the ability to utilize organs otherwise

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unsuitable for transplant, and leaving the native organ *in situ* with the potential for regeneration. Hepatocytes are fully differentiated cells, but they demonstrate some capacity for pluripotency and regeneration, with the ability to change into cholangiocytes under the right conditions. Primary hepatocytes are contact-dependent cells, which can make them difficult to expand *in vitro*.[1] However, current animal and human studies demonstrate that they can propagate in areas other than the liver, such as the splenic bed, abdominal subcutaneous tissue, mesentery, and omentum. Some studies have looked at the development of biologic scaffolds for propagation.[2] Other cellular therapies being evaluated include the use of mesenchymal stromal and other multidirectional stem cells to develop induced hepatocyte-like cells. However, efforts to fully replicate all enzymatic functions of primary hepatocytes have not been clinically demonstrated.[3]

Although primary hepatocytes lack the differentiation potential of stem cells, there are fewer ethical dilemmas associated with use of these cells. Animal models date back to the 1960s, with the first clinical trials performed in 1993. However, advancement of clinical application has been slow due in part to limitations of suitable tissue supplies and reproducible robust techniques. This led to a series of recommendations by a consortium of experts in 2009, in an attempt to address areas of improvement for the clinical application of this therapy.[4] Many of the recommendations that came from this meeting will be discussed in the following sections. As of 2017, only four US institutions and fewer than 10 throughout the remainder of the world have programs established for the safe performance of HCT, with the first having been performed in Pittsburgh. At present, there are about 80 articles on the topic, about the same as the number of human subjects that have undergone the procedure. Each case served as a bridge to definitive orthotopic liver transplantation (OLT) or delay in death. At its core, the success of these efforts hinges on a strong multidisciplinary support system, comprising basic science and clinical staff and strong adherence to general good practice guidelines. The purpose of this review is to examine and consolidate the current literature on this topic.

Clinical indications

Many of the currently described indications for HCT are similar to those for OLT. However, what has not been clearly identified is the patient who would most benefit from HCT. The focus has traditionally centered on correction of congenital metabolic liver disorders, with a few trials examining its potential utility in cirrhotics and more recently acute liver failure. Ultimately, clinical benefits must balance the risks of the procedure, as well as the severity and overall prognosis of the disease.

Congenital metabolic disease

Congenital metabolic diseases comprise 10%−15% of primary indications for OLT in children and less than 5% of indications for adults. There have been a number of studies examining the feasibility of HCT as a therapeutic option. The most common conditions that have been evaluated for HCT include the following: Crigler-Najjar (CN), familial hypercholesterolemia, and alpha-1 antitrypsin deficiency (A1AD), among others. When considering therapy for these patients, it is important to be cognizant of the different

potential requirements for therapeutic treatments. Much of what has been described are patients with severe manifestations of their respective diseases with HCT representing a potential bridge or salvage effort. It has been suggested that that the number of cells needed to reconstitute the function of one deficient enzyme is less than that for a patient with acute liver failure.[5]

Crigler-Najjar—CN is an autosomal recessive disorder caused by a mutation in the UDP-glucuronosyltransferase 1A1 gene, important in the metabolism of bilirubin. This leads to high serum levels of unconjugated bilirubin that often result in poor mentation, lack of coordination, slurred speech, and brain damage. For both CN-type 1 (no functional UDP-glucuronosyltransferase 1A1 gene product) and CN-type 2 (some functionality, but < 20% of normal), several studies have shown that one would need 10% of normal function to reduce serum bilirubin below levels that lead to brain injury.[3,6] Phototherapy (less effective in older patients) and OLT (definitive) are two established treatments. Currently, 10 patients are reported to have undergone allogeneic HCT. Both adult and fetal sources for liver cells were used. All patients underwent HCT without complications and experienced a 20%−50% reduction in serum bilirubin levels. Although 90% of the patients were found to have a benefit from HCT, all eventually underwent referral for OLT.[3,6,7]

Familial hypercholesterolemia—Familial hypercholesterolemia is a spectrum of low-density lipoprotein (LDL) receptor gene deficiency disorders. This results in elevated blood serum LDL, associated with early onset cardiovascular disease. The severity of symptoms depends on whether the patient is homozygous or heterozygous and often manifests as early as the third decade of life. Current medical management consists of the use of statins, lipid reducing, and bile sequestering agents. Studies in rabbits demonstrate that with as little as 5% gene expression, there is a 20%−40% decrease in the serum LDL. In a pilot study by Grossman, five cases were described with an average reduction of up to 20% of LDL levels. In these patients, a left lateral liver segment resection was performed to harvest tissue for hepatocyte isolation and processing with recombinant retrovirus. The cells were subsequently transfused back into their respective donors. All patients tolerated the exchange, with no obvious complications.[8]

Alpha-1 antitrypsin deficiency—A1AD is an autosomal co-dominant genetic disorder involving the alpha-1 antitrypsin (A1AT) protease inhibitor produced by the *SERPINA1* gene found in the liver. Without the actions of this enzyme, enzymes like neutrophil elastase break down elastin excessively and degrade lung elasticity leading to emphysema and chronic obstructive pulmonary disease. Defective A1AT can also accumulate in the liver and result in hepatocyte death and cirrhosis. The degree of deficiency creates a spectrum of severity. Neonatal homozygotes develop early onset jaundice quickly leading to liver failure, making it the leading indication for liver transplantation in newborns. In adults (heterozygotes of varying penetrance), in addition to liver dysfunction, severe pulmonary damage can develop, especially in smokers. Serum A1AT levels that are 10%−15% of normal have been correlated with clinically significant disease.[9,10] Another study evaluated autologous transfers with some success as demonstrated by a reduction in ammonia levels. Unlike the other previously described conditions, A1AD with time leads to parenchymal

damage, which complicates potential cell engraftment due to destruction of the innate liver structure.[11]

Other conditions that have been evaluated with similar results include glycogen storage diseases, urea cycle disorders, and factor VII deficiency.[12,13] In each condition, the goal has been reduction in the symptoms seen with severe manifestations of the disorder. Petrowsky *et al.* were able to demonstrate that OLT as a whole is a highly effective form of gene therapy. In their cohort, children and individuals transplanted early in their disease course had better outcomes. Given these findings, it would stand to reason that HCT could potentially play a significant role in the treatment. As previously alluded to, there has been no documentation of cure, but some efforts have worked as a bridge to OLT and potential for improvement of quality of life and cost improvement.[14]

Acute and chronic liver failure

Patients who experience acute liver failure often die while awaiting liver transplantation. Although OLT is the only treatment shown to improve mortality, HCT is being evaluated as a potential option to sustain the rapidly decompensating patient. There have been over 35 patients worldwide who have undergone HCT for acute liver failure (from ages 3 wk to 69 y) due to drug induced, viral, mushroom poisoning, postliver resection, alcohol abuse, and idiopathic causes.[15] All patients underwent allogeneic HCT using either adult or fetal hepatocytes. The majority of patients died before receiving a solid organ transplant, but seven survived. The general consensus appears to be that there may be some benefit for this therapy with documented small improvements in ammonia levels, encephalopathy, cerebral perfusion, and cardiovascular instability.[16–18] These findings parallel those seen in animal models; however, due to the lack of randomized studies, it is not clear if results are due to the transplanted liver cells.

The evidence supporting the use of HCT in patients with cirrhosis is even less clear. Unlike many metabolic diseases and acute liver failure, the liver architecture is disrupted in chronically damaged liver. The subsequent fibrosis makes it difficult for cells to diffuse across the sinusoids, leading to poor engraftment.[19] A range of 5%−15% of liver cell mass has been estimated to provide sufficient metabolic support to produce a clinical therapeutic effect.[18,20,21] Mito *et al.* evaluated ChildPugh A-C cirrhotics and saw improvements in encephalopathy but were unable to say if it was due to the HCT. Other studies had borderline improvements in encephalopathy, protein synthesis, and kidney function without improved clinical outcomes.[22,23] Although there appears to be some promise in patients with cirrhosis secondary to A1AT, chronic liver failure has seen the least success for all indication (Table 1).

Hepatocyte sources, isolation techniques, preservation, and viability testing

One draw of cellular therapy is its potential to utilize tissue otherwise not suitable for whole organ transplantation. However, there remain certain features that must be considered to optimize the yield and quality of the cells before transplantation. Liver cells are usually

obtained from organs considered not suitable for solid organ transplantation due to steatosis, prolonged warm or cold ischemia times, gross injury to the organ, vascular or biliary lesions, or blood group incompatibility. Much like traditional transplant criteria, donors should not have evidence of infection or history of neoplasia (although this is being challenged), history of hepatitis B or C, human immunodeficiency virus, human T-cell lymphotropic virus, syphilis, no liver disease (cirrhosis, cholestasis, or hemophilia), and cold ischemia time less than 12 hours (there is some evidence that there may be no difference up to 24 h).[19,24] Given the increasing incidence of obesity and metabolic syndrome, ways to adequately utilize fatty livers should be further evaluated.[1,25]

Although the greatest success with quality has been found with neonatal donors, there is significant controversy surrounding their use.[23,24,26] Neonatal hepatocyte donors are too small, have immature liver function or technical difficulties related to vessel or biliary tree size, to be adequately utilized in whole organ transplantation.[24,27]

Conversely, livers from older donors have shown diminished viability. Problems appear to arise with cell integrity, likely due to the use of collagenase to disrupt the liver matrix, which often leads to lower yields.[24] The process of disintegrating the liver in this case results in yields lower than those obtained otherwise. Therefore, livers from older donors are being called into question as a source of hepatocytes.[19,24,25,28]

Over the years, several techniques have been described. Many institutions use a technique similar to that described by Strom. In brief, the major liver vessels, and if feasible bile duct, are cannulated and perfused with a chelating agent, which disrupts the cells. To speed up the collagenase time, the liver may be divided into right and left lobes. Caudate lobes discarded from OLT may be independently used as a source as well.[24] After digestion, the hepatic tissue is disrupted, and the suspended cells are filtered and centrifuged.[19,24]

On isolation, the cells are assessed for quality, most commonly using the Trypan blue exclusion assay. Viability greater than 60% is considered the minimum for reliable engraftment.[17,24] If preservation is planned, the cells are stored at 140°C; otherwise, they do not survive longer than10–14 d. Known adverse effects of cryopreservation include mitochondrial damage, loss of capacity for adenosine triphosphate synthesis and abnormal respiratory chain enzyme activity, increased mitochondrial permeability, and impaired respiration.[4] How it affects engraftment is unknown in the clinical setting.[4,24,28] (Table 2)

Transplantation

Whether with fresh or frozen cells, the HCT process is significantly less invasive than OLT and puts less physiologic stress on the recipient. Its overall safety and tolerance has been consistently demonstrated.[4,12,20,21,29] During infusion, it is standard to have cardiopulmonary monitoring, abdominal ultrasound to examine the portal venous flow, and catheter-based portal pressure measurements.[4,30] With some studies suggesting the use of transthoracic echocardiography to evaluate for pulmonary emboli.[31] Pulmonary capillary microembolism has been reported, but it has not been associated with appreciable morbidity or mortality[17,29]

As for the rate of infusion, the general consensus is that cell infusion should be limited to at most 8 mL/kg/h and 100 million cells/kg recipient weight, with the administration of anticoagulation (heparin).[4,23,32] This is to decrease the risk of hepatocyte thrombus formation. A safety protocol for the titration of the infusion has been suggested, where an increase in portal vein pressure or a decrease of portal vein flow velocity of more than 50% would lead to a temporary discontinuation of the infusion. If these values returned to less than a 25% decrease, the infusion was resumed. No adverse effects were identified with this protocol. Transient increased portal pressures are commonly seen yet tend to correct within 24 h and elevation in liver enzymes, also short lived, correct within 1 wk, without clinical issues.[22,32,33] Infusion durations range from 30 to 60 min.[12,23] Repeat injections are often performed in the intensive care unit setting with time between injections ranging from several hours to days.[34] In planning multiple injections, careful consideration should be given for the placement of permanent *versus* temporary catheters for ease of access. The vast majority of these patients are immunocompromised, and line-related infection has been reported.[13] Overall, no consensus has been reached on the method of injection, which may be performed with or without surgical assistance or general anesthesia.

Portal system injection

The portal system is by far the preferred route for patients with acute and metabolic conditions. As a direct route into the hepatic sinusoids, it has the theoretical advantage of delivering a larger number of cells. In adults, access via internal jugular vein (with cannulation of the right portal vein similar to intrahepatic portosystemic shunt procedure), femoral vein, and splenic vein has most often been described.[4,23] When compared with other modes of injection, the portal approach has a higher reported incidence of transient increase in portal pressures. A transhepatic portal venous approach has also been described in pediatric patients without complication.[34] With neonates, umbilical vessels make convenient access points for the portal system.[20,32] In general, for patient without fibrosis, the portal vein approach appears to be well tolerated and readily accessible in patients who do not have fibrosis.

Splenic artery (intrasplenic) injection

In patients with fibrosis, as manifested by elevated portal pressures, splenic artery injection has become the preferred route of administration. The splenic artery is readily accessible through a femoral artery approach.[3] Early clinical trials have demonstrated that cells administered via the spleen eventually transition to liver sinusoids.[19,24] It is theorized that the lower flow associated with this route is less likely to lead to mechanical cell destruction and thus increase opportunity of embedding. There is also a reduced risk of systemic embolization or portal vein thrombosis in coagulopathic and chronically inflamed patients.[3] This approach, especially through direct cannulation of the splenic artery, has been associated with risk of gastric and splenic necrosis due to thrombotic events.[35]

Intraparenchymal/Intraperitoneal injection

Intraparenchymal injection has not been well described and carries the risk of injection into hepatic veins. This may theoretically lead to entry of cells into pulmonary capillaries with subsequent embolism.[3] Intraperitoneal (omental) transfusion has also been described. The

few documented cases demonstrate improved metabolic activity and donor cell implantation in the liver and spleen.[4,5,23,36] Even, the inoculation of lymph nodes is less commonly evaluated. Komori et al. demonstrated in mouse studies that hepatocytes injected into jejunal, popliteal, axillary, or periportal lymphatic tissue generated statistical and clinically significant ectopic hepatic mass to salvage lethal liver failure.[37] This has yet to be reliably demonstrated in human subjects. Of the potential sites, only splenic and peritoneal injections appear to have the potential to accommodate the cell volume needed for clinically significant HCT.[3] However, due to the host immune response and the lack of potential resting sites, the cells do not typically survive for long in this environment.[3,36,38] (Table 3)

Liver preconditioning

Uptake and survival of transplanted cells after HCT are severely limited by multiple factors including the endothelial lining of sinusoids, innate and adaptive immune responses, and apoptosis. Specific factors that hinder the uptake of these cells include cytokines and chemokines, primarily from Kupffer cells and monocytes.[39] In an effort to counteract this physical defense, to minimize apoptosis, and to optimize the recipient livers' ability to support the transplanted cells, methods to "precondition" the recipient liver have been evaluated. Animal models have demonstrated that given regenerative stimuli such as partial hepatectomy, embolization, or irradiation and anti-inflammatory drugs (tumor necrosis factor-alpha inhibitor etanercept), many of the deleterious effects from the innate system and cytokine/chemokine response are reduced and engraftment improves.[4,19,28,40] Interestingly, activation of vascular endothelial growth factor from native hepatocytes, Kupffer cells, monocytes, and hepatic stellate cells has also shown to increase the permeability of endothelial cells and allow greater penetration of the transplanted hepatocytes.[39,41] Currently, the techniques that show the most promise are irradiation of the liver, partial embolization, and more recently novel microbead and ischemic preconditioning therapy.[3,31]

Irradiation

The human liver is very radiosensitive. A single 30 Gy dose of whole liver radiation can cause liver failure due to endothelial damage and consequent veno-occlusive disease. However, a third of the liver can be irradiated with doses as high as 90–100 Gy with no adverse consequences to the whole organ,[4] indicating that the volume of liver irradiated is important in the process. Low-dose single-lobe irradiation has been shown to improve cell engraftment and proliferation.[42] This is thought to be mediated by a short-term disruption of hepatic sinusoidal endothelial cells and by suppression of the phagocytic activity of Kupffer cells. Optimal timing for HCT after hepatic irradiation ranges from 1 to 7 d, yet a minimal dose has not been established.[4,33] Considerations with radiation therapy include the long-term risk of radiationinduced liver fibrosis, through activation of stellate cells, and avoidance in cirrhotic patients, as they have increased radiation sensitivity.[4,31,43]

There is even more hesitation about the use of hepatic irradiation in young children undergoing HCT. Infants aged <2 y are likely at greater risk of developing complications, but results are inconclusive.[4] In children aged >2 y, on the other hand, single fractions of 3–5

Gy have been found to be safe, but it is unclear whether it would be enough to promote sufficient repopulation.[4,19]

Portal embolization

Reversible partial embolization of the portal vein later followed by HCT was successfully demonstrated in Macaque monkeys.[19,31] This resulted in replacement of 10% of liver mass with donor cells. The temporary embolization, using absorbable material, generated ischemia/reperfusion injury and stimulated a regenerative response. A transient increase in portal pressure and mild inflammatory reaction are known side effects of portal vein embolization. In general, reversal of embolization is seen to occur within 14 d.[4] Portal vein embolization in nonhuman primates with occlusion of the left and right anterior portal branches significantly improved the engraftment of hepatocytes after autologous cell transplantation and resulted in repopulation of 10% of the nonembolized lobe.[4,19]

Microbeads

The use of microbeads is another form of nonselective embolization of distal branches of presinusoidal vessels. The intent is to induce regenerative signals without the expense of significant liver parenchyma. This contrasts with proximal partial portal embolization, which completely occludes portal circulation in some anatomical segments of the liver. This technique has been newly described by Pourcher *et al.*, where they were able to demonstrate stimulation of regenerative factors with preserved parenchyma and maximum number of hepatic vessels, yielding three times improved engraftment of hepatocytes, compared with hepatocyte transplant alone.[44] Complications they identified in mice were due to necrosis of greater than 70% of the liver parenchyma, thought to be due to major obstruction of sinusoids (>80%) and vascular hemorrhage leading to acute liver failure and death. This group plans to expand their research to larger animal models before clinical implementation.

Ischemic preconditioning

A potential area for consideration is the use of ischemic preconditioning. This involves a brief ischemic period followed by reperfusion before the prolonged inflow occlusion. The thought is that the brief ischemic exposure period stimulates protective mechanisms against repetitive stress on cells. The majority of studies have demonstrated effectiveness in other organs such as the heart, brain, intestine, and skeletal muscle. The few studies that have evaluated use in the liver have found similar beneficial effects in both animal and human studies. No studies have evaluated its utility in cirrhotic livers or in the context of cellular therapy. However, it would be reasonable to hypothesize a potential role given evidence that occlusion and injury of sinusoids lead to increased risk of parenchyma necrosis and therefore poor graft uptake.[45,46]

Chemical therapy

The use of medications takes advantage of our understanding of the physiological barriers we have uncovered. Agents such as cyclophosphamide, doxorubicin, and nitric oxide accelerate and increase entry into liver sinusoids and engraftment of cells in liver parenchyma. This is without significantly increasing intrapulmonary cell translocation, a

previously mentioned theoretical concern with HCT.[31,39,41] Other less common alternatives include the use of cytotoxic pyrrolizidine alkaloids, retrorsine and monocrotaline, toxic bile salts, or ischemiareperfusion injury in combination with additional injuries such as liver resection, carbon tetrachloride, or other drugs.[4,23] Unfortunately, none of these therapeutic options are well suited for clinical applications (Table 4).

Engraftment detection and immunosuppression

Methods to determine how well cells have incorporated into the liver are challenging in the clinical setting.[3,34] Techniques include short tandem repeat analysis, Fluorescence *in situ* hybridization, Indium-111- or 99m-technetium radiolabeled cells for short-term tracking, polymerase chain reaction, and liver biopsies. Liver biopsies have been found to be overall safe, but only questionably useful, as often donor cells are not identified in the samples.[17,38] Additional markers for success have included improvement in lab values such has bilirubin, specific enzymatic activity, encephalopathy, ammonia clearance, and so forth. Results have been modest, yet clinically significant, at least in the short term as most documented patients proceeded to OLT.[3]

Up to 70% of transplanted cells are reported to be cleared by the innate system within the first 3 days.[3,39,41] A higher risk of rejection has been seen with hepatocytes, compared with whole organs, accounting for a significant amount of graft cell loss seen with this therapy.[41,47,48] Therefore, most institutions use some form of immunosuppressive therapy. Usually, it is the same or similar protocol utilized for their whole organ transplantation.[5,23,31] However, there is no consensus on the best protocol. The most common combinations include tacrolimus with or without steroids and/or monoclonal antibodies, such as interleukin-2 receptor antibodies basiliximab or daclizumab.[4,39]

Rejection appears to occur due to a combination of the innate (Kupffer cells and neutrophils) and the adaptive (T cells) response systems.[39,41] Animal studies have consistently demonstrated improvement in cell longevity and engraftment with the use of tacrolimus, sirolimus, mycophenolate, depletion of T cells, use of COX-2 inhibitors (naproxen and celecoxib), and tumor necrosis factor-alpha antagonist (etanercept). In most described cases, steroids (usually methylprednisolone) are administered intravenously during cell infusion, with a subsequent taper to oral.[3,39] Oral tacrolimus would then follow with serum level goals ranging from 7e10 ng/mL (depending on age).[34] Others have recommended adding oral mycophenolate mofetil to enhance immunosuppression during the early post- transplant period. The only unifying recommendation was the use of a calcineurin inhibitor.[4,39,48]

Conclusion

The advancements in cellular therapy, though slow in progress, are highly promising in the ability to alleviate the burden faced in solid organ transplantation. Current obstacles span all aspects of HCT, from the acquisition of cells to their maintenance. Based on current literature, it would appear that the ideal recipient may be found in those individuals deficient in selective liver enzymatic functions (congenital or acquired) without structural parenchyma damage. In combination with newer techniques to “precondition” the liver, HCT may very

well become a viable long-term option, beyond that of a bridge or salvage therapy. By examining each phase, we hope to fuel critical thought into ways to improve and expand clinical applicability.

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Table 1 –

Most indications are similar to that of OLT. Metabolic conditions have had the best documented support.

Clinical indications for HCT
Congenital metabolic
Crigler-Najjar
Familial hypercholesterolemia
Alpha-1 antitrypsin deficiency
Glycogen storage disease
Urea cycle disorders
Factor VII deficiency
Acute Liver Failure
Drug induced
Alcoholic hepatitis
Viral hepatitis
Poisoning
Post resection failure
Mushroom poisoning
Idiopathic
Chronic liver failure

Table 2 –

Many qualities that make a donor organ unsuitable for whole organ transplantation may still be acceptable for cell harvest and transplantation.

Whole organ donor contraindication	Hepatocyte donor contraindication
Steatosis >50%	Absolute
Gross organ injury Vascular or biliary lesions	Evidence of infection (Hep B/C, HIV, HTLV syphilis)
Blood group incompatibility	Blood group incompatibility
Evidence of infection (Hep B/C, HIV, HTLV, syphilis)	Relative
History of malignancy	Steatosis >50%
Cold ischemia time >12 h	History of malignancy Cold ischemia time >12 h Elderly donors

Hep B/C = hepatitis B or C; HIV = human immunodeficiency virus; HTLV = human T-cell lymphotropic virus.

Table 3 –

Most commonly described routes of administration of hepatocytes.

Route of Transfusion	Advantage	Disadvantage
Portal vein	• Preferred for acute and metabolic conditions • Can deliver a large quantity directly • Ease of access	• Avoid in fibrosis • Transient increase in portal pressures
Splenic artery (intrasplenic)	• Preferred when there is evidence of fibrosis • Less likely to lead to mechanical cell destruction • Reduced risk of portal vein thrombosis	• Potential for gastric or splenic infarction
Intraparenchymal/Intraperitoneal	• Improved metabolic activity and implantation • Can accommodate a large volume of cells	• Increased risk of microemboli to lungs • Reduced longevity due to lack of anchorage

Table 4 –

Techniques to prepare the liver to allow improved uptake and engraftment of cells.

Liver preconditioning	
Irradiation	• Low dose may improve cell engraftment and proliferation • Suppresses phagocytic activity of Kupffer cells • Optimal timing for transplant 1–7 days after radiation • Long-term risk of fibrosis • Low doses may be used for children
Portal embolization	• Reversible with absorbable material • Ischemia/reperfusion injury stimulates the regenerative response • Can cause transient increased in portal pressure and mild inflammatory reaction • Replacement of up to 10% of liver mass with donor cells has been demonstrated
Microbeads	• Nonselective embolization of distal presinusoidal vessels • Induces regenerative signals and spares significant liver parenchyma • Major obstruction and hemorrhage can potentially lead to acute liver failure and death • Animal studies only
Ischemic preconditioning	• Short period of ischemia followed by a brief period of reperfusion before planned insult • The brief reperfusion period stimulates protective mechanisms against repetitive stress • Clinical application has not been performed with hepatic cell transplants
Chemical therapy	• Various agents accelerate and increase entry into liver sinusoids and engraftment in the parenchyma • Numerous side effects make these options ill-suited for clinical application