

## Review Article

### Stem Cells in Hepatobiliary Diseases

Aejaz HM<sup>1\*</sup>, Khan AA<sup>1</sup>, Parveen N<sup>1</sup>, Baskar S<sup>3</sup>, Mahaboob V<sup>1</sup>, Khaja MN<sup>1</sup>, Lakshmi NM<sup>2</sup> and Habibullah CM<sup>1</sup>

<sup>1</sup>Centre for Liver Research and Diagnostics, Deccan College Of Medical Science, Hyderabad, India

<sup>2</sup>Jawaharlal Nehru Technological University, Hyderabad, India

<sup>3</sup>Nichi-In Centre for regenerative Medicine, Chennai. India.

\*Dr Aejaz Habeeb M, Dept. of Hepatology, Owaisi Hospital, Kanchanbagh, Santosh Nagar, Hyderabad 500058, India. E. Mail; m\_aejazhabeeb@rediffmail.com

Published online on 26 Dec 2006

The hepatobiliary system refers to the liver, gall bladder and bile ducts – organs that are involved with the production, storage, transport and release of bile, a secretion that prepared fats for further digestion

There are numerous conditions that can harm the hepatobiliary system, some of which are life threatening and ultimately, require surgery and/or liver transplantation. Liver damage can occur from a variety of sources: infections with viruses (hepatitis A, B, and C viruses), exposure to toxic drugs or chemicals, excessive use of alcohol, genetic disorders, diabetes, heart failure, cancer and shock

In many cases, the liver is able to repair itself; in others, a variety of treatments may be effective. However, if liver damage is severe, the organ may not recover, resulting in liver failure which is life threatening. Once this happens, the patient may need a new liver.

#### **Cirrhosis (Chronic liver disease)**

Cirrhosis describes a condition in which damaged liver cells are replaced by scar tissue. Over time, the build-up of scar tissue becomes so great that it impacts blood flow through the liver, destroying more liver cells and ultimately leading to greater and greater loss of liver function.

Almost any liver disease can result in cirrhosis, but excessive use of alcohol remains the most common cause.

#### **Hepatitis**

Inflammation of the liver is called hepatitis. Inflammation (or soreness) of the liver can be traced to many different causes, including viral infections, alcohol, fat accumulation in the liver, an incorrectly functioning immune system, exposure to chemicals and other toxins, and certain drugs.

It may be caused due to the following viruses C, B, A and E.

#### **Acute liver failure**

Fulminant hepatic failure is defined as the development of hepatic encephalopathy and profound coagulopathy within 8 weeks of the onset of symptoms in patients without preexisting liver failure disease. The various causes of this devastating condition include Hepatitis A, B and C, ingestion of various hepatotoxins and metabolic liver disorders such as Wilson's disease. There is rapid development of cerebral edema and multiorgan failure within days of clinical presentation. Liver transplantation in fulminant hepatic failure patients is considered in grade III and IV encephalopathy.

The survival rate for these patients without transplantation is less than 20%. Survival with transplantation is 60-80%.

#### Inherited metabolic disorders

Many metabolic diseases cause liver injury in infants and children. In many cases the liver is the sole organ affected clinically. Metabolic liver disease may present as an acute, life-threatening illness in the neonatal period or may be manifested as chronic liver disease, presenting in adolescence or adulthood and progress to liver failure, cirrhosis or hepatocellular carcinoma. Some of the diseases are relatively common. Alpha-1-antitrypsin deficiency occurs in approximately 1 in 1,800 live births, Wilson disease which occur in 1 in 30,000, Tyrosinemia occurs 1 in 100,000 individuals, Porphyrria occurs 1 in 100,000 persons, or OTC deficiency where only 1 individual is affected out of 40,000-80,000.

**Alpha1-antitrypsin deficiency.** This deficiency is the most common inherited cause of liver disease for which liver transplantation is performed in children. Although the frequency of this co dominant recessive disorder is 1: 2,000 to 1:7,000, only a minority of individuals with the Pi ZZ phenotype develop liver disease. Children with alpha1-antitrypsin deficiency often present with neonatal cholestasis. In most of these children, the jaundice gradually resolves, but within the first decade of life, 25% develop cirrhosis. Cirrhosis secondary to alpha1-antitrypsin deficiency can have its first presentation at any age.

**Wilson's disease.** Wilson's disease is an autosomal recessive disorder of copper excretion that can result in either severe acute or chronic hepatitis with liver failure. Other complications of the disease include neurological dysfunction, hemolytic anemia, and renal involvement. Most patients presenting with chronic liver disease respond dramatically to chelation therapy with penicillamine or trientine and have long-term sustained remissions of the disease. In contrast,

patients who present with FHF invariably die unless urgent liver transplantation can be performed. In a study it was reported that Wilson's disease patients who underwent transplantation showed 77% survival with improved neurologic symptoms. Thus liver engraftment appears to cure the underlying biochemical defect.

**Tyrosinemia:** Hereditary tyrosinemia is a genetic inborn error of metabolism associated with severe liver disease in infancy. The disease is inherited in an autosomal recessive fashion, which means that in order to have the disease; a child must inherit two defective genes, one from each parent. In families where both parents are carriers of the gene for the disease, there is a one in four risk that a child will have tyrosinemia. 80% of all the patients with tyrosinemia will die prior to 2 years of age from an acute liver crisis.

**Ornithine transcarboxylase:** OTC deficiency is a serious metabolic disorder caused by a missing enzyme in the liver cells affecting one in 40,000 – 80,000 births. In affected babies the OTC gene in the liver is abnormal and does not make enough of the OTC enzyme, so the liver is not able to clear toxic substances from the blood. Nitrogen accumulates and is converted into ammonia rather than urea. When ammonia reaches the brain through the blood it may cause irreversible brain damage and/or death. The cause of OTC has been identified as a single gene defect.

**Porphyrias:** Porphyrria is a diverse group of diseases in which production of heme is disrupted. These porphyrin disorders usually are found in the liver and are called coproporphyrinuria when limited to coproporphyrins. Physical symptoms may be chronic, evident only during periods of acute attack, both chronic and acute, or they may be absent altogether.

**Biliary atresia:** Biliary atresia is a serious but rare disease of the liver that affects newborn infants. It occurs in about one in 10,000 children. The cause of biliary atresia is not

known, and treatments are only partially successful. Biliary atresia is the most common reason for liver transplantation in children in the United States and most of the Western world. The liver damage incurred from biliary atresia is caused by injury and loss (atresia) of the bile ducts that are responsible for draining bile from the liver. Bile is made by the liver and passes through the bile ducts and into the intestines where it helps digest food, fats, and cholesterol. The loss of bile ducts causes bile to remain in the liver. When bile builds up it can damage the liver, causing scarring and loss of liver tissue. Eventually the liver will not be able to work properly and cirrhosis will occur. Once the liver fails, a liver transplant becomes necessary. Biliary atresia can lead to liver failure and the need for liver transplant within the first 1 to 2 years of life.

Orthotopic (solid or whole organ) liver transplantation (OLT) has significantly improved the prognosis in patients with liver diseases. Because the production of various metabolic enzymes takes place in the liver, a liver transplant can be an effective treatment for metabolic liver disease if advanced liver disease has developed.

It is a highly successful treatment for biliary atresia and the survival rate after surgery has increased dramatically in recent years. Children with biliary atresia are now living into adulthood, some even having children of their own. Because biliary atresia is not an inherited disease, the children of survivors of biliary atresia do not have an increased risk of having it themselves. Improvements in transplant surgery have also led to a greater availability of livers for transplantation in children with biliary atresia. In the past, only livers from small children could be used for a child with biliary atresia because the size of the liver had to match.

However, the number of patients requiring OLT far exceeds the number of solid livers available for transplant and the risks and complications of OLT and the financial costs are high.

### **Hepatocyte transplantation**

Hepatocyte transplantation has been attempted in patients with acute liver failure, end-stage

liver disease and certain liver-based metabolic disorders such as Wilson's disease, Tyrosinemia, Alpha anti trypsin deficiency and urea cycle defects. The rationale behind the technique is that donated hepatocytes will support the failing liver, thus bridging patients to whole organ transplantation or, in some cases, avoiding transplantation while the native liver regenerates. The transplanted cells will produce the missing enzyme in patients with inborn errors of metabolism, correcting the underlying defect. The advantages of hepatocytes transplantation over the conventional method are:

#### **Non-surgical procedure**

Better utilization of donor organs, as cells from one donor could be used for more than a single patient

Native liver is still present as a safety net

Future option of gene therapy, as the native liver cells will be available for genetic manipulation.

#### **Much cheaper procedure**

The hepatocytes are isolated from either cadaver liver or human fetal liver. The donated cells are transplanted as a suspension in to the portal vein via a transhepatic approach and integrate into the recipient liver cell plates. The major limitation of hepatocytes transplantation is the shortage of donor organs, liver cells have to be blood group matched and also there is a need for lifelong immunosuppression as in conventional liver transplantation.

Hepatocyte transplantation has been successful in many animal models of acute liver failure and clinical attempts have been made in humans with encouraging results. The potential advantages of cell transplantation include a simpler, safer, less costly procedure. It is now widely accepted that liver cells have great regenerative capacity in vivo. This proliferative capacity enables transplanted cells to reconstitute injured, or metabolically defective, liver tissue. Liver cells can be infused into the portal vein/ spleen or intraperitoneally. The transplantation of liver cells in ectopic sites (other than liver) is advantageous because of the safety and less

invasive procedure, further in all the above mentioned ectopic sites cells engraft well to perform the synthetic and metabolic functions.

### **Pre-clinical studies on hepatocyte transplantation**

Efficacy of hepatocyte transplantation has been studied in several animal models of ALF. The most commonly used models include galactosamine-induced liver failure in rats, rabbits, guinea pigs and dogs, and thioacetamide-induced liver failure in rabbits and rat. In these experiments, hepatocyte transplantation has shown survival rates of more than 60%. Of the various sites (like intraportal, intrasplenic, intraperitoneal) used for transplantation by different groups, intraperitoneal location appears more appropriate, in view of the large number of cells required to support the liver. In our study we transplanted  $60 \times 10^6$  cells per Kg body weight in D-galactosamine-induced ALF animal model with more than 60% survival rate in treated animals as compared to no survival in untreated controls.

### **Clinical Studies**

Based on the pre-clinical data clinical trials were initiated at different centers. Mito and Kusano were the first to attempt hepatocytes transplantation in cirrhotic patients. Hepatocytes were isolated from the segments of the cirrhotic livers of the patients and transplanted by injection into the splenic pulp, splenic artery, splenic vein, or portal vein. Although the injections were tolerated well and there was some evidence of improvement in encephalopathy, protein synthesis, and renal function, the ultimate clinical outcome was not altered significantly in chronic liver failure. This study was a landmark for taking hepatocyte transplantation into clinics. The clinical study was done at our centre (1994) where 7 acute liver patients with grades III and IV hepatic encephalopathy underwent intraperitoneal human fetal hepatocyte transplantation. The results showed that hepatocyte transplantation might be beneficial in patients with ALF in grade III

or IV encephalopathy. These beneficial effects could be related to their metabolic and detoxification function. Also, the transplanted hepatocytes may proliferate under the influence of hepatotropic factors hereby increasing their total metabolic and detoxifying capacity. Soriano and co-workers transplanted hepatocytes in three patients by infusing the hepatocytes through portal vein. Out of the three only one responded to the therapy. In another study Bilir and co-workers from University of Colorado infused isolated hepatocytes into portal vein via transjugular catheterization. Intraperitoneal transplantation of hepatocytes was done in a 26-year old acute fatty liver of pregnancy patient (Habibullah et al 2005) at our centre, patient recovered within two days of transplantation.

### **Hepatocyte transplantation into the liver led to correct deficiency.**

The engraftment and function of transplanted hepatocytes in a 10 year girl with Crigler-Najjar Syndrome, an inherited disease in which the individual is deficient in the enzyme UDP glucuronosyltransferase which conjugates bilirubin, leading to severe jaundice (Fox et al 1998). The patient was given  $7.5 \times 10^9$  allogenic donor hepatocytes by infusion via portal vein catheter. For 18 months post-transplant, this individual demonstrated significant increases in excretion of conjugated bilirubin in her bile, increased enzyme activity in her liver biopsies and a reduced need for UV light phototherapy.

Similarly, a 5-year-old child with urea cycle disorder, ornithine transcarbamoylase deficiency, received 1 billion hepatocytes and showed clinical improvement. Recently, a 4-year-old patient with infantile Refsum disease received hepatocyte transplantation, which led to partial clearance of abnormal bile acids with pipercholic acid being reduced to 60% of pre-transplantation levels. The child was able to stand and walk 6 months after hepatocyte transplantation.

The limitations of hepatocytes transplantation are

Shortage of donor livers

Large number of hepatocytes is required to treat a single patient

Big size of the hepatocytes that may cause embolization.

Stem cell biology holds great promise in transplantation and has attracted global attention as can be judged by the number of recent papers in many reputed journals on this subject.

### Hepatic stem cells

It was noticed during transplantation that a proportion of hepatocytes undergoes clonal expansion. These cells thus act in a limited sense as stem cells. During severe liver injury, activation of bi-potential small oval shaped cells has been observed, which can differentiate into hepatocytes and biliary epithelial cells (Forbes *et al* 2002). Hepatocytes can also be derived from bone marrow cell populations in humans. Oval cells/hepatocytes could be derived from bone marrow cells in the rat (Peterson *et al*, Alison *et al*, Theise *et al*). Multipotent adult progenitor cells (MAPCs) isolated from bone marrow can give rise to hepatic progenitors and hepatocytes (Schwartz *et al* 2001, Jiang *et al* 2002).

### Sources of hepatic progenitors

Isolation of hepatic progenitors from human source is a major challenge for the clinical application of this therapy. Recently hepatic progenitors have been isolated from the following sources:

#### Sources of stem cells:

**Autologous bone marrow:** Autologous bone marrow will be aspirated in sterile condition from the patient's iliac crest.

**Cadaver liver:** Cadaver livers that had been considered insufficient quality for organ transplantation are the one of the source of hepatic progenitors.

**Aborted fetuses:** Hepatocytes isolated from aborted human fetuses are potential source for the hepatic progenitor cells.

### Hepatic progenitors from fetal liver

Hepatic progenitors originate from endodermal cells that form the so-called hepatic diverticulum. In human embryos hepatic progenitors can be detected by the end of the fourth week. These cells can differentiate into either hepatocytes or biliary cells.

However, specific selection markers for hepatic progenitors from fetal liver need to be still confirmed. In several studies hematopoietic markers like CD34, Thy-1, and c-kit, mRNA, for the flt-3 receptor, previously reported restricted to hematopoietic stem cells have been used for identification/isolation of hepatic progenitors. Sharing of hematopoietic markers by hepatic progenitors is supported by the fact that the liver remains hematopoietic organ during the entire fetal period and for approximately the first week after birth in the neonates.

Malhi *et al* (2002) isolated Stem/progenitor cells from the human fetal liver has demonstrated high differentiation and liver population capacity in the animals, and in under culture conditions, these progenitor cells proliferated for several months, with each cell undergoing more than forty divisions, but they retained normal karyotypes. In our center also we have isolated the progenitor population using counter flow elutriation system, which in culture condition isolated hepatic progenitor cells differentiate to biliary and hepatocytes and have high proliferation capacity.

**Routes of Transplantation: The following routes have been proposed in various liver diseases.**

**1. Acute liver failure patients:** Intra peritoneal

- Percutaneous intra hepatic artery catheterization.

**2. Inherited metabolic disorders patients:**

- Umbilical Vein
- Percutaneous intra hepatic route
- intra hepatic artery

**3. Chronic liver failure patients:**

- intrasplenic

The ability of animal models of human disease and insights into how transplanted cells engraft, function and proliferate in the rodent liver offers unique opportunities to analyze stem cell biology. The ability to work with enriched or highly purified population of human stem/ progenitor cells will help translate this area into exciting clinical applications

## Reference

1. Strom SC, Chowdhury JR, Fox IJ. Hepatocyte transplantation for the treatment of human disease. *Semin Liver Dis* 1999;19:39-48.
2. Fox IJ, Chowdhury JR, Kaufmann SS, et al. Treatment of the Crigler-Najjar syndrome type I with hepatocyte transplantation. *N Engl J Med* 1998;388:1422-6.
3. Riordan SM, Williams SR. The Wilson's disease gene and phenotypic diversity. *J Hepatol* 2001;34:165-71.
4. Brewer GJ. Recognition, diagnosis, and management of Wilson's disease. *Proc Soc Exp Biol* 2000;223:39-46.
5. Sveger T. Prospective study of children with  $\alpha$ -antitrypsin deficiency: eight-year follow-up. *J Pediatr* 1984;104:91-4.
6. Sveger T. The natural history of liver disease in  $\alpha$ -1-antitrypsin deficient children. *Acta Pediatr Scand* 1995;77:847-51.
7. Ryder SD, Beckingham IJ. ABC of diseases of liver, pancreas, and biliary system: acute hepatitis. *Br Med J* 2001;322:151-3.
8. Balistreri WF, Grand R, Hoofnagle JH, et al. Biliary atresia: current concepts and research directions. Summary of a symposium. *Hepatology* 1996;23:1682-92.
9. Bates MD, Bucuvalas JC, Alonso MH, Ryckman FC. Biliary atresia: pathogenesis and treatment. *Semin Liver*