

## Review Article

### **Cell Therapy for Acute Liver Failure - Ideal source of cell**

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Liver is the central metabolic organ that regulates body's energy supply, secretes several essential compounds and clears substances by several methods, including recycling, inactivation and excretion. Global loss of the liver function results in profound metabolic instability and disruption of essential functions such as acid-base balance and thermoregulation leading to acute liver failure (ALF). If this is not rapidly reversed, complications such as uncontrolled bleeding occur, and dependent organs such as brain and kidneys begin to fail, reducing the chance of recovery even further. Acute liver failure (ALF) carries high morbidity and mortality (>80%) even in the best centres.

Presently, orthotopic liver transplantation (OLTx) is the only treatment that improves the survival rate in patients with ALF. Advent of various immunosuppressive agents has improved the success rate of this procedure by preventing rejection. Non availability of donor organs however remained a major limitation.

Two approaches viz., (i) hepatocyte transplantation (ii) extracorporeal liver support system, have been attempted to provide temporary liver support to failing liver till a suitable organ becomes available. These approaches have demonstrated their efficacy in the pre-clinical and clinical studies.

#### **Preclinical studies- Hepatocyte transplantation in experimental models of Acute Liver Failure**

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<sup>1-6</sup>, and thioacetamide-induced liver failure in rabbits and rat<sup>7-11</sup>. In these experiments, hepatocyte transplantation has shown survival rates of more than 60 percent<sup>12-15</sup>. 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 failing liver. We transplanted  $60 \times 10^6$  cells per kg body weight in D-galactosamine induced ALF animal model with more than 60 per cent survival rate in treated animals as compared to no survival in untreated controls<sup>16</sup>.

### **Clinical studies- Hepatocyte transplantation in patients with acute and chronic liver failure**

Based on the pre-clinical data clinical trials were initiated at different centres. Mito and Kusano<sup>17</sup> were the first to attempt hepatocyte 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. This study was a landmark for taking hepatocyte transplantation into clinics. This was followed by the report from our centre in 1994 where seven acute liver failure patients were infused human foetal hepatocytes intraperitoneally<sup>18</sup>. We used allogenic hepatocyte transplantation in human patients with ALF using human foetal hepatocytes. Seven patients with ALF of less than two weeks duration and having grades III or IV hepatic encephalopathy without complicating systemic illnesses underwent hepatocyte transplantation; the preliminary results showed that hepatocyte transplantation may be beneficial in patients with ALF in grade III or IV encephalopathy. Recently at our centre we have performed intraperitoneal transplantation of hepatocytes in a 26 yr old acute fatty liver of a pregnant patient who recovered within two days of transplantation<sup>24</sup>.

Storm *et al*<sup>9</sup> reported successful bridging of patients to OLTx through hepatocyte transplantation. Three of five patients in the study had acute decompensation of chronic liver failure. Soriano and coworkers<sup>20</sup> treated three patients by infusing the hepatocytes through portal vein. Of the three only one

responded to the therapy. In another study, Bilir and coworkers<sup>21</sup> from University of Colorado infused isolated hepatocytes into portal vein via transjugular catheterization. None of the acute liver failure patients survived for long term.

### **Hepatocyte transplantation in metabolic diseases**

In a landmark study reported in 1998<sup>22</sup>, a child with Crigler-Najjar type I, suffering from dangerous hyperbilirubinaemia, was given  $7.5 \times 10^9$  allogenic donor hepatocytes by infusion via portal vein catheter. This procedure resulted in reduction of serum bilirubin levels for more than six months. Similarly, a 5 yr old with urea cycle disorder, ornithine transcarbamylase deficiency, received 1 billion hepatocytes and showed clinical improvement. Hepatocyte transplantation in a 4 yr old patient with infantile Refsum disease, which led to partial clearance of abnormal bile acids with pipercholic acid being reduced to 60 per cent of pretransplantation levels. Hepatocyte transplantation was performed in this case showed that child was able to stand and walk 6 months after hepatocyte transplantation<sup>23</sup>.

Very recently we have demonstrated the efficacy of the hepatic progenitors transplantation managing hyperbilirubinemia in the treatment of crigler-najjar syndrome type 1 by hepatic progenitor cell transplantation<sup>26</sup>. Patient reporting the confirmed case of Crigler Najjar syndrome type 1 of age 2 year female with unconjugated hyperbilirubinemia and bilirubin of  $>30\text{mg/dl}$  was treated with hepatic progenitor cell infusion through hepatic artery. No procedure related complications encountered. No kernicterus was observed. Total Bilirubin started falling 10 days after cell infusion. After 2 month of cell infusion, bilirubin starts decreasing from  $29.0\text{mg/dl}$  to  $16\text{ mg/dl}$ , conjugated bilirubin increasing approximately 5 fold, unconjugated bilirubin decreasing nearly 2 fold and SGPT also decreasing from  $210\text{ U/L}$  to  $64\text{ U/L}$ . This study demonstrates the efficacy of hepatic progenitor cell in

management of hyperbilirubinemia in these patients. As the procedure is very simple and the patient has tolerated the cell therapy, infusion can be repeated as and when required to manage hyperbilirubinemia which often causes lethal kernicterus. This study was developed to assess safely, feasibility and efficacy of hepatic progenitor cell transplantation in a child of Crigler-Najjar Syndrome type-I.

Similarly we have also demonstrated the efficacy of the hepatic progenitors in management of hyperbilirubinemia in confirmed case biliary atresia (BA) with raised bilirubin of 28.5 mg/dl and PELD score 20. Biochemical liver functional tests of this patient show cholestasis (elevated cholesterol & gamma-GTs) and increased ALT, total bilirubin, conjugated bilirubin and ALP. Patient was treated with hepatic progenitor cell infusion through hepatic artery. The total bilirubin and conjugated bilirubin started decreasing during first month after cell infusion. The level of total bilirubin has shown and maintaining its three fold decrease after months of cell infusion. After 2 months of cell infusion, Hepatobiliary scintigraphy showed increased liver cell function. This study demonstrates the efficacy and functionality of hepatic progenitor cell in the management of biliary Atresia in this patient. Further, as there is decrease in the serum bilirubin it shows that there is some percentage of the engraftment of the infused cells. As the procedure is very simple and the patient has tolerated the infusion therapy can be repeated as when required to manage BA<sup>27</sup>.

#### **Autologous bone marrow stem cell transplantation in chronic liver disease (liver cirrhosis)**

Autologous bone marrow derived stem cells also provide important source of cells for treatment of liver diseases. In our centre we have demonstrated the safety and tolerability of injecting autologous bone marrow stem cells (CD34+) in four patients with liver insufficiency. The study was based on the hypothesis that the CD34+ cell population in G-CSF mobilized blood and autologous bone

marrow contains a subpopulation of cells with the potential for regenerating damaged tissue. We separated CD34+ stem cell population from the bone marrow. The potential of the bone marrow cells to differentiate into hepatocytes and other cell lineages is already reported. Besides this, several reports demonstrated the plasticity of the hematopoietic stem cells to differentiate into hepatocytes. Recently Sakaida demonstrated the reduction of fibrosis in chemically induced liver cirrhosis following bone marrow stem cell transplantation. From therapeutic point of view chronic liver cirrhosis is one of the targets where BMC transplantation can be employed. In this condition, there is excessive deposition of extracellular matrix and necrosis of hepatocytes. Encouraged by this evidence that the CD34+ cell population contains cells with the potential to form hepatocyte-like cells, four patients with liver insufficiency were given G-CSF to mobilise their stem cells. 0.1X10<sup>8</sup> CD34+ cells were injected into the hepatic artery. No complications or specific side effects related to the procedure were observed. All the four patients showed improvement in serum albumin, bilirubin, ALT after first month of cell infusion<sup>25</sup>.

#### **Xenogenic liver cells: Alternative source of cells for treatment of acute liver failure**

Use of freshly isolated hepatocytes seems to be a practical approach for obtaining a large number of viable cells. Primary human hepatocytes can be harvested for surgical samples, biopsies, or from liver grafts; however, their availability is limited. To obtain a large number of hepatocytes, several sources have been tried, *viz.*, transformed hepatocyte cell lines, cultured hepatocytes and freshly isolated hepatocytes. Cell line has the advantage of ability to sustain cell growth indefinitely, which is not possible with primary hepatocyte culture; however, alteration of gene expression under culture conditions may pose a problem<sup>28</sup>.

Thus, most groups have used hepatocytes from other species, more often of porcine, goat and rabbit origin. Metabolic, detoxification and

synthetic functions of porcine hepatocytes have been studied extensively.

### **Extracorporeal bioartificial liver support system (BALSS) for the management of Acute liver Failure**

Hepatocytes are major component for the development of any BALSS. Approximately  $25 \times 10^9$  hepatocytes are needed to provide adequate artificial liver support system for patients with ALF<sup>29</sup>.

#### **Immunomodulation of hepatocytes**

The xenogenic source of the hepatocytes offers the use of freshly isolated hepatocytes for development of BALSS and isolated hepatocyte transplantation. To overcome these limitations, two techniques, namely (i) Microencapsulation in alginic acid poly-L-lysine membrane, (ii) UV-B irradiation have been used.

#### **Microencapsulation of goat cells for the development BAL**

Encapsulation of the hepatocytes in alginate poly-L-lysine membrane has been shown to provide protection against such damage<sup>30</sup>. Hence the use of microencapsulated hepatocytes in bioreactor module provides a way for using xenogenic hepatocytes.

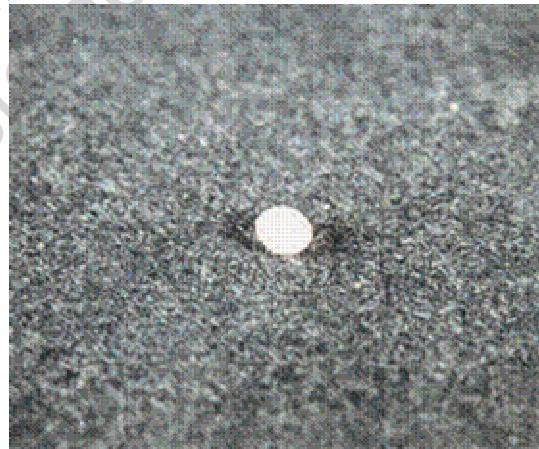
In our study, goat hepatocytes were encapsulated in alginate poly-L-lysine- with the ultimate goal of developing an ideal BAL device and reconfirmed the immunoisolation provided by the encapsulation of hepatocytes and have also evaluated a hollow bioreactor module using these encapsulated hepatocytes and shown its ability to detoxify ammonia to urea<sup>31</sup>. (fig 1)

#### **Xenogenic transplantation of microencapsulated rat hepatocytes in ALF animal model**

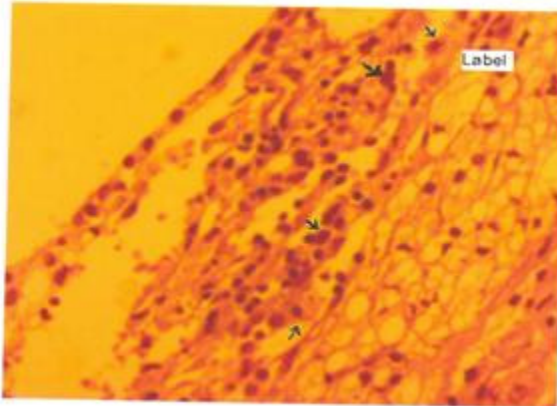
We studied the longevity of the cells after encapsulation following intraperitoneal

administration of encapsulated xenogenic hepatocytes intraperitoneally in a D-galactosamine animal model of fulminant hepatic failure (FHF). All the animals that did not receive hepatocytes died within 36 h. The animals that received encapsulated hepatocytes had better survival rate (73%) whereas only 25 per cent of those that received non-encapsulated hepatocytes survived. The transplanted capsules were found to be intact even 60 days after transplantation with retrieval rate as high as 75 per cent, and 80 per cent of viable cells had normal capacity to produce urea. This suggested that microencapsulation was effective in maintaining functional capacity of and preventing immune destruction of the transplanted hepatocytes.

**Fig 1: Showing single alginate poly-L-lysine microcapsule**



**Fig 2: The histopathology of retrieved gel showing viable cells on day 30**



### **Xenotransplantation of UV-B irradiated hepatocytes in ALF animal model**

Ultraviolet irradiation has emerged as a modality with profound immunomodulatory effect. UV-B irradiation is useful, since it alters the cell surface properties and reduces the immunogenicity of the grafts *in vivo*, while sparing the function of specialized cells. Xenogenic transplantation of UVB irradiated hepatocytes in drug-induced ALF animal model have shown 60 per cent survival rate in treated animals as compared to 23 per cent in untreated animals. No significant change in cellular and humoral immune response was observed in the animals, which received UV-B-irradiated xenogenic hepatocytes<sup>16</sup>.

### **Role of xenoantibodies against galactosyl (alphagal) epitope in (HAR)**

The gal epitope is produced in large amounts in marsupials, in placental non-primate mammals, in prosimians, and in New World monkeys, but not in Old World monkeys, apes or humans. Anti-gal antibodies, on the other hand, are produced abundantly in humans, apes and Old World monkeys. These xenoantibodies recognize the gal epitope formed due to the activity of (α-1-3 β 1-4 GlcNac- R), which is a terminal disaccharide on glycoproteins and glycolipids<sup>33-35</sup>. The gal epitope is formed due to the activity of α-(1-3)-galactosyltransferase. Absence of this enzyme leads to non-expression of the gal epitope in humans, apes, and Old World

monkeys. The interaction of anti gal antibodies with cells carrying the gal epitope leads to lysis (HAR). Several strategies to eliminate the contribution of anti-gal antibodies to HAR have been proposed. These include depletion of the antibodies either nonspecifically by plasmapheresis or specifically by immunoadsorption. Other strategies depend on the depletion or eliminating the Gal epitope from donor<sup>36</sup>. Inactivation of the *GalT* gene has the potential advantage of eliminating anti-gal antibody involvement in HAR permanently and completely. However, it has been suggested that the deletion of the Gal epitope may be lethal. The application of immunoisolation technologies to prevent host sensitization to implanted cells is a feasible approach to avoid xenograft rejection.

### **Longterm survival of intraperitoneal transplanted TGP immobilized cells in acute liver failure rat animal model**

Hepatocytes are anchorage dependent for their long-term survival and functions. Various techniques are being used successfully for providing a substratum for long-term survival of the transplanted cells such as microcarriers, microencapsulation, extracellular matrices but these are derived from animal source. Hence not appropriate for clinical use. There is a need of chemically synthesized biocompatible polymer for clinical application.

Mebiol Gel, an aqueous solution of thermo reversible gelation polymer (TGP) which is a biocompatible polymer. The intraperitoneal transplantation of hepatocytes embedded in TGP (Mebiol Gel) resulted in prolonged survival and function of the cells and was able to support acute liver failure in animal models thus giving a hope that when applied in humans, it could successfully provide liver support in severe acute liver failure when transplanted intraperitoneally<sup>32</sup> Fig (2).

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